Perennial Ryegrass for Dairy Cows: Grazing Behaviour, Intake, Rumen Function and Performance

Hassan Z. H. Taweel

Promotor: Prof. Dr. ir. Seerp Tamminga
Hoogleraar Diervoeding
Wageningen Universiteit

Co-promotoren: Dr. ir. Jan Dijkstra
Universitair docent, leerstoelgroep Diervoeding
Wageningen Universiteit

Dr. ir. Anjo Elgersma
Universitair hoofddocent, leerstoelgroep Gewas- en Onkruidecologie
Wageningen Universiteit

Promotiecommissie:

Dr. J. L. Peyraud (INRA, France)
Prof. Dr. H. H. T. Prins (Wageningen Universiteit)
Dr. A.M. van Vuuren (ASG, Lelystad)
Dr. Dennis Poppi (The University of Queensland, Australia)

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

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Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. Dr. ir. L. Speelman
in het openbaar te verdedigen
op dinsdag 14 september 2004
des namiddags te 16.00 uur in de Aula

Taweel H. Z., 2004. Perennial Ryegrass for Dairy Cows: Grazing Behaviour, Intake, Rumen Function and Performance. Ph.D. Thesis, Wageningen University, Wageningen Institute of Animal Sciences, Wageningen, The Netherlands.

ISBN: 90-8504-096-5

Abstract

Taweel H. Z., 2004. Perennial ryegrass for dairy cows: grazing behaviour, intake, rumen function and performance. In temperate environments, perennial ryegrass (*Lolium perenne* L.) is the most widely used species for feeding dairy cows. That is because of its high productivity, palatability, digestibility and nutritive value. However, perennial ryegrass suffers from two main disadvantages: firstly, its unbalanced nutrient content in terms of crude protein (CP) and energy availability at rumen level and secondly, its low dry matter intake (DMI) by high producing dairy cows. In ruminants, DMI is, in addition to metabolic constraints, a function of the balance between eating motivation, which is strongly related to palatability, on one hand and rumen capacity on the other. This thesis was divided into three parts. The first part aimed to investigate the possibility of improving pasture DMI by increasing rumen capacity. Rumen capacity is related to the rate of clearance (Kcl) of material from the rumen, which is the summation of both rates of degradation (kd) and passage (kp). Six varieties of perennial ryegrass were screened for their clearance and degradation rates using *in vitro*, *in vivo* and *in situ* techniques. The results suggested that there is a narrow range for selection with respect to clearance and degradation rate. The second part of this thesis aimed at investigating the possibility of improving pasture DMI by improving its palatability. Palatability is mainly a function of flavour and taste, which arise from certain compounds in the grass, especially water-soluble carbohydrates (WSC). High sugar grass varieties were fed to dairy cows in mid lactation under stall-feeding and grazing conditions. The results showed that feeding high sugar grass did not lead to an improved DMI and milk yield under either stall-feeding or grazing conditions. Moreover, milk composition in terms of fat, protein and lactose, rumen pH, fibre clearance and degradation rates were not influenced. However, rumen ammonia and milk urea concentrations were significantly reduced when feeding high sugar grass. The third part of this thesis aimed to investigate the role rumen fill and fermentation end products may play in signalling the termination of a grazing bout, especially the dusk grazing bout of dairy cows under continuous stocking. It was found that dairy cows interrupted the first two main grazing bouts at morning and afternoon long before their maximal rumen capacity was reached. Moreover, rumen fill was always maximal at the time when the dusk grazing bout ceased, indicating that rumen fill is more likely to play a major role in signalling the termination of the dusk grazing bout.

Key words: Dairy cows, Perennial ryegrass, Rumen fill, Clearance, Degradation, Grazing behaviour.

Preface

After obtaining my MSc in Animal Nutrition from Wageningen University, and despite the fact that the first words of my supervisors about me during the graduation ceremony were, that I was never in time when the lecture started at 8:00 am, they went on hiring me for a Ph.D. position. I must have made a good impression on them that made them hire me, knowing that I hate to wake early in the morning, which is sometimes required when performing research with dairy cows that need to be milked at 6:00 am. Another reason why they hired me maybe that they did not have any applicants who were willing to take on the job, knowing that most Dutch graduates by then preferred to work for the industry, which offered higher salaries. One of these two reasons could be why I was hired to do my PhD degree in Animal Nutrition. Regardless which one was it, although I hope it was the first one, I am grateful that I was allowed to purse my goals and I was given the opportunity to obtain a PhD degree without having to pay any fees, instead I was paid quite well to do that. This is one of the reasons why I consider myself a lucky person.

The research reported in this thesis focused around two main ideas. The first main idea of this research project was born when Anjo Elgersam (co-promotor) had a meeting with the director of research of Barenbrug seed company in The Netherlands (Laurens Beerepoot). The focus was mainly on perennial ryegrass varieties differences in morphological, physical and chemical characteristic and yield, but upon meeting Seerp Tamminga (head of Animal Nutrition Group), the project was extended to include all kinds of animal digestion, utilisation and performance measurements. Therefore, the project carried all the fingerprints of a multidisciplinary approach, which is considered to be the best way to perform research these days and is promoted by all kinds of funding organisations and bodies. Two departments (Plant and Crop Science and Animal Science) and one private company (Barenbrug Holland BV) were involved in the creation, planning, troubleshooting and conduction of the project. The advantages of multidisciplinary research projects and approach are numerous and obvious, however, there are some disadvantages to multidisciplinary research, which for whatever reason are never highlighted or mentioned. These disadvantages arise mainly from the fact that different groups have different interests and targets, which may lead to combining many targets within one experiment. This may compromise the experimental design and complicate the interpretation of the results. As a junior researcher (PhD fellow), I was involved in the project long after the experimental design was agreed upon and long after the grass was sown in plots based on that design, however, at later stages of the project I was given the opportunity to have my own input and initiatives.

The second main idea of this research project was born when my colleague (Bart Tas) and I were evacuating the rumens of grazing dairy cows. We started the evacuations at 20:00 h and went on till 24:00 h, because we had to evacuate four cows consecutively. We noticed that the rumens of the cows evacuated at around 24:00 h were totally full to such a degree that we had troubles re-filling them back, whereas, the rumens of cows evacuated earlier were not full and had some empty space. We started digging in the literature for research that focused on the role of rumen fill and distension in regulating and controlling short-term intake by grazing dairy cows. We found that, most of the research concerning the role of rumen fill in regulating short-term intake was done under in-doors feeding conditions. In addition, most of that research focused on the influence of rumen fill on daily intake, and was conducted by inserting balloons or inert objects into the rumen. The influence of rumen fill on intake, at different eating sessions or grazing bouts, was rarely investigated. One experiment was conducted with grazing dairy cows, where the researchers (Chilibroste et al., 1997) studied the morning grazing session intensively, and investigated the role rumen fill may play in signalling the termination of this session. They found that dairy cows interrupted the morning grazing bout long before reaching their maximum rumen fill capacity. This was not surprising to us, as from our observations we noticed that the rumens of grazing dairy cows were totally full only around midnight when darkness falls in The Netherlands during summer time. Therefore, we wanted to investigate the role rumen fill plays in signalling the termination of the three main grazing bouts of dairy cows, especially the dusk grazing bout. To do that we tried to be inventive and coupled new and old techniques and technologies together. We used the sophisticated newly developed jaw recorders to know what the animals were doing at all times during the day. We used the rumen evacuation procedure to measure the fluctuation in rumen fill during the day and to be able to obtain an intake value at the different grazing bouts. We utilised dynamic modelling to get a better insight of our collected data. We constructed a small dynamic model that predicts the fluctuation in rumen fill during the day based on intake rate, eating time, eating pattern and clearance data. One of the major conclusions of this experiment was that experimenting with free ranging animals is complex, but at the same time it is a lot more challenging and much more fun than experimenting with stall-fed animals.

Hassan Z. H. Taweel
Wageningen, 8 July 2004

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General Introduction

General Introduction

The goal of most dairy operations in the western world is to maximise milk production in a cost-effective manner while sustaining the health and welfare of the animals and the environment. Feed costs represent a major share of the total milk production costs. To minimise production costs it is important to maximise forage intake, minimise concentrates intake and improve the efficiency of forage utilisation by dairy cows, especially in early lactation. The intense genetic selection for higher milk production during the past decades has resulted in increased genetic potential for milk yield in the modern dairy cow. This has increased the demand for a sufficient energy supply and accentuated the problem of negative energy balance (NEB) in early lactation. It is very likely that cows selected for high milk yield were also indirectly selected for a higher potential to mobilise body reserves and to ingest more feed. However, high producing dairy cows in early lactation fail to consume enough feed to fulfil their energy requirements when offered forage only diets, even if the forage is of a high quality (Van Vuuren, 1993; Kolver and Muller, 1998; Gibb et al., 1999). This has led to the intense use of concentrate rations with high energy density in the dairy industry in order to maximise total dry matter intake (DMI), energy intake and thus alleviate the NEB. Feeding high energy density rations constitutes a financial burden on the producer. This is because such rations are expensive compared to home-grown forages and their feeding might also lead to more rumen health problems and consequently nutritional and metabolic disorders and extra health and medication costs.

Forages are the natural feed source for ruminants and are required for an optimal rumen environment and function. Therefore, maximising daily total energy intake through maximising forage intake and forage energy density rather than through concentrate rations supplementation is very desirable. Maximising intake of young leguminous forages, although beneficial economically, is associated with nutritional and metabolic disorders, such as bloat and alkalosis. However, maximising intake of high quality grasses is the most economical and nutritionally safe way to alleviate the negative energy balance of high producing dairy cows.

Because of its high productivity, palatability, digestibility and nutritive value, perennial ryegrass is the most widely used forage for feeding dairy cattle in temperate environments. At an early stage of maturity, young and leafy perennial ryegrass contains 18 to 24 % DM, 18 to 25 % CP, 40 to 50 % NDF and 6.3 to 7.1 MJ NE_L/kg DM (Clark and Kanneganti, 1998). However, dairy cows fed perennial ryegrass only, either grazed pasture or fed-freshly-cut, fail to achieve a high milk production (kg/d). Reported milk production values of cows fed only pasture grass rarely exceed 25 kg/d (Van Vuuren, 1993; Dalley et al., 1999). The low DMI, rather than energy content of the grass has been identified as the main factor responsible for the lower total energy intake and milk production (Kolver and Muller, 1998). Reported DMI of early lactating dairy cows grazing high quality grass pasture, or fed on freshly cut grass, rarely exceeds 19 kg/d, or 3.25 % of body weight (BW) (Bargo et al., 2003). At this level of pasture intake and energy content, energy input (135 MJ NE_L/d) will be sufficient for a 600-kg grazing cow to cover its maintenance requirements (0.3934 MJ NE_L/kg BW $^{0.75}$) and produce no more than 28 kg of milk per day (3.2 MJ NE_L/kg milk). If such a cow has the potential to produce 40 kg/d of milk, it will require 173 MJ NE_L/d and will be short of 38 MJ NE_L/d, unless it increases its grass DMI to 25 kg/d, or 4.2 % BW. Otherwise, such a cow will lose approximately 1.6 kg live BW per day, assuming that each kg live BW contains 24 MJ NE_L (AFRC, 1995). The amount and rate of tissue mobilisation depend on several factors such as body condition of the cow at calving, milk production level, age, parity, diet composition and level of intake. Although mobilised body tissue contributes a significant amount of milk energy, excessive mobilisation can lead to metabolic disorders, such as ketosis and pregnancy toxaemia (Kronfeld, 1970) and to poor reproductive performance (Butler and Smith, 1989; Staples et al., 1990).

Many interventions to maximise grass DMI have been proposed, studied and implemented. These interventions were mostly related to pasture and grazing management because these factors can be controlled effectively by the producer. Such interventions include manipulating herbage allowance, feeding frequency and timing, time of cutting, pasture maturity, and fertilisation rate. Herbage allowance (kg herbage/cow/day) was identified as one of the most important factors influencing pasture DMI by dairy cows (Hodgson and Brookes, 1999). Pasture DMI increased as herbage allowance increased, but at a progressively declining rate, indicating that the relationship between herbage allowance and pasture DMI is curvilinear asymptotic (Peyraud et al., 1996; Dalley et al., 1999; Bargo et al., 2002). Research results are very variable with regard to the plateau value of herbage allowance at which no extra increase in DMI is expected. Peyraud et al. (1996) reported an increased DMI from 15.3 to 18.5 kg/d as herbage allowance increased from 20 to 54 kg DM/cow/d, with a plateau occurring at an allowance of 33 kg DM/cow/d. Dalley et al. (1999) reported an increased DMI from 11 to 19 kg/d as herbage allowance increased from 20 to 70 kg DM/cow/d, with a plateau occurring at an allowance of 55 kg DM/cow/day. Moreover, Hodgson and Brookes (1999) reported an increased DMI as herbage allowance increased, with a plateau occurring at allowances of 60 to 72 kg DM/cow/d. Despite the fact that increasing herbage allowance increases DMI, the level of increase at the highest herbage allowance did not reach the level needed to satisfy energy requirements of the high producing dairy cow (4.2 % BW). In addition to that increasing herbage allowance constitutes a waste of resources and increases production cost due to the increased need for pasture and land.

It has been suggested that pasture DMI may be increased by more frequent allocation of new pasture, as more frequent feeding maintains a fresher, more palatable pasture. In addition to the fact that such an intervention is labour intensive and results in increased labour and thus production costs, it was found that more frequent allocation of pasture did not increase pasture DMI by dairy cows (Dalley et al., 2001). Changing the time at which the new pasture is allocated was also suggested as a measure to improve DMI by dairy cows. Under strip-grazing systems, allocating the new pasture after the evening milking when DM and water-soluble carbohydrates (WSC) contents are at their maximal level was suggested to be advantageous for increasing pasture DMI. Orr et al. (2001) reported a non-significant increase in DMI from 17 to 18 kg/d when allocated the new pasture after the evening milking as compared to after the morning milking, and they attributed the non-significant level to the low number of replicates in their study. Under a cut and carry system, cutting time was suggested as a measure to increase DMI by dairy cows. Mayland et al. (1998) reported higher DMI by cattle offered alfalfa cut in the afternoon rather than in the morning. Allocating a young and leafy grass has shown to increase DMI when compared with older more mature grass. Parga et al. (2002) reported that at the same level of herbage allowance, dairy cows consumed 2 kg of DM more, when offered 18-day old compared to 38-day old perennial ryegrass.

Altering the N fertilisation rate of grass pasture has been shown to be one of the managerial measures that can be used to improve DMI of dairy cows. On one hand, reducing N fertilisation is known to reduce crude protein (CP) content and elevate DM and WSC contents of the grass, which may lead to a higher DMI due to the lower uptake of water within the grass. However, on the other hand, reducing N fertilisation is known to reduce the green leaf mass per unit of land, which has a major influence on pasture DMI under grazing, either continuous stocking or rotational (Hodgson, 1986; Peyraud et al., 1996). Van Vuuren et al. (1992) reported an increase in DMI for stall-fed dairy cows with grass fertilised at a low rate (250 kg N/ha) compared to a high rate (500 kg N/ha), which was attributed to the higher DM content of the grass at the low fertilisation rate (22 vs. 14 %). In contrast, Delagarde et al. (1997) reported that at the same level of herbage allowance, dairy cows consumed less DM when grazed unfertilised pasture compared to fertilised pasture and attributed that to the lower green leaf mass of the unfertilised pasture. Extremely lowering the N fertilisation results in a reduced grassland productivity through a reduction of the growth-rate, increasing the demand for pasture land and thus increasing production costs. Moreover, a very low fertilisation rate may drastically reduce CP content of the grass and adversely influence DMI due to N shortage for the microbes at rumen level. Most of the managerial interventions mentioned above have the tendency to improve grass DMI by dairy cows, however, none of them has been able to increase grass DMI to the level required to satisfy the energy requirement of the early lactating high producing dairy cow (4.2 % BW). Two questions arise at this point; firstly, what is preventing the cow from consuming more grass per day to a level that satisfies its energy requirement for maximal genetic potential for milk? And secondly, can we manipulate grass quality through selection and breeding or through the choice of ryegrass variety in such a way that it encourages the high producing cow to increase its grass DMI?

Assuming perfect herbage allowance, feeding frequency, environmental and management conditions and a good palatability, feed intake in ruminants is most likely controlled by both physical and physiological factors. Physical factors include the cow's rumen holding capacity (rumen fill) for DM or fibre. Physiological factors include end products of rumen fermentation and intestinal digestion, rumen pH and osmolality, hormones secreted by the endocrine system such as insulin and glucagons, or secreted by the gastrointestinal tract such as gastrin and cholecystokinin (Grovum, 1981). It is generally believed that, as energy density in the ration increases and fibre content decreases, physical factors pose less of a constraint on feed intake and physiological factors become more important in regulating feed intake. Therefore, one may tentatively conclude that the intake of low to medium quality forage may be limited mainly by distension and fill of the rumen, but that when good quality forage is fed, additional factors, mainly physiological, may become important in signalling satiety and consequently limit intake. Within this framework, many factors related to rumen function could influence (not control) DMI. Anything that increases the rate of breakdown of the plant material in the rumen would be expected to increase the throughput and hence the intake that can be attained at a given distension level which would signal satiety (Grovum, 1984). Moreover, anything that contributes to the dilution of fermentation end products in the rumen would also be expected to increase intake at a given concentration level that would signal satiety. Improving microbial activity in the rumen, loosening plant cell wall structure, increasing saliva flow to the rumen and increasing the frequency and strength of rumen contractions would all be expected to positively influence DMI. Conversely, influences which inhibit microbial activity in the rumen and reduce rumination, saliva flow and frequency of rumen contractions (antibiotics, low pH) would be expected to slow down the rate of breakdown of plant material and decrease throughput and consequently intake for a given distension level.

Altering the chemical, physical and mechanical characteristics that contribute to the low DMI of perennial ryegrass pasture through breeding, selection and the choice of variety may be the way forward in trying to maximise its DMI. The main aim of the research reported in this thesis was to verify if altering certain characteristics of perennial ryegrass through the choice of variety would lead to improvements in its DMI and utilisation by dairy cows. Modifying characteristics that contribute to physical limitations such as water and fibre content, degradation rate by microbes in the rumen, or rate of clearance of fibre from the rumen may lead to a considerable improvement in DMI. Choosing varieties that have fast fibre clearance and degradation rates may reduce the residence time of material in the rumen, allow more space for extra material to be ingested and increase DMI. Therefore, an *in vitro* gas production experiment (Chapter 1), an *in vivo* rumen evacuation experiment (Chapter 2) and an *in situ* nylon bag experiment (Chapter 3) were conducted to test if different varieties of perennial ryegrass differ in their fibre clearance and degradation rates. In Chapter 3 also a comparison between the three techniques is reported.

Palatability is a major determinant of what and how much a healthy non-starved animal will eat of a given feed. Palatability includes all of the oral pharyngeal and olfactory sensations arising from the feed such as flavour, taste, smell and texture but does not include any of its post ingestive effects. Flavour and taste provide the primary information for food preference, tolerance or rejection, while visual and olfactory messages function as secondary reinforcers (Chiy and Phillips, 1999). Cattle have been shown to be sensitive to the same principal flavours (sweet, sour, salt and bitter) as humans, but they have different sensation thresholds (Phillips, 1993). Foods with strong bitter, salty and sour flavours were avoided by cattle (Nombekela et al., 1994) or had reduced intakes (Frederick et al., 1988), indicating that these flavours negatively influenced the palatability of the food. In contrast, sweeteners have the potential to enhance palatability at high concentrations and thus increase DMI. Numerous authors have reported the relation between sweet foods and increased intake (Chiy and Philips, 1999; Siever-Kelly et al., 1999). Therefore, breeding perennial ryegrass varieties to have a higher concentration of WSC and sugars, to a level that can be sensed by dairy cows, may improve their palatability further and hence improve their DMI. High sugar grass may also have a lower fibre content, as the increased sugar content has to come on the expense of other components. This may lead to a faster DM degradation and better digestibility and hence improved DMI. However, high sugar grass may increase the acid load in the rumen and reduce pH to a level that is not optimal for cell wall degrading bacteria, counterbalancing the advantages of increased palatability on DMI. A Low rumen pH severely reduces the activity of cell wall degrading bacteria, leading to a lower clearance rate of fibre and particles and hence a decreased throughput and DMI. Lower fibre degradability and intake may also lead to milk fat depression. To examine this apparent controversy, in this thesis we investigate the effect of feeding high sugar grass on DMI, rumen function and parameters and milk production and composition in lactating dairy cows under stall-feeding (Chapter 4) and under grazing (Chapter 5). In Chapter 5, grazing behaviour was also measured to examine the effect of high sugar grass on eating time, ruminating time, bite rate, chewing rate and bite mass of dairy cows.

Most of the research concerning the role of rumen fill and fermentation end products in regulating DMI in dairy cows has been performed with animals kept and fed indoors. Few studies about rumen fill and fermentation end products in grazing dairy cows have been conducted, probably due to methodological limitations. Under grazing many plant and environmental factors in addition to the animal factors may influence DMI. Herbage allowance, sward surface height, tiller density, leaf strength, eating time, time allocated for rumination and chewing are all known to influence the capacity of the animal to consume herbage pasture. A huge amount of research was directed towards understanding and quantifying the effect of plant characteristics on grazing behaviour and DMI of dairy cattle (Gibb et al., 1997; McGilloway et al., 1999; Rook, 2000). However, when plant factors are not limiting, such as under continuous stocking systems where herbage availability is not limited at any time during the day, animal behaviour factors and rumen factors (physical and physiological) may become the prime controllers of DMI. Under such conditions rumen fill and/or fermentation end products may play a major role in signalling the termination of a grazing event. Dairy cows have shown to exercise three main grazing events (bouts) during the day: at morning, afternoon and dusk time (Rook et al., 1994; Gibb et al., 1998; Soriano et al., 2000). During each of these grazing bouts, the dairy cow grazes continuously (non-stop) for more than one hour. Many experiments have shown that the dusk grazing bout is much longer than the other grazing bouts during the day (Rook et al., 1994; Rook and Huckle, 1997; Soriano et al., 2000). Moreover, eating behaviour of dairy cows has shown not to be constant during the day, indicating that dairy cows manipulate their eating behaviour in terms of biting rate and chewing rate as the day progresses and may have a different eating behaviour at dusk time (Phillips and Leaver, 1986; Gibb et al., 1998). In Chapter 6, attention is given to the role rumen fill and fermentation end products may play in signalling the termination of any of the three main grazing events (bouts) especially the dusk grazing bout, and hence controlling DMI under grazing. Moreover, in this Chapter the changes in cow's eating behaviour among the three main grazing bouts were investigated and quantified. Furthermore, based on eating behaviour and grass quality at the different bouts, a dynamic model that predicts the fluctuation in rumen pool sizes during the day was developed (Chapter 6).

The general discussion focused on evaluating the concepts of this thesis and was divided into four sections: the first section evaluated the effect of variety, management, season and time of the day on composition and nutritive value of perennial ryegrass. The second section discussed the concept of improving grass DMI by choosing varieties that have a fast clearing and/or degrading fibre. The third section dealt with the concept of improving perennial ryegrass DMI by feeding varieties that are characterised by their high sugar content. The last section of the general discussion evaluated the effect of management (grazing system), weather conditions (temperature), sward surface height and lactation status on daily grazing behaviour and on grazing behaviour at the three main grazing bouts during the day.

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Chapter 1

The Effect of Grass Variety on Fermentation Characteristics of Perennial Ryegrass Assessed Using the Cumulative Gas Production Technique

H. Z. Taweel[1], B. A. Williams[1], B. M. Tas[1], A. Elgersma[2], J. Dijkstra[1], and S. Tamminga[1]

[1] Wageningen University, Department of Animal Science, Wageningen Institute for Animal Sciences (WIAS), Animal Nutrition Group, P. O. Box 338, 6700 AH Wageningen, The Netherlands.

[2] Crop and Weed Ecology Group (CWE), Department of Plant Science, Wageningen University, P. O. Box 430, 6700 AK, Wageningen, The Netherlands

Submitted to Journal of Animal Science

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Abstract

The effect of grass variety on fermentation characteristics of perennial ryegrass was assessed using the automated in-vitro gas production technique. The experiment aimed to investigate varietal differences and involved six diploid perennial ryegrass varieties. The varieties were sampled three times between early July and late August and fermented using the automated gas production technique with rumen liquid from grass-fed cows as an inoculum. Gas curves were fitted using a di-phasic logistic model, which assumes two fractions, which will result in a mathematically determined division of the gas production curves into two phases. The first phase is assumed to arise from the rapidly fermentable fraction, and the second phase from the slowly fermentable fraction. Variation in fermentation characteristics of perennial ryegrass appeared to be mainly due to variation in the rapidly fermentable fraction, as varieties differed ($P < 0.05$) in the lag time of the first phase (λ_1). Moreover, fermentation of varieties with a higher water-soluble carbohydrates (WSC) content resulted in a higher ($P < 0.05$) propionate proportion, a lower ammonia concentration and a lower ($P < 0.05$) nonglugogenic to glucogenic ratio (NGR). The efficiency of microbial N utilisation as a percentage of N input was higher for varieties with a higher WSC and a lower protein content. The slowly fermentable fraction of the grass was less variable, and less influenced by the variety, as the gas production parameters of the second phase were not different among varieties. Although this study showed that variation in fermentation characteristics and N utilisation efficiency among perennial ryegrass varieties was not large enough, selecting for a high WSC and a low protein content may be beneficial in reducing N losses to the environment.

Key Words: Perennial ryegrass, Variety, Fermentation, Gas production, Ruminants

1. Introduction

Perennial ryegrass is the most widely used grass species for grazing in temperate environments. Many varieties of perennial ryegrass exist. New varieties are being bred, tested, and introduced to the market to be used by dairy farmers. The chemical composition and nutritive value of perennial ryegrass have been shown to be affected by genetic factors (Hannaway et al., 1997), season (McDonald et al., 1995) and management factors, e.g. N fertilisation level (Van Vuuren et al., 1991). Improving the quality of perennial ryegrass is considered to be desirable, especially in terms of increasing protein utilisation and voluntary intake. The unbalanced rumen-available crude protein to energy ratio in perennial ryegrass causes high N losses (Valk et al., 1990). Several ways to decrease N losses associated with grass feeding have been proposed, such as: decreasing N fertilisation (Valk et al., 1996), omitting N fertilisation (Peyraud et al., 1997), supplementing with a rapidly fermentable source of energy-containing concentrates (Bach et al., 1999). Although effective in reducing N losses these proposed ways are associated with negative impact on grass growth and yield and product cost. Therefore, it was thought that grass quality and utilisation may be improved by selection and breeding. For the latter to be feasible, information on fermentation characteristics of different varieties of perennial ryegrass is needed. Therefore, the main objective of this work was to investigate whether there is variation in fermentation characteristics and microbial N utilisation efficiency among perennial ryegrass varieties.

2. Materials and Methods

2.1. Experimental design

The experiment was conducted with six diploid varieties (cv1 to cv6) of perennial ryegrass (*Lolium perenne* L.). These six varieties were selected based on their chemical composition, especially their water-soluble carbohydrates (WSC) content, and on their heading date. One of the varieties (cv1) was expected to have a high WSC content as it was selectively bred for this trait. Of the six varieties, three (cv1, cv2 and cv6) had an intermediate heading date (25 to 28 May), and three (cv3, cv4 and cv5) had a late heading date (7 to 9 June). The varieties were sown in randomised blocks on the same paddock in the autumn of 1999, and received the same management and N fertilisation level (300 kg/ha/yr). Herbage samples from each variety were taken at 6 cm cutting height on 5 July, 2 August and 30 August 2000, at 4 weeks of re-growth stage and approximately 2000 kg DM/ha of target yield. Immediately after sampling, the samples were placed in plastic bags, sealed and stored at –20 °C pending the gas experiment and chemical composition analysis.

2.2. Samples and chemical analysis

Grass samples were freeze-dried and ground to pass a 1-mm screen. Air-dry matter was determined after freeze-drying. Dry matter (DM) content of the samples (previously freeze-dried) was determined after oven drying (4 + 2 h at 104 °C). Organic matter (OM) content was determined by ignition (3 + 1 h at 550 °C). Neutral detergent fibre (NDF) was determined as described by Van Soest et al. (1991). Acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to

Van Soest (1973). Nitrogen was determined using the Kjeldahl method (ISO 5983) and crude protein (CP) was calculated (N × 6.25). Water-soluble carbohydrate content was determined according to Thomas (1977).

2.3. Gas production measurements

Rumen fluid was obtained from three high-producing Holstein-Friesian dairy cows kept on a grass pasture predominated by perennial ryegrass. These cows were offered a minimal amount of concentrates, 1 to 2 kg/d. Rumen fluid was collected 2 h after the morning milking into pre-warmed vacuum flasks, which had been flushed with CO_2. Approximately 500 mg of the ground grass was fermented in 100-ml serum bottles containing 81 ml of a semi-defined medium (Lowe et al., 1987) and 5 ml of inoculum. The inoculum was prepared as described by Theodorou et al. (1994) and comprised 2.5 ml of strained rumen fluid and 2.5 ml of saline. Samples from the inoculum were taken for VFA and ammonia-nitrogen (NH_3-N) analysis. Fermentation kinetics was measured using an automated gas production system as described by Davies et al. (2000) and gas production over 72 hours recorded. All samples were incubated in quadruplicate. Bottles containing no substrate were included as blanks. As four different runs were needed, a standard herbage sample was incubated in the different runs to allow correction for variation in rumen fluid between runs. Gas production curves were fitted using the logistic di-phasic model described by Schofield et al. (1994) and shown in equation 1.

$$V = V_{1F}\ \{1 + \exp(2 + (4\mu_{m1} / V_{1F})(\lambda_1 - t))\}^{-1} + V_{2F}\ \{1 + \exp(2 + (4\mu_{m2} / V_{2F})(\lambda_2 - t))\}^{-1} \ldots(\text{Eq } 1)$$

Where V is the gas volume at any time during the incubation in ml, V_F is the final gas volume in ml, μ_m is the maximum rate of gas production, which occurs at the point of inflection of the gas curve, λ is the lag time in h; the time elapsed between incubation and the start of gas production, and t is the time in h. The numbers (1 and 2) following V, μ_m, and λ, represent the first and second phases arising from the first and second substrate pools, respectively. The term μ_m/V_F has the dimension of time^{-1}, and is referred to as the specific rate of degradation (SR) and is comparable to the rate constant (k) in the exponential equation $V = V_0 \exp^{-kt}$ (Schofield et al., 1994).

In this model the first phase is assumed to arise from the first substrate pool, which is assumed to be the rapidly fermentable fraction. The second phase is assumed to arise from the second substrate pool, which is assumed to be the slowly fermentable fraction. The gas production parameters were corrected for the standard. To correct for the standard, a correction factor (CF) was created for every run by dividing the grand average of the standard sample in the four runs by the observed value of the standard in that run, as shown in equation 2.

CF run1 = average of standard in the 4 runs / observed standard value in run 1…(Eq 2)

After 72 h of fermentation, samples from the fermentation fluid were taken and analysed for VFA and NH_3-N concentrations. For VFA analysis, 10 ml of the buffered rumen fluid was added to a glass bottle that contained 0.5 ml of 85 % phosphoric acid (Merck Art. 573, Amsterdam, The Netherlands). Samples were centrifuged at 10,000 × *g* for 10 min. After centrifugation, 0.5 ml of the supernatant, 0.2 ml water and 0.3 ml internal standard (4 g of 4-methyl-valeric acid per liter) (Merck Art. 806088, Amsterdam, The Netherlands) were transferred to an autosampler glass vial for VFA analysis by gas chromatography (GC). Afterwards, 1 µl of the mixture was injected into a 1.8 m × 2 mm column in a GC type Fisons HRGC MEGA2, fitted with a flame ionisation detector. The temperature of the injector was 180 °C and of the detector 225 °C; the carrier gas flow was pure nitrogen saturated with formic acid. VFA values were corrected for the inoculum values and for the standard. The correction for the inoculum was done simply by subtracting the amount of VFA present in the inoculum before incubation, from the observed VFA value of every bottle.

For NH_3-N analysis, 5 ml of the buffered rumen fluid was added to a glass bottle that contained 5 ml of 10 % Trichloroacitic acid (TCA). The concentration of NH_3-N was determined by the indophenol method, or the Berthelot reaction as described by Searle (1984). The values of NH_3-N concentrations were corrected for the values of the blank and the standard samples. The correction for the blank (bottles without substrate) was done by subtracting the amount of NH_3-N produced by the blank, from the observed NH_3-N value of every bottle.

The OM loss from the bottles was estimated by filtering the residue remaining in the bottles through sintered crucibles and incinerating at 550 °C for 4 h. The OM fermentability was calculated as the OM loss divided by OM input to the bottles and was corrected to the blank and standard.

2.4. Statistical Analysis

The data (chemical composition, gas parameters, VFA, NH_3-N) were subjected to a two-way ANOVA analysis using general linear model procedures of SPSS (SPSS for Windows, Rel. 10.1.0. 2000. Chicago: SPSS Inc.). The sources of variation in the analysis were the variety and the sampling date. The interaction between variety and sampling date could not be accounted for in the models, as the degrees of freedom were not sufficient to test for it. Therefore the assumption that no interaction occurred was added as an additional requirement for the models. For gas data, average values of the four bottles (replicates) were used in the models, as samples were not randomly allocated to the bottles. The multiple comparison procedure Tukey was used to separate least square means. Unless stated otherwise, differences of $P < 0.05$ were considered to be significant.

3. Results and Discussion

Table 1 shows mean chemical composition of the six grass varieties used in the experiment. The varieties differed significantly in their DM, OM, CP, WSC, NDF, and ADF content. Varieties (cv1 and cv4) had a high WSC content compared to the other varieties. The high WSC content of cv1 was expected, as this variety was selectively bred for an elevated concentration of WSC. However, cv4 also had an elevated concentration of WSC although it was not bred for that trait. Variety (cv3) had the highest CP content and cv6 had the highest fibre content. Wilkins et al. (2000) also reported highly significant differences among perennial ryegrass varieties in DM and CP content. Moreover, Radojevic et al. (1994) and Smith et al. (1998) reported differences among perennial ryegrass varieties in terms of WSC, CP and NDF content. The chemical composition of the grass varieties in our experiment at 300 kg N/ha/yr agrees with what was reported by Valk et al. (1996) and Peyraud et al. (1997), who worked with similar herbage in terms of re-growth stage and fertilisation level. However, they all reported a higher NDF content of approximately 500 g/kg DM, which may reflect a difference in the drying procedure (freeze-drying vs. oven-drying) of the samples and/or differences in weather conditions (radiation, temperature, humidity, and rainfall). Goering (1972) showed a significant effect of heating on the cell wall content and characteristics.

Table 1. Mean chemical composition (g/kg DM) of six perennial ryegrass varieties

	Variety							
	cv1	cv2	cv3	cv4	cv5	cv6	SEM[1]	*P* value
DM [2]	167^{ab}	168^{ab}	177^{a}	175^{ab}	173^{ab}	166^{b}	3	0.02
OM	888^{a}	881^{ab}	882^{ab}	880^{ab}	877^{b}	877^{b}	2	0.05
CP	159^{ab}	159^{ab}	166^{a}	151^{b}	155^{ab}	159^{ab}	6	0.01
NDF	415^{a}	430^{ab}	426^{ab}	414^{b}	422^{ab}	436^{b}	3	0.03
ADF	241^{ab}	250^{bc}	244^{ab}	240^{a}	245^{abc}	254^{c}	1	0.00
ADL	16.8	16.4	15.9	15.2	17.0	17.0	0.3	0.18
WSC	181^{a}	160^{ab}	153^{b}	180^{a}	157^{ab}	141^{b}	8	0.00
OM residue [3]	111	110	115	113	122	122	3	0.38

[1] SEM is the standard error of the mean (n = 18)

[2] DM is expressed as g/kg fresh material

[3] OM residue is calculated as OM - CP - WSC - Fat - NDF

[a, b, c] Means in the same raw with different superscripts differ significantly ($P < 0.05$)

Table 2 shows mean gas production parameters, based on a di-phasic logistic model fit of the six varieties. This model allows the separation of the material into two substrate pools, by mathematically dividing the gas curves into two phases. The model fitted the curves well, as the R^2 values were higher than 0.995 and the mean squares of the error (MSE) around 1.05.

Table 2. Mean gas production parameters of six perennial ryegrass varieties, based on a di-phasic logistic model fit of their gas production curves

	Variety							
Parameter [1]	cv1	cv2	cv3	cv4	cv5	cv6	SEM[2]	*P* value
V_{1F}, ml	82.1	82.8	85.7	89.4	80.9	80.2	1.4	0.29
SR_{1}, % h^{-1}	18.0	16.0	16.6	16.2	18.1	16.5	0.4	0.46
λ_{1}, h	1.3^{ab}	1.4^{a}	1.1^{ab}	1.3^{ab}	1.0^{b}	1.1^{ab}	0.1	0.02
V_{2F}, ml	57.3	57.0	53.0	54.6	59.7	59.4	0.9	0.20
SR_{2}, % h^{-1}	6.5	5.9	5.9	5.7	6.6	6.0	0.1	0.27
λ_{2}, h	4.6	5.4	4.9	5.3	4.2	4.6	0.2	0.23

[1] V_{1F} and V_{2F} are the total amounts of gas in ml produced by the first and the second phase, respectively. SR_1 and SR_2 are the specific rates of degradation of the first and second phase, respectively. λ_1 and λ_2 are the lag times: time elapsed between the inoculation and the start of gas production in hours, for the first and second phase, respectively.

[2] SEM is the standard error of the mean (n = 18)

[a, b] Means in the same raw with different superscripts differ significantly ($P < 0.05$)

The total amount of gas (V_{1F}) and the specific rate of degradation (SR_1) of the first phase were not significantly different among varieties, whereas the lag time of the first phase (λ_1) was significantly different among varieties. Variety (cv5) had a shorter ($P < 0.05$) λ_1 when compared with cv2 (Table 2). This might indicate that the rapidly fermentable fraction of different grass varieties might differ in the time needed for it to become available for the microbes in the rumen. In contrast, Miller et al. (2001) reported, based on an *in vitro* gas production experiment, no differences in lag time between two perennial ryegrass varieties differing in their WSC content. They also observed a significant difference in degradation rates between those two varieties, which was attributed to the lower fibre fraction within the high WSC variety. In their experiment they used a mono-phasic model to fit the gas curves, which represented the whole material as one fraction, therefore, distinguishing fermentation kinetics for the rapidly and slowly fermentable fractions was not possible.

The gas parameters of the second phase (V_{2F}, SR_2, λ_2) did not differ significantly among varieties, indicating that the slowly fermentable fraction of grass is less variable and less influenced by variety than the rapidly fermentable fraction. Williams et al. (2000) divided Italian ryegrass leaves into their cell walls and cell contents and fermented them separately. They concluded that cell contents were more variable in fermentation characteristics than cell walls.

In the present experiment, the first phase produced 60 % of the total gas. This supports the observations of Williams et al. (2000), who also reported that 60 % of the gas was produced by the first phase when fermenting whole leaves of Italian ryegrass and using a di-phasic logistic model to fit the gas curves. In contrast, other authors (Groot et al., 1996; Cone et al., 1997), who worked with perennial ryegrass, found that the first phase produced much less gas in comparison with the second phase. These authors, however, used a different multi-phasic model to fit their gas production curves. In general, 4-week old perennial ryegrass contains 880 g OM/kg DM, of which 423 is NDF and the remaining 457 is non-fibre-OM. The WSC and CP make up 320 g, and fat makes up 30 g, leaving 107 g unaccounted for. Fernandez-Rivera (1998) reported that fermentation of one gram of protein, cellulose, glucose and starch yielded 202, 524, 393 and 497 ml of gas, respectively. Calculating the hypothetical gas production by each phases using these values assuming that no interaction between substrates occurs, which could not be necessarily the case, showed that the first phase produces a maximum of 45 % of the total gas. This value is lower than the 60 % observed in the present study, therefore, this might point to the direction that part of the NDF may be rapidly fermentable and is accounted for in the first phase by the logistic di-phasic model. Williams et al. (2000) fermented isolated Italian ryegrass cell walls and concluded that their fermentation exhibited two phases, of which one was rapidly fermentable.

Table 3 shows the mean VFA and NH_3-N concentrations in the fermentation fluid after 72 h of fermentation of the six varieties. Only the propionate proportion and the non-glucogenic to glucogenic ratio (NGR) differed significantly among varieties. Varieties with a high WSC content (cv1 and cv4) produced less acetate and more ($P < 0.05$) propionate, therefore they had a lower ($P < 0.05$) NGR than the other varieties. Moreover, fermentation of varieties with a high WSC content resulted in a lower NH_3-N concentration. It has been shown in several studies (Stokes et al., 1991; Bach et al., 1999) that adding sugars or easily fermentable carbohydrates to in-vitro rumen systems, elevates propionate proportion and decreases NH_3-N concentration and NGR. Rumen microbes tend to shift to fermentation pathways that result in propionate as an end product when they have an excess of easily and rapidly fermentable substrates (Strobel & Russell 1986). Moreover, when energy availability improves, rumen microbes' capability to utilise NH_3-N available in the medium increases, resulting in a lower NH_3-N concentration (Russell et al., 1983; Dahlberg et al., 1988; Bach et al., 1999). The fact that varieties with a high WSC content also had a lower CP content (Table 1) might have contributed to the lower observed NH_3-N concentration in their fermentation fluid. As differences were only detected among varieties that differed significantly in their WSC content, this might lead to the conclusion that variation in fermentation characteristics within perennial ryegrass is mainly due to variation in the rapidly fermentable fraction.

Table 3. Mean VFA molar proportions (mol/100 mol) and NH_3-N concentration (mg/L) produced by fermentation of six perennial ryegrass varieties

	Variety							
	cv1	cv2	cv3	cv4	cv5	cv6	SEM[1]	*P* value
Total VFA [2]	46.2	45.0	44.1	45.4	46.1	45.3	0.4	0.70
Acetate, %	58.5	58.9	58.5	58.3	59.0	59.4	0.2	0.06
Propionate, %	23.6^{a}	23.1^{ab}	23.0^{ab}	23.6^{a}	22.8^{b}	22.8^{b}	0.2	0.04
Butyrate, %	9.4	9.4	9.6	9.2	9.4	9.4	0.1	0.40
Isobutyrate, %	1.5	1.5	1.6	1.6	1.6	1.6	0.0	0.80
Isovalerate, %	3.6	3.7	3.9	3.7	3.7	3.6	0.1	0.50
Valerate, %	3.2	3.3	3.4	3.6	3.4	3.2	0.1	0.09
NGR [3]	3.0^{ab}	3.1^{b}	3.1^{b}	2.9^{a}	3.1^{b}	3.1^{b}	0.0	0.01
NH_3-N, mg/L	44.3	48.1	54.0	37.6	49.5	44.3	4.1	0.19

[1] SEM is the standard error of the mean (n = 18)

[2] Total VFA is expressed as mM

[3] NGR is the nonglucogenic to glucogenic ratio, calculated as (Acetate + (2 × Butyrate) + Valerate) / (Propionate + Valerate)

[a, b] Means in the same raw with different superscripts differ significantly ($P < 0.05$)

Nitrogen utilisation efficiency and microbial N synthesis efficiency are presented in Table 4. Nitrogen utilisation efficiency was calculated using N input to the bottle in mg (CP/6.25) × 0.5 g) and N output from the bottle as ammonia (NH_3) in mg (NH_3-N mg/L × 0.086 L). The difference between input and output was assumed to have been utilised by the microbes to grow. As shown in Table 4, the amount of N utilised in mg was rather similar for all varieties at around 8.6 mg. However, when this amount was expressed as a percentage of N input, it appeared that cv4 had a higher ($P > 0.05$) nitrogen utilisation of 74 % compared to 65 to 70 % for the other varieties. Variety (cv4) had the lowest CP content, therefore the lowest N input, and the highest non-CP-OM input to the bottles. This means that more energy generating substrate was available to utilise less N when this variety was fermented. However, cv1 also had a higher non-CP-OM input, but at the same time it had a higher N input, therefore, the same amount of energy generating substrate was available to utilise more N. Satter and Slyter (1974) showed that microbes have a maximum capacity to utilise N with the available energy.

The efficiency of microbial protein synthesis (g microbial N/kg available OM) was calculated based on N input and output and OM input to the bottle using two approaches. In the first approach, it was assumed that 80 % of the OM was available to the microbes. In the second approach it was assumed that microbes generate energy only from the fermentable non-CP-OM. For this purpose the OM fermentability (OMF) was calculated using the remaining OM in the bottles after 72 hours of incubation (Table 4). It was shown that microbial protein synthesis was constant (P = 0.68) at around 25 g microbial N/kg available OM for all the varieties. This same value is used in the Dutch and French protein evaluation systems to calculate microbial protein synthesis in the rumen, based on fermentable OM (FOM) (Tamminga et al., 1994).

Table 4. Nitrogen utilisation and microbial N synthesis efficiency (g microbial N/kg FOM) for six perennial ryegrass varieties

	Variety							
	cv1	cv2	cv3	cv4	cv5	cv6	SEM[1]	*P* value
N input, mg	12.7^{ab}	12.7^{ab}	13.2^{a}	12.1^{b}	12.4^{ab}	12.7^{ab}	0.5	0.01
N output, mg	3.8	4.1	4.6	3.2	4.3	3.8	0.4	0.19
N Utilised, mg	8.9	8.6	8.6	8.8	8.2	8.9	0.3	0.56
N utilised, %[2]	70.8	68.1	65.3	73.8	66.5	70.9	2.0	0.31
OM input, mg	444^{a}	441^{ab}	441^{ab}	440^{ab}	438^{b}	439^{ab}	1.0	0.05
Non-CP-OM input, mg	365	361	358	365	361	359	2.8	0.12
OMF, % [3]	92	91	91	91	92	92	0.3	0.72
Microbial N synthesis 1 [4]	25.0	24.4	24.4	25.1	23.3	25.4	0.7	0.63
Microbial N synthesis 2 [5]	26.7	26.4	26.4	26.7	24.7	27.0	1.0	0.68

[1] SEM is the standard error of the mean (n = 18)

[2] is N utilised as % of N input

[3] OMF is the fermented organic matter as a percentage of organic matter that was incubated in the gas bottles after 72 h of fermentation

[4] is microbial N synthesised as g/kg FOM, assuming 80 % availability of OM to the microbes (OM input in mg × 0.8)

[5] is microbial N synthesised as g/kg FOM, assuming that microbes obtain energy from the fermentable non-CP-OM (Non-CP-OM × OMF %).

[a, b] Means in the same raw with different superscripts differ significantly ($P \leq 0.05$)

4. Conclusions

The variation in fermentation characteristics among perennial ryegrass (*Lolium perenne* L.) varieties appeared to be mainly due to variation in the rapidly fermentable fraction. The slowly fermentable fraction was less variable and less influenced by the variety. Higher protein utilisation was observed for varieties with a lower protein and a higher water-soluble carbohydrate content.

Acknowledgements

The authors wish to extend their gratitude to H.Y. Negash for the lab work and to Harm Smit for WSC analysis. This research was financed by Barenbrug Holland BV and the Ministry of Economic Affairs (Economische Zaken) in The Netherlands.

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Chapter 2

Improving the Quality of Perennial Ryegrass (*Lolium perenne* L.) for Dairy Cows by Selecting for Fast Clearing or Degrading Neutral Detergent Fibre

H.Z. Taweel [1], B.M. Tas [1], J. Dijkstra [1], S. Tamminga [1]

[1] Wageningen Institute for Animal Sciences, Wageningen University and Research Centre, Department of Animal Science, Animal Nutrition Group, P. O. Box 338, 6700 AH, Wageningen, The Netherlands

Submitted to Livestock Production Science

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H.Z. Taweel, B.M. Tas, J. Dijkstra, S. Tamminga

Abstract

Neutral detergent fibre (NDF) fractional clearance rate (Kcl) and fractional degradation rate (kd) of six varieties of perennial ryegrass were measured to examine the possibility of selecting for varieties with fast degradable NDF. The experiment was conducted in 2000 and repeated in 2001. In each year, six multiparous rumen-cannulated dairy cows were stall-fed with six varieties of perennial ryegrass during three two-week periods, using a double 3 × 3 Latin square design. The NDF fractional clearance rate (Kcl_{NDF}) and the acid detergent lignin fractional clearance rate (Kcl_{ADL}) were estimated using two consecutive rumen evacuations, separated by a 12-hour period of feed deprivation assuming a first order kinetics. The NDF fractional degradation rate (kd_{NDF}) was calculated based on the assumption that NDF fractional passage rate (kp_{NDF}) was equal to Kcl_{ADL}. The Kcl_{NDF} of the different grass varieties was in the range of 5 to 6 %/h. The kd_{NDF} was lower than reported *in situ* values and ranged from 2 to 3 %/h. The Kcl_{NDF} and kd_{NDF} were not significantly different among grass varieties in both years. The difference between the fastest disappearing and degrading variety and the slowest one was less than 1 %/h. This may indicate that within perennial ryegrass varieties there is a narrow range of improvement with regard to clearance and degradation rates.

Key words: dairy cow; clearance rate; degradation rate; perennial ryegrass; fibre

1. Introduction

Grass is a very important source of nutrients for domesticated ruminants as it forms a big portion of their diet for a large part of the year. In temperate environments, perennial ryegrass is the most widely used species for grazing because of its high palatability and digestibility (Delagarde et al., 2000). However, perennial ryegrass on it is own, cannot sustain a high milk production because of its limited intake and nutritional composition, and it may result in high losses of N to the environment (Valk et al., 1990; Tamminga, 1992; Van Vuuren et al., 1993). Improvements in the utilisation of grass by ruminants in terms of maximizing its intake and optimising its microbial conversion in the rumen are highly desirable.

Grass quality in terms of intake and protein utilisation may be improved by selection and breeding. One way to improve the utilisation of grass by breeding could be by selecting for fast degradable fibre. This would decrease the residence time of the course material in the rumen and, therefore, improve herbage intake and the nutritional status of the high producing dairy cow. Moreover, it would improve the availability of carbohydrates for the micro-organisms in the rumen and decrease N losses. However, at present it is not yet known if there is sufficient genetic variation within perennial ryegrass to allow selection for fast degradable fibre. *In vitro* research results are not consistent; Lantinga et al. (1996) suggested that perennial ryegrass varieties might differ in their cell wall (fibre) degradation rates. Whereas, Taweel et al. (submitted) (Chapter1) suggested, based on an *in vitro* gas production experiment, that variation within perennial ryegrass varieties is mainly due to variation in the rapidly fermentable fraction and that the slowly fermentable fraction of grass is less variable and less influenced by the variety. Therefore, the objective of this study was to examine if different varieties of perennial ryegrass differ in their NDF fractional clearance rate (Kcl_{NDF}) and NDF fractional degradation rate (kd_{NDF}) *in vivo*.

2. Materials and methods

2.1. Experiments

In two experiments, performed in 2000 between 24th June and 1st September, and in 2001 between 29th June and 7th September, lactating dairy cows were stall-fed with six varieties of perennial ryegrass. Between the two years, the experiments differed slightly in that; different animals were used for each year, N fertilisation rate was 300 and 400 kg/ha per year and the animals received 4.6 and 2.8 kg of concentrates per day in 2000 and 2001, respectively.

2.2. Animals and Feeding

In the year 2000, six multiparous high producing Holstein-Friesian dairy cows (625 ± 6 kg BW) in mid lactation (126 ± 21 DIM) were used in the experiment. The cows produced 28.8 ± 1.8 kg/d of milk at the start, and 21.9 ± 1.4 kg/d at the end of the experiment. In 2001, six multiparous high-producing Holstein-Friesian dairy cows (563 ± 5 kg BW) in early-mid lactation (77 ± 14 DIM) were used. These cows produced 28.4 ± 1.2 kg/d of milk at the start, and 24.2 ± 0.8 kg/d at the end of the experiment. In both years, all six cows were fitted with a large rumen cannula (Type 1C, 10 cm id, Bar Diamond, Inc., Parma, Idaho, U.S.A.). Surgical procedure and experimental layout were approved by an ethical committee, and executed in accordance with Dutch legislation on the use of experimental animals. Two weeks before the experiment started, the cows were housed individually in the tie-stall and were fed on fresh grass. This was done to adapt the cows to the tie-stall and grass feeding. During the experiment, the cows were fed *ad libitum* with different varieties of fresh perennial ryegrass. Feeding took place daily at 6:00, 9:00, 12:00, 16:00, 18:00, and 22:00 h taking care that the animals always had an ample amount of grass in front of them. Grass was mown daily, starting at 13:00 h, and carried to the shed where it was sampled for dry matter (DM) determination, filled in boxes and weighed. Each box was filled with 10 to 12 kg of fresh grass. The boxes to be fed at 16:00 and 18:00 h were placed in the tie-stall next to the animals, whereas the other boxes to be fed at 22:00, 6:00, 9:00 and 12:00 h were placed in a cooling unit at 4 ºC. In addition to *ad libitum* grass, the cows received daily 4.6 kg concentrates in 2000, and 2.8 kg in 2001. The composition of the concentrates is shown in Table 1. Concentrates were fed twice daily, after the morning (6:00 h) and the evening (16:00 h) milking. The temperature in the tie-stall was maintained below 21 ± 2 °C, using cooling fans. Fresh water and mineral blocks were available all the time to the animals. Feed refusal (residue) was collected once daily at 16:00 h, weighed, and sampled for DM determination.

Table 1. Chemical composition (g/kg as fed) and ingredients of the concentrates used in the experiments, as given by the feed company (Rijnvallei, Wageningen, The Netherlands)

Ingredients		Chemical composition	
Feedstuff	Percentage	DM (g/kg)	875
Beet pulp	21 %	Ash (g/kg)	46
Maize	21 %	Fat (g/kg)	27
Barley	21 %	Starch (g/kg)	261
Soya hulls	11.5 %	Sugars (g/kg)	106
Coconut peels	10 %	CP (g/kg)	126
Beet molasses	8 %	NDF (g/kg)	238
Argentinean Soya	7.5 %	ADL (g/kg)	20

2.3. Diet and Experimental Periods

Six diploid varieties (cv1 to cv6) of perennial ryegrass (*Lolium perenne* L.) were sown in the autumn of 1999. They received the same amount of N fertilisation (300 kg/ha in 2000, and 400 kg/ha in 2001) and the same management. The varieties were cut daily at the same re-growth stage (4 weeks) and target yield (2000 kg DM/ha) and stall-fed to the six cows in three periods, using a double 3 × 3 Latin square design. Varieties (cv1 to cv3) were used in the first Latin square, whereas, varieties (cv4 to cv6) were used in the second Latin square. Each period consisted of two weeks: one week for adaptation and the other for measurements.

2.4. Rumen Evacuation and Sampling

Two rumen evacuations were done for each animal at the end of the second week of each period. The first evacuation was done on Thursday evening at 19:30 h and the second on Friday morning at 9:00 h. The animals did not receive any feed between the two evacuations, but water was freely available during this time. All rumen contents that could be removed by hand (called mat) were emptied into a big rectangular insulated container. During removal of rumen contents a 10 % sample was taken by putting each 10th hand-full in a separate small (20 L) plastic container. Material not removable by hand (bailable liquid) was bailed into another 20-L plastic container using a 1-L plastic bottle. Rumen contents in the big and small containers were weighed and the bailable liquid was proportionally added to the big and the small containers. The contents of the small container were strained through cheesecloth by hand squeezing, and the liquid to solids ratio was determined. Based on this ratio, three representative samples of total rumen contents (solids + liquid) of 400 g were reconstituted and stored in a freezer (-20 °C) pending further analysis. The animals were rumen-evacuated one at a time; therefore, the time when the evacuation started and ended for each animal in both days (Thursday and Friday) was registered. At the evening evacuation on Thursday, while one animal was being rumen-evacuated the other animals had access to fresh grass until 15 minutes before their rumen evacuation.

2.5. Grass Sampling

As rumen evacuations were done on Thursday and Friday of each measurement week in each period, grass samples from the different varieties on offer were collected on Wednesday and Thursday, as these will best represent what is in the rumen on Thursday and Friday. These grass samples were stored in the freezer (-20 °C). At the end of the experiment these grass samples were freeze-dried and the two days samples of each variety in each period were pooled for further analysis.

2.6. Samples and Chemical Analysis

To calculate daily dry matter intake (DMI), DM was determined in the daily grass and refusal samples by sampling approximately 300 g using a grass-core, and oven drying at 70 °C for 24 h.

Pooled grass and rumen content samples were freeze-dried and ground to pass a 1-mm screen. Air-dry matter was determined after freeze-drying. The DM content of the samples (previously freeze-dried) was determined after oven drying (4 + 2 h at 104 °C). Organic matter (OM) content was determined by ignition (3 + 1 h at 550 °C).

Neutral detergent fibre (NDF) was determined as described by Van Soest et al. (1991). Acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest, (1973). Nitrogen was determined using the Kjeldahl method (ISO 5983) and crude protein (CP) was calculated (N × 6.25). Water-soluble carbohydrate (WSC) was determined only in the grass samples according to the procedure described by Thomas (1977).

2.7. Calculations

Daily grass DMI (kg/d) was calculated for each cow as the difference between the daily grass DM offered and the daily DM refused. To get an estimate of the DMI by each animal in each period, the daily DMI was averaged over the last eight days of each period neglecting the last day when the evacuation was performed. The daily intake of the different components (OM, N, WSC, NDF and ADL) was estimated from the daily DMI and the percentages of these components in the grass varieties. Rumen pools (kg) of the different components (DM, OM, NDF and ADL) were estimated from the weight of the total fresh rumen contents in the first evacuation and the percentages of these components in the rumen contents. Rumen pools were also expressed in g/kg BW. Rumen NDF pool was divided into potentially digestible NDF (DNDF) and indigestible NDF (INDF) pools, based on the equation presented by Chandler (1980) in which the INDF fraction is predicted based on the lignin concentration as shown in equation 1.

$\mathrm{INDF} = 2.4 \times (\mathrm{ADL}/\mathrm{NDF})$……………………………………..(Eq 1)

Where INDF is the proportion of NDF remaining after 144 hours *in vitro* fermentation and ADL/NDF is the proportion of lignin contained in the NDF.

Rumen fractional clearance rate (Kcl) (%/h) for the different pools was calculated based on the assumption of first order kinetics, using the logarithmic transformation of the exponential equation: $R_{(t)} = R_{(0)} \times e^{-\mathrm{Kcl} \times t}$, where $R_{(t)}$ is the amount present at any time t (the second evacuation), $R_{(0)}$ is the amount present at time zero (the first evacuation), Kcl is the fractional clearance rate in %/h, and t is the time between the end of the first evacuation on Thursday and the start of the second evacuation on Friday in hours.

The clearance rate represents both passage and degradation, as these are the only processes by which the different pools leave the rumen. The production of DM, OM, and CP by the microbes in the rumen complicates the calculation of DM, OM, and CP clearance rates. However, rumen microbes do not produce any NDF allowing a good estimation of the NDF fractional clearance rate ($\mathrm{Kcl}_{\mathrm{NDF}}$) and the DNDF fractional clearance rate (Kcl_{DNDF}). To calculate the fractional degradation rate of NDF (kd_{NDF}) and DNDF (kd_{DNDF}) an estimate of the fractional passage rate is needed. This was done using the ADL as an internal marker, assuming that it is completely undegradable in the rumen and its clearance is through passage only. In this approach a second assumption was made, that is, NDF and DNDF pass out of the rumen at the same rate as ADL, therefore kd_{NDF} and kd_{DNDF} were calculated as shown in equations 2 and 3.

$kd_{NDF} = Kcl_{NDF} - Kcl_{ADL}$……………………………………….(Eq 2)

$kd_{DNDF} = Kcl_{DNDF} - Kcl_{ADL}$………………….……………….(Eq 3)

2.8. Statistical Analysis

The two Latin squares were analysed separately. Therefore, a comparison was possible between varieties (cv1 to cv3) in the first Latin square and between varieties (cv4 to cv6) in the second Latin square. Moreover, the two years' data were analysed separately. Animal data were subjected to a three-way ANOVA analysis using general linear model procedures of SPSS (SPSS for Windows, Rel. 10.1.0. 2000. Chicago: SPSS Inc.). Sources of variation in the statistical analysis were the variety, the period, and the cow. Grass data were subjected to a two-way ANOVA in which sources of variation were the variety and the period. When significant differences due to the variety were detected, the multiple comparison procedure Tukey was used to separate least square means.

The effect of the year was studied for the grass data only, by pooling the two years grass data and analysing them statistically adding the year to the model as an extra source of variation. For animal data the year effect could not be analysed statistically because six different cows were used in each year.

3. Results and Discussion

Table 2 shows the chemical composition of the different grass varieties in two years. In 2000, the varieties differed ($P < 0.05$) in their DM and WSC content. Varieties (cv1 and cv4) in the first and second Latin squares, respectively, had a higher WSC contents compared to the other varieties. The other constituents (OM, NDF and ADL) were not significantly different among varieties. In 2001, the varieties differed significantly only in their WSC content (Table 2, 1st Latin square). The fact that the varieties did not differ in their ADL content in both years facilitated the comparison between them, as any observed differences in the Kcl_{NDF}, Kcl_{ADL} or kd_{NDF} could not be attributed to differences in ADL content.

The year seemed to have a large influence on grass chemical composition (Table 2). In 2001, the CP, NDF and ADL contents were higher ($P < 0.05$) and DM and WSC contents were lower ($P < 0.05$) than in 2000. The high CP and low DM and WSC contents in 2001 can be explained by the higher N fertilisation rate in this year, as 400 kg N/ha were applied compared to 300 kg N/ha in 2000. McGrath (1988) noted that the addition of N fertiliser reduced WSC concentration in grass through an increase in growth rate. Valk et al. (1996) observed an increase in CP and a decrease in DM content with higher N fertiliser levels, which was in agreement with Wilman and Wright (1983). The higher NDF and ADL contents in 2001 cannot be attributed to the higher N fertilisation rate. Wilman et al. (1977) and Valk et al. (1996) reported that NDF content in freeze-dried grass samples tended to decrease at higher N fertilisation rates. Other factors (viz. climatic and weather conditions, age of the sward) might have contributed to this observed difference between years in NDF and ADL content, but this will not be discussed further as it is out of the scope of this paper.

Table 2. Mean chemical composition of six perennial ryegrass varieties in two Latin squares in two years (2000 and 2001)

		Latin square								Total	
		1st Latin square				2nd Latin square					
		Variety				Variety					
Year		cv1	cv2	cv3	*P*	cv4	cv5	cv6	*P*	Mean	SEM
2000	DM[1]	167^{a}	168^{a}	177^{b}	0.02*	175	173	166	0.12	171	3
	OM	888	881	882	0.23	880	877	877	0.49	881	1
	CP	159	159	166	0.20	151	155	159	0.09	158	6
	WSC	181^{b}	160^{a}	153^{a}	0.00*	180^{f}	157^{e}	141^{d}	0.00*	162	8
	NDF	415	430	426	0.13	414	422	436	0.10	424	3
	ADL	17	16	16	0.20	15	17	17	0.14	16	0.3
2001	DM[1]	156	157	155	0.86	151	156	156	0.76	155	6
	OM	889	886	886	0.59	885	885	883	0.52	886	3
	CP	204	191	197	0.15	185	194	199	0.18	195	5
	WSC	125^{b}	111^{ab}	86^{a}	0.02*	108	97	97	0.17	104	4
	NDF	422	442	465	0.12	462	456	443	0.60	448	8
	ADL	21	20	22	0.43	20	22	20	0.44	21	1

[a,b,c] Means in the same row within the 1st Latin square with different superscripts differ significantly ($P < 0.05$).

[d,e,f] Means in the same row within the 2nd Latin square with different superscripts differ significantly ($P < 0.05$).

[1] DM is expressed in g/kg fresh grass whereas the other fractions are expressed as g/kg DM.

*$P < 0.05$

Between years, the chemical composition of the different grass varieties was not consistent. Varieties with a low CP content in 2000 (cv1) had a high CP content in 2001 and varieties with a low NDF content in 2000 (cv 4) had a high NDF content in 2001. However, varieties seemed to be consistent in their WSC content over years, as cv1 and cv4 showed a higher WSC content than the other varieties in both years.

Table 3 shows the DM, OM, NDF, DNDF and ADL intake of the different grass varieties. In both years, the intake of DM and OM, either expressed as kg/d or as g/kg BW, was not significantly different among varieties. In contrast, the intake of NDF and DNDF, expressed either in kg/d or in g/kg BW, was different among varieties. In 2000 in the 2nd Latin square, cv4 had a lower NDF and DNDF intake compared to other varieties in the same Latin square. Similarly, in 2001 in the 1st Latin square, cv1 had a lower (P = 0.03) DNDF intake (g/kg BW) compared to other varieties in the same Latin square. In 2000, total DMI, total organic matter intake and grass DMI were higher than in 2001. The reasons are threefold. Firstly, more concentrates were fed in 2000 than in 2001 (4.6 vs. 2.8 kg/d). Secondly, the cows used in 2000 were heavier than in 2001 (625 vs. 563 kg BW); the relation between body weight and daily DMI is well established. Thirdly, the grasses in 2000 had higher DM content (171 vs. 155 g/kg fresh) and lower NDF content (424 vs. 448 g/kg DM) compared to the grasses in 2001. The negative influence of high moisture content (Lahr et al., 1983) and high NDF content (Allen, 2000) on daily intake is well established. Total NDF intake and total ADL intake were similar in both years, regardless the differences in concentrate supplementation and cows' weight and ranged around 7.8 and 0.35 kg/d respectively. Other authors (Peyraud et al., 1997) reported similar values of NDF intake when fed fresh 30-day old perennial ryegrass to dairy cows in mid lactation. However, when the intake values were expressed in g/kg BW, it appeared that cows in 2001 consumed more grams of NDF, DNDF, ADL and grass DM per kg BW than in 2000 (Table 3). This might be related to the fact that cows in 2001 were in an earlier lactation stage than cows in 2000 (77 vs. 126 DIM at the start of the experiment). Intake of multiparous cows peaks up at around 9 weeks after calving and persists for several weeks, then starts to decline around 18 weeks after calving.

Table 4 shows the rumen pool sizes of DM, OM, NDF, DNDF, INDF and ADL for dairy cows fed different varieties of perennial ryegrass in two years. Despite differences in chemical composition (Table 2) and NDF intake (Table 3) among varieties, the rumen pools of cows fed different grass varieties were not significantly different. Although different and smaller animals were used in the experiment of 2001, rumen pools (in kg) appeared to be similar for both years (Table 4). However, when rumen pools were expressed as g/kg BW it appeared that cows in 2001 had larger rumen pool sizes than in 2000. This might be related to the earlier stage of lactation of cows used in 2001, which resulted in higher intakes in g/kg BW of these

Table 3. The mean intake in kg/d and in g/kg BW of different chemical components of six perennial ryegrass varieties in two years (2000 and 2001)

		Latin square								Total	
		1st Latin square				2nd Latin square					
		Variety				Variety					
Year		cv1	cv2	cv3	*P*	cv4	cv5	cv6	*P*	Mean	SEM
2000		(kg/d)									
	GDMI	16.2	16.5	16.6	0.73	16.2	17.3	16.8	0.26	16.6	0.4
	TDMI	20.2	20.5	20.7	0.73	20.3	21.4	20.9	0.26	20.7	0.4
	OMI	18.2	18.3	18.5	0.81	18.1	19.0	18.6	0.24	18.4	0.3
	NDFI	7.6	7.9	7.9	0.48	7.6^{d}	8.1^{e}	8.2^{e}	0.01*	7.9	0.2
	DNDFI	6.8	7.2	7.2	0.41	6.9^{d}	7.3^{e}	7.4^{e}	0.00*	7.1	0.2
	ADLI	0.35	0.35	0.34	0.81	0.33	0.38	0.37	0.10	0.35	0.01
		(g/kg BW)									
	GDMI	26.8	27.2	27.1	0.80	25.6	27.3	26.5	0.38	26.7	0.9
	TDMI	33.6	33.9	33.7	0.83	32.1	33.7	32.9	0.43	33.3	0.9
	OMI	30.2	30.2	30.1	0.93	28.6	29.9	29.2	0.45	29.7	0.8
	NDFI	12.5	13.1	12.9	0.47	12.0	12.8	12.9	0.08	12.7	0.4
	DNDFI	11.3	11.8	11.7	0.40	10.8	11.6	11.6	0.11	11.5	0.3
	ADLI	0.59	0.58	0.56	0.56	0.52	0.59	0.58	0.05	0.57	0.02
2001		(kg/d)									
	GDMI	15.7	16.0	15.0	0.45	15.2	15.2	15.7	0.66	15.5	0.2
	TDMI	18.2	18.4	17.5	0.45	17.7	17.7	18.1	0.66	17.9	0.2
	OMI	16.3	16.5	15.6	0.44	15.8	15.8	16.2	0.65	16.0	0.2
	NDFI	7.3	7.7	7.6	0.14	7.7	7.5	7.6	0.09	7.6	0.1
	DNDFI	6.4	6.8	6.7	0.07	6.8	6.6	6.7	0.09	6.7	0.1
	ADLI	0.38	0.36	0.37	0.66	0.36	0.38	0.36	0.47	0.37	0.01
		(g/kg BW)									
	GDMI	28.3	28.6	26.6	0.31	26.9	27.4	27.9	0.56	27.6	0.3
	TDMI	32.7	33.0	31.0	0.29	31.3	31.8	32.3	0.54	32.0	0.4
	OMI	29.3	29.4	27.6	0.27	27.9	28.4	28.8	0.52	28.6	0.3
	NDFI	13.1	13.8	13.5	0.07	13.6	13.6	13.5	0.81	13.5	0.2
	DNDFI	11.4^{a}	12.2^{b}	11.9^{ab}	0.03*	12.1	12.0	11.9	0.62	11.9	0.2
	ADLI	0.68	0.65	0.66	0.54	0.63	0.68	0.64	0.37	0.66	0.03

[a,b] Means in the same row within the 1st Latin square with different superscripts differ significantly ($P < 0.05$).

[d,e] Means in the same row within the 2nd Latin square with different superscripts differ significantly ($P < 0.05$).

[1] GDMI is the grass dry matter intake, TDMI is the total dry matter intake (grass + concentrates); OMI, NDFI, DNDFI, and ADLI are the total intake for organic matter, neutral detergent fibre, digestible neutral detergent fibre and acid detergent lignin, respectively.

*$P < 0.05$

cows (Table 3). These findings suggest that rumen pools are influenced by the lactation status of the cows. Similarly, Bosch et al. (1992) reported larger rumen pool sizes in early lactation compared to late lactation which was related to the higher rumen capacity at early lactation (Baile and Forbes, 1974). Most of the varieties were consistent in their rumen pools over years, cv5 and cv6 had a large DM and NDF rumen pools in both years, and cv1 had a small DM and NDF pools in both years.

Studies reporting rumen pool sizes of *ad libitum* grass-fed dairy cows are very limited. Van Vuuren et al. (1992) reported rumen pool sizes of grass-fed dairy cows. Their values were lower than rumen pool sizes observed in the present study (14 vs. 21 g/kg BW rumen DM pool size), which might be related to the advanced stage of lactation of the cows used in their experiment and to the methodology employed to calculate rumen pool sizes. They rumen-evacuated the cows six times during two days and reported the average pool size based on all evacuations. Bosch et al. (1992) measured rumen pool sizes of dairy cows fed grass silage differing in maturity and reported a range between 17 and 22 g/kg BW rumen DM pool, which is similar to the range observed in the present study.

Table 5 shows the DM, OM, NDF, DNDF and ADL clearance rates (Kcl), and NDF, DNDF degradation rates (kd) of the different grass varieties, measured using two consecutive rumen evacuations separated by a 12-hour period of feed deprivation and equations 2 and 3. In both years, Kcl_{DM}, Kcl_{OM} and Kcl_{NDF} were not significantly different among grass varieties. The values fluctuated around 5.5 %/h for all varieties, and the difference between the fastest and the slowest disappearing variety was less than 1.0 %/h. The Kcl_{DNDF} was higher than the Kcl_{NDF} in both years and ranged around 6.2 %/h. In both years, Kcl_{DNDF} was not different among grass varieties, as the difference between the fastest and slowest variety was less than 1.0 %/h. The two-year data showed that Kcl_{NDF} and Kcl_{DNDF} for the different varieties were consistent over years, as cv2 had a high Kcl_{NDF} and Kcl_{DNDF} and cv5 and cv6 had a low Kcl_{NDF} and Kcl_{DNDF} in both years. This might indicate that NDF clearance behaviour for different grass varieties is consistent over years.

Kcl_{ADL} was not different among grass varieties in both years; however it seemed to be different between years. Kcl_{ADL} was higher in 2001 (3.3 %/h) than in 2000 (2.9 %/h) (Table 5). These values are higher than reported values for the passage rate of particles of fresh 28-day old perennial ryegrass (2.3 %/h) (Mambrini and Peyraud, 1994), and lower than the ADL passage rate (3.5 %/h) reported by Van Vuuren et al. (1993) for perennial ryegrass.

In 2001, Kcl were slightly higher than in 2000 (Table 5), this might be related to the earlier stage of lactation of the cows used in 2001, as the Kcl seems to decrease as cows advance in lactation. Van Vuuren et al. (1992) measured Kcl of OM, NDF and ADL in grass-fed dairy cows in June, July, September and October using rumen evacuation technique and external markers and concluded that Kcl decreases as cows advance in lactation.

Table 4. Mean rumen DM, OM, NDF, DNDF, INDF and ADL pools in kg and in g/kg BW of six perennial ryegrass varieties in two years (2000 and 2001)

		Latin square								Total	
		1st Latin square				2nd Latin square					
		Variety				Variety					
Year	Pool[1]	cv1	cv2	cv3	*P*	cv4	cv5	cv6	*P*	Mean	SEM
2000					(kg)						
	RDM	10.9	11.2	12.9	0.30	12.3	13.1	14.3	0.46	12.4	0.5
	ROM	9.7	9.8	11.2	0.31	10.8	11.4	12.4	0.51	10.9	0.4
	RNDF	5.1	5.4	5.9	0.26	5.3	5.8	6.2	0.58	5.6	0.2
	RDNDF	4.1	4.4	4.9	0.18	4.2	4.6	4.9	0.68	4.5	0.2
	RINDF	0.98	0.99	0.97	0.96	1.06	1.22	1.36	0.27	1.1	0.1
	RADL	0.41	0.41	0.41	0.96	0.44	0.51	0.57	0.26	0.46	0.02
					(g/kg BW)						
	RDM	18.2	18.3	21.0	0.31	19.4	20.7	22.4	0.55	20.0	0.8
	ROM	16.1	16.2	18.4	0.32	16.9	18.0	19.4	0.59	17.5	0.7
	RNDF	8.6	8.8	9.7	0.30	8.3	9.1	9.8	0.64	9.0	0.4
	RDNDF	6.9	7.2	8.1	0.20	6.6	7.2	7.6	0.74	7.3	0.3
	RINDF	1.7	1.6	1.6	0.86	1.7	1.9	2.1	0.31	1.8	0.1
	RADL	0.69	0.68	0.66	0.87	0.70	0.80	0.89	0.31	0.74	0.04
2001					(kg)						
	RDM	11.2	12.3	12.0	0.79	12.2	13.1	13.4	0.29	12.4	0.5
	ROM	10.0	10.8	10.6	0.80	10.8	11.6	11.8	0.34	10.9	0.4
	RNDF	4.8	5.5	5.5	0.46	5.9	6.3	6.3	0.64	5.7	0.2
	RDNDF	3.6	4.3	4.3	0.31	4.7	5.0	4.9	0.88	4.5	0.2
	RINDF	1.24	1.19	1.20	0.92	1.13	1.34	1.34	0.21	1.2	0.1
	RADL	0.52	0.50	0.50	0.92	0.47	0.56	0.56	0.20	0.52	0.02
					(g/kg BW)						
	RDM	20.3	22.0	21.3	0.79	21.3	23.4	23.7	0.31	22.0	0.7
	ROM	17.9	19.4	18.8	0.80	18.9	20.7	21.0	0.37	19.5	0.6
	RNDF	8.7	9.9	9.9	0.44	10.3	11.3	11.0	0.63	10.2	0.4
	RDNDF	6.5	7.7	7.7	0.30	8.3	8.9	8.7	0.81	8.0	0.3
	RINDF	2.2	2.1	2.1	0.88	2.0	2.4	2.4	0.29	2.2	0.1
	RADL	0.93	0.89	0.89	0.88	0.83	1.00	0.98	0.28	0.92	0.03

[1] RDM is rumen DM pool; ROM is rumen OM pool; RNDF is rumen NDF pool; RDNDF is rumen digestible NDF pool; RINDF is rumen indigestible NDF pool and RADL is rumen ADL pool.

Table 5. Mean fractional clearance (Kcl) and degradation (kd) rates of the different components (DM, OM, NDF, DNDF, ADL) of six perennial ryegrass varieties in two years (2000 and 2001)

		Latin square								Total	
		1st Latin square				2nd Latin square					
		Variety				Variety					
Year	Rate[1]	cv1	cv2	cv3	*P*	cv4	cv5	cv6	*P*	Mean	SEM
2000	Kcl_{DM}	5.5	5.7	5.6	0.72	5.2	4.9	5.2	0.26	5.3	0.2
	Kcl_{OM}	5.6	5.9	5.7	0.63	5.4	5.1	5.4	0.24	5.5	0.2
	Kcl_{NDF}	5.4	5.9	5.7	0.57	5.3	4.9	5.1	0.17	5.4	0.2
	Kcl_{DNDF}	6.3	6.6	6.4	0.88	6.0	5.8	5.8	0.35	6.1	0.2
	Kcl_{ADL}	2.7	3.5	3.0	0.24	2.8	2.4	3.1	0.37	2.9	0.2
	kd_{NDF}	2.7	2.4	2.7	0.83	2.4	2.5	2.0	0.43	2.5	0.2
	kd_{DNDF}	3.6	3.1	3.4	0.81	3.2	3.4	2.7	0.43	3.2	0.3
2001	Kcl_{DM}	5.6	5.5	5.4	0.71	5.4	5.2	5.3	0.76	5.4	0.1
	Kcl_{OM}	5.8	5.7	5.6	0.80	5.5	5.3	5.4	0.82	5.6	0.1
	Kcl_{NDF}	5.5	5.6	5.4	0.93	5.5	5.2	5.4	0.81	5.4	0.1
	Kcl_{DNDF}	6.3	6.5	6.1	0.83	6.1	6.0	6.0	0.95	6.2	0.2
	Kcl_{ADL}	3.6	3.1	3.4	0.10	3.0	2.9	3.4	0.58	3.3	0.2
	kd_{NDF}	1.9	2.5	2.0	0.41	2.4	2.3	1.9	0.79	2.2	0.2
	kd_{DNDF}	2.7	3.4	2.7	0.45	3.1	3.1	2.6	0.81	2.9	0.3

[1] Kcl is the fractional clearance rate expressed in %/h and calculated using two consecutive rumen evacuations separated by a 12-hour period of feed deprivation using first order kinetics; kd is the fractional degradation rate expressed in %/h and calculated using the clearance rate of ADL (Kcl_{ADL}) to correct for the passage rate.

The degradation rates (kd_{NDF} and kd_{DNDF}) reported in Table 5, were calculated using Kcl_{ADL} to correct for fractional passage rate as shown in equations 2 and 3. In both years, kd_{NDF} and kd_{DNDF} did not differ significantly among grass varieties. The difference between the fastest degrading variety and the slowest one was less than 1.0 %/h. The kd_{NDF} and kd_{DNDF} were higher in 2000 than in 2001 (2.5 vs. 2.2 %/h and 3.2 vs. 2.9 %/h respectively), maybe due to the higher Kcl_{ADL} observed in 2001 (Table 5), keeping in mind that it was used to correct for passage. In this experiment, the kd_{NDF} measured using rumen evacuation was much lower than reported values of kd_{NDF} for 28-day old perennial ryegrass measured using the *in situ* nylon bag technique in other experiments (Van Vuuren et al., 1993; Valk et al., 1996). This is believed to be partly due to the use of ADL as a passage marker, and partly due to the fact that the rumen evacuation technique estimates the degradation rate for the total NDF pool (degradable and undegradable), whereas nylon bag technique only describes the potentially degradable NDF fraction. When correcting for the INDF fraction and calculating the kd_{DNDF} the values were elevated to 3.2 %/h but were still lower than reported *in situ* values of kd_{NDF} for perennial ryegrass. In other experiments (Van Vuuren et al., 1992 and 1993) it has been shown that Kcl_{ADL} and ADL fractional passage rate (KP_{ADL}) are higher than the NDF fractional passage rate (KP_{NDF}). Thus, using the Kcl_{ADL} or KP_{ADL} to estimate KP_{NDF} will underestimate kd_{NDF}. They also measured kd_{NDF} using both rumen evacuation and nylon bag techniques and concluded that the kd_{NDF} estimated by the rumen evacuation technique was always lower than that estimated by the nylon bag technique.

The higher Kcl_{NDF} and Kcl_{DNDF} in 2001, which were probably due to the earlier stage of lactation, were coupled with a higher Kcl_{ADL}, resulting in a slightly lower kd_{NDF} and kd_{DNDF} in this year, as Kcl_{ADL} was used to calculate the kd. The kd_{NDF} and kd_{DNDF} for the different varieties were not as consistent over years as the Kcl_{NDF} and Kcl_{DNDF}. Some varieties (cv1 and cv3) had high kd values in 2000 and low ones in 2001. However, other varieties (cv6) were consistent over years (Table 5).

4. Conclusions

In these experiments, six diploid varieties of perennial ryegrass were compared for their neutral detergent fibre fractional clearance and degradation rates. The different varieties did not differ significantly in their neutral detergent fibre fractional clearance and fractional degradation rates. The difference between the fastest degrading variety and the slowest one was less than 1.0 %/h. Similarly, the difference between the fastest clearing (disappearing) variety and the slowest one was less than 1.0 %/h. This suggests that there is a narrow range of less than 1.0 %/h of improvement for these traits. Moreover, it has been shown that neutral detergent fibre clearance behaviour of grass varieties is consistent over years.

Acknowledgements

The authors wish to extend their gratitude to Barenbrug Holland BV and The Ministry of Economic Affairs (Economisch Zaken) in The Netherlands, for financing this work.

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Chapter 3

Fibre Degradation Rate of Perennial Ryegrass Varieties Measured Using Three Techniques: *in situ* Nylon Bag, *in vivo* Rumen Evacuation and *in vitro* Gas Production

H.Z. Taweel, B.M. Tas, B. A. Williams, J. Dijkstra and S. Tamminga

Wageningen Institute for Animal Sciences, Wageningen University and Research Centre, Department of Animal Science, Animal Nutrition Group, P. O. Box 338, 6700 AH, Wageningen, The Netherlands

Fibre Degradation Rate of Perennial Ryegrass Varieties Measured Using Three Techniques: *in situ* Nylon Bag, *in vivo* Rumen Evacuation and *in vitro* Gas Production

H.Z. Taweel, B.M. Tas, B. A. Williams, J. Dijkstra and S. Tamminga

Abstract

This study had two aims: firstly, to examine whether perennial ryegrass varieties differ in their NDF degradation rates (kd_{NDF}). Secondly, compare grass kd_{NDF} obtained using *in situ* nylon bag, *in vivo* rumen evacuation and *in vitro* gas production techniques. The samples for the nylon bag and gas production measurements originated from an indoor feeding experiment, in which six diploid varieties of perennial ryegrass were fed to six high producing rumen-cannulated dairy cows using a double 3 × 3 Latin square design. In this experiment, NDF clearance rate (Kcl_{NDF}) and kd_{NDF} of each variety were estimated using two rumen evacuations separated by a 12-h period of feed deprivation. For the *in situ* measurements, freeze-dried grass samples were chopped to a 1-cm length, weighed into nylon bags and incubated in the rumen for 2, 4, 8, 12, 24, 48 and 336 h. For the *in vitro* gas measurements, freeze-dried grass samples were ground to pass a 1-mm sieve and incubated with inoculum from grass-fed cows for 72 hours using an automated gas production system. The resulting gas curves were fitted using the two-phase logistic model. The three techniques showed that the different varieties of perennial ryegrass used in this study did not differ significantly in their kd_{NDF}. Moreover, the three techniques showed that there is a small range of less than 1 %/h in kd_{NDF} within perennial ryegrass varieties. On average, the absolute values of kd_{NDF} estimated with *in situ* (nylon bag) and *in vivo* (rumen evacuation) were similar at around 2.5 %/h, whereas, *in vitro* gas production estimates were much higher at around 6.0 %/h. The correlation between the three techniques in kd_{NDF} was very poor and the ranking of the varieties based on their kd_{NDF} was different for the three techniques.

1. Introduction

In Western Europe, perennial ryegrass is the most widely used grass species for grazing cattle, because of its high productivity, palatability and nutritive value. However, the low dry matter intake (DMI) of perennial ryegrass pasture has been identified as a major factor limiting milk production of high producing dairy cows (McGilloway and Mayne, 1996; Kolver and Muller, 1998). The DMI of dairy cows on perennial ryegrass pasture rarely exceeds 3.25 % of body weight (BW), which is much lower than what cows can consume of a total mixed ration based on grass-maize silage and concentrates (4.2 % BW). This is probably related to physical constraints, rate of removal from the rumen through degradation and passage, and water consumption associated with pasture (Beever and Thorp, 1997).

Altering the chemical, physical and mechanical characteristics that contribute to the low DMI of perennial ryegrass pasture through breeding, selection and the choice of variety may be a way forward in trying to maximise its DMI. Modifying characteristics that contribute to physical limitations such as fibre content, degradation rate by microbes in the rumen, or rate of clearance of fibre from the rumen may lead to a considerable improvement in DMI. Choosing varieties that have a fast rate of fibre degradation may reduce the residence time of material in the rumen, allow more space for extra material to be ingested and thus increase DMI. However, it is not yet known if perennial ryegrass varieties differ in their fibre degradation rate to allow such a choice.

Research results, based on *in vitro* gas production experiments, are not consistent. Lantinga et al. (1996) suggested that perennial ryegrass varieties might differ in their cell wall (fibre) degradation rates. Taweel et al. (submitted) (Chapter 1) on the other hand, reported that variation in fermentation characteristics within perennial ryegrass varieties is mainly due to variation in the rapidly fermentable fraction and that fermentation characteristics of the slowly fermentable fraction are not different among grass varieties. Moreover, based on the results of an *in vivo* rumen evacuation experiment, Taweel et al. (submitted) (Chapter 2) reported no differences in neutral detergent fibre (NDF) clearance and degradation rates among perennial ryegrass varieties.

The rumen evacuation technique estimates the clearance rate of NDF from the rumen, which represents both degradation and passage rates. Therefore, the NDF degradation rate measured using the rumen evacuation technique is normally based on an estimate of NDF passage rate and is calculated by subtracting passage rate from clearance rate. In this technique the NDF passage rate is normally assumed to be equal to the acid detergent lignin (ADL) clearance rate.

The *in vitro* gas production technique relies on the relationship between gas production (accumulation) and forage degradation. The anaerobic fermentation of forage by rumen microbes produces VFA, CO_2, CH_4 and traces of H_2. A major criticism of the technique is the production of indirect gas that is not directly related to substrate fermentation but is produced as a result of the reaction between VFA and bicarbonate buffer. One complicating factor in describing forage NDF degradation characteristics using the gas production technique is that forage is naturally a mixture of components that ferment at different rates. Therefore, when fermenting the whole forage using the gas production technique it can be difficult to obtain an accurate estimate of the NDF degradation rate. One way to obtain an estimate of the NDF degradation rate from gas production curves could be by dividing the obtained curves into two phases that correspond to the rapidly and slowly fermentable fractions of the forage. However, the slowly fermentable fraction may constitute other components in addition to the NDF, which may limit the value of such an approach. Another way to obtain a good estimate of NDF degradation rate from gas production curves is by isolating or extracting the NDF of the forage and fermenting it alone (Pell et al., 2000). For that to be successful, the extraction procedure and detergent should not influence or modify NDF fermentation characteristics and should not remove compounds that inhibit digestion and fermentation (Pell et al., 2000).

Degradation characteristics of forage NDF can be measured using the *in situ* nylon bag technique, which relies on the measurements of disappearance of NDF from the nylon bag. Contrary to the other techniques, nylon bag measurements are done directly to the pool in question and yield accurate estimates of the NDF fractional degradation rate without the need to estimate the NDF passage rate. Therefore, this study aimed at: firstly, examining if perennial ryegrass varieties differ in their NDF degradation rates using three techniques: *in situ* nylon bag, *in vivo* rumen evacuation and *in vitro* gas production. Secondly, comparing grass NDF degradation rates obtained using *in situ*, *in vivo* and *in vitro* techniques.

2. Materials and Methods

2.1. Experimental design and sample collection

The samples for the *in situ* and *in vitro* measurements originated from an indoor feeding experiment, in which six diploid varieties of perennial ryegrass were fed to six high-producing rumen-cannulated dairy cows using a double 3 × 3 Latin square design. In each Latin square three varieties were fed to three cows during three periods. Each period consisted of two weeks: one for adaptation and the other for measurements of intake, digestibility, milk production, and rumen pools and fermentation kinetics. Procedural details that describe the experiment, varieties, measurements, rumen evacuations and sample analysis have been previously described (Taweel et al., submitted) (Chapter 2). In this experiment, NDF clearance (Kcl_{NDF}) and degradation (kd_{NDF}) rates of each variety were estimated using two rumen evacuations separated by a 12-h period of feed deprivation. The Kcl_{NDF} was calculated based on the assumption of first order kinetics, using the logarithmic transformation of the exponential equation:

$$R_{(t)} = R_{(0)} \times e^{-Kcl \times t}$$

where $R_{(t)}$ is the amount of NDF present in the rumen at the second evacuation, $R_{(0)}$ is the amount of NDF present in the rumen at the first evacuation, and t is the time between the two evacuations in hours.

To estimate kd_{NDF}, an estimate of the NDF fractional passage rate was needed. This was done by assuming that ADL is completely undegradable and its clearance (Kcl_{ADL}) from the rumen is only via passage. Moreover, it was assumed that NDF passes out of the rumen at the same fractional rate as ADL.

2.2. Nylon bag and gas production measurements

For the *in situ* nylon bag and *in vitro* gas production measurements, grass samples from each variety in each period were collected on Wednesday and Thursday of each measurement week immediately after mowing. Grass samples were collected in those days as they would best represent what is in the rumen on Thursday and Friday when the evacuations were performed. These grass samples were stored in the freezer at -20 °C. At the end of the experiment, these grass samples were freeze-dried and the samples from the two days of each variety in each period were pooled.

For nylon bag measurements, samples were chopped to approximately 1-cm length using a paper cutter and weighed into nylon bags (8 × 15 cm inner size; pore size = 40 µm; PA 40/30, Nybolt, Switzerland). To obtain a sample:surface area ratio of approximately 20 mg/cm^2, 5 g of DM of each sample was weighed into each bag. Rumen incubations were carried out using 12 rumen-cannulated dairy cows. Samples were incubated in the rumen for 2, 4, 8, 16, 24, 48 and 336 h. Six cows grazing on a pasture predominated by perennial ryegrass were used for the short time incubations (2 to 48 h), whereas six cows fed silage indoors were used for the long time incubations (336 h). The incubations were carried out according to the all-out procedure. For incubation times 2, 4 and 8 h, one bag of each sample was incubated in each cow, whereas, for incubation times 16 and 24 h two bags and for incubation time 48 h three bags of each sample were incubated in each cow. This was done to minimise or eliminate, as much as possible, the effect of the cow on degradation characteristics. For the determination of the zero time point, samples were washed in a washing machine for 50 minutes with 70-L cold water without centrifuging (wool program). After rumen incubations, bags were immediately placed in ice for 5 minutes to stop fermentation and further rinsed with tap water. Then bags were washed in a washing machine using the procedure described earlier. After washing, bags were placed in the freezer at –20 °C, freeze-dried and weighed. Residues were pooled over incubation time of each variety in each period and ground through a 1 mm sieve. Grass and residue samples were analysed for NDF as described by Goelema et al. (1998).

The resulting NDF disappearance curves were fitted using the model described by Robinson et al. (1986) to determine the degradation parameters of NDF in the rumen. The non-linear iterative procedure of SPSS was used to fit the equation:

$R = U + D \times \exp^{(-kd \times t)}$,

where R is the percentage of NDF recovered at time t (h), U is the undegradable fraction of NDF, D (100 – U) is the potentially degradable fraction of NDF, kd is NDF fractional degradation rate in h^{-1}, and t is the time in hours.

For gas measurements, samples were ground to pass 1-mm sieve and incubated with inoculum from grass-fed cows for 72 hours, using an automated gas production system as described by Davies et al. (2000). The resulting gas curves were fitted using the logistic di-phasic model described by Schofield et al. (1994). In this model, the first phase is assumed to arise from the first substrate pool, which is assumed to be the rapidly fermentable fraction. The second phase is assumed to arise from the second substrate pool, which is assumed to be the slowly fermentable fraction. Procedural details of the gas production measurements, curve fitting and analysis have been described previously (Taweel et al., submitted) (Chapter 1).

2.3. Statistical analysis

To examine if NDF degradation characteristics differ among varieties of perennial ryegrass, *in situ* and *in vitro* data were subjected to a two-way ANOVA analysis using general linear models of SPSS (SPSS for Windows, Rel. 10.1.0. 2000. Chicago: SPSS Inc.). The sources of variation in the analyses were the variety and the period. The interaction between variety and period could not be accounted for in the model as the degrees of freedom were not sufficient to test for it; therefore, it was added as a requirement for the model. The clearance and degradation rates obtained from the *in vivo* data were analysed statistically as two separate Latin squares using general linear models of SPSS. Within each Latin square, three varieties could be compared. The sources of variation within each Latin square in these analyses were the variety, period and the cow. To study the correlation between the NDF degradation rates measured using the different techniques, the two-tailed Pearson bivariate correlation procedure of SPSS was employed.

3. Results and Discussion

Table 1 shows the NDF degradation characteristics of the six grass varieties measured using the *in situ* (nylon bag), the *in vivo* (rumen evacuation) and the *in vitro* (gas production) techniques. The *in situ* NDF degradation kinetics parameters (U, D and kd) did not differ significantly among varieties, which was in agreement with the *in vivo* and *in vitro* data. On average, the U-fraction of NDF was around 14 % and the D-fraction around 86 % with a degradation rate of around 2.5 %/h for all varieties. Other authors (Miller et al., 2001) reported significant differences in degradation characteristics of DM and OM between two perennial ryegrass varieties which differed in their water-soluble carbohydrate content. However, they did not report the *in situ* degradation characteristics of NDF. In their experiment, the two varieties differed largely in their NDF content leading to differences in OM degradation characteristics. In grasses, NDF is degraded more slowly than the other OM components, therefore, grasses with high NDF content are expected to have a lower OM digestibility and a slower OM degradation rate compared to grasses with low NDF content.

Table 1. Estimates of NDF degradation characteristics of six varieties (cv1 to cv6) of perennial ryegrass measured using the *in situ* nylon bag, *in vivo* rumen evacuation and *in vitro* gas production techniques

	Variety								
Variable	cv1	cv2	cv3	cv4	cv5	cv6	Mean	SD[1]	P
			In situ nylon bag[2]						
U_{NDF}, %	14.2	13.8	12.5	13.7	14.5	14.5	13.9	1.4	0.3
D_{NDF}, %	85.8	86.2	87.5	86.3	85.5	85.5	86.1	1.4	0.3
kd_{NDF}	2.5	2.4	2.5	2.5	2.7	2.4	2.5	0.2	0.2
			In vivo rumen evacuation[3]						
kcl_{NDF}	5.4	5.9	5.7	5.3	4.9	5.1	5.4	0.9	0.2
kd_{NDF}	2.7	2.4	2.7	2.4	2.5	2.0	2.5	0.8	0.4
			In vitro gas production[4]						
Gas, ml	57	57	53	55	60	59	57	3.9	0.2
kd_2	6.5	5.9	5.9	5.7	6.6	6.0	6.1	0.5	0.3
Lag, h	4.6	5.4	4.9	5.3	4.2	4.6	4.8	0.6	0.2

[1] SD is the standard deviation

[2] *in situ* nylon bag degradation kinetics parameters, U_{NDF} is the undegradable fraction of NDF in %, D_{NDF} is the potentially degradable fraction of NDF in % and kd_{NDF} is the fractional degradation rate of NDF in %/h.

[3] *in vivo* rumen evacuation degradation kinetics parameters, kcl_{NDF} is the fractional clearance rate of NDF, kd_{NDF} is the fractional degradation rate of NDF

[4] *in vitro* gas production kinetics parameters of the second phase, Gas is the total volume of gas produced by the second phase in ml, kd_2 is the specific rate of degradation of the second phase and Lag is the lag time of the second phase in hours.

The three techniques showed that the different varieties of perennial ryegrass used in this study did not differ significantly in their NDF degradation rates and characteristics. Moreover, the three techniques showed that there is a small range of less than 1 %/h in the NDF degradation rate within perennial ryegrass varieties. On average, the *in situ* and the *in vivo* estimates of kd_{NDF} were similar at around 2.5 %/h, whereas, the *in vitro* gas production estimates were much higher at around 6.0 %/h. This could be related to sample preparation, as the samples for *in vitro* gas measurements were ground through a 1-mm sieve, whereas the *in situ* samples were chopped to 1-cm size before incubation. In the *in vivo* experiment the grass was mown at 6-cm above ground level and fed fresh to the cows. The effect of sample preparation on degradation characteristics is well documented and established (Uden and Van Soest, 1984; Michalet-Doreau and Ould-Bah, 1992). Mould et al. (2000) showed that grinding the samples to 1-mm particle size before incubation led to higher estimates of degradation rate compared to samples ground to 5-mm size. Grinding reduces the particle size of the material leading to increments in the surface area that will be exposed to microbial colonisation and action during incubation leading to faster degradation rates. In addition to that, gas measurements were done on the whole grass samples and estimates of the degradation rate of the second phase (slowly fermentable fraction) were assumed to correspond to kd_{NDF}. The fact that the slowly fermentable fraction may have contained other components than NDF could also explain the higher degradation rates obtained when using the *in vitro* gas production technique. Moreover, the fact that the logistic model, fitted to the gas curves, estimates the maximum degradation rate, which occurs at the point of inflection of the curve, whereas the *in situ* and *in vivo* models estimate the average fractional degradation rates may explain the high values observed *in vitro*. In agreement with our observations, Cone et al. (1998) also reported that the absolute level of kd_{NDF} estimated by the *in vitro* gas production technique was two to three times higher than the *in situ* values. They attributed that to sample preparation, as in their study the *in vitro* samples were freeze-dried and ground to 1-mm particle size whereas, the *in situ* samples were thawed, fresh and chopped to 1-cm size.

The coefficient of variation (standard deviation/mean) of estimating kd_{NDF} was similar for the *in situ* and the *in vitro* techniques (8 %) and was lower in comparison to that of the *in vivo* technique (32 %). This may indicate that the *in situ* nylon bag and *in vitro* gas production predict kd_{NDF} with a similar precision and that they are more repeatable and less susceptible to error compared with the *in vivo* technique. The high coefficient of variation in the *in vivo* measurements is most likely due to the animal variation, which was eliminated for both the *in situ* and *in vitro* technique.

Table 2 shows the correlation coefficients between NDF degradation rates and characteristics estimated using the *in situ* nylon bag, *in vivo* rumen evacuation and *in vitro* gas production techniques. Although the *in situ* and *in vivo* techniques yielded similar average estimates of the kd_{NDF} for perennial ryegrass at around 2.5 %/h, the correlation between both techniques was very low. The correlation coefficients between kd_{NDF} estimated *in vivo* and *in situ* NDF degradation characteristics (U, D and kd_{NDF}) were not significant ($r < 0.50$; $P > 0.05$). When comparing the *in vivo* with the *in vitro* technique, low correlation coefficients were also observed between kd_{NDF} and the fermentation parameters of the second phase. However, the correlation between kd_{NDF} *in vivo* and gas volume produced by the second phase was significant ($r = 0.60$; $P = 0.01$). A very poor non-significant correlation ($r = 0.14$) was observed between kd_{NDF} *in vivo* and the *in vitro* degradation rate of the second phase. When comparing *in situ* with *in vitro*, poor correlations were observed between NDF degradation kinetics parameters estimated *in situ* and gas production parameters of the second phase estimated *in vitro*. Once again, a poor non-significant correlation was observed between the rates of degradation measured *in situ* and *in vitro* ($r = 0.09$).

Table 2. Correlation coefficient for NDF degradation characteristics and rates measured using the *in situ* nylon bag (NB), *in vivo* rumen evacuation (RE) and *in vitro* gas production (GP) techniques

Variable[1]	kd_RE	U_NB	D_NB	kd_NB	V_GP	kd_GP	Lag_GP
kd_RE	1	0.45	0.45	0.07	-0.60*	0.14	0.36
U_NB		1	1	0.32	0.50*	0.04	0.13
D_NB			1	0.32	-0.50*	0.04	0.13
kd_NB				1	0.01	0.09	0.26
V_GP					1	0.54*	-0.58*
kd_GP						1	-0.87*
Lag_GP							1

[1] kd is the fractional degradation rate of NDF, U is the undegradable fraction of NDF, D is the potentially degradable fraction, V is the total gas volume produced by the second phase and Lag is the lag time of the second phase in hours.

* Correlation is statistically significant ($P < 0.05$) (2-tailed test)

Similarly, Khazaal et al. (1993) and Ichinohe et al. (1998) reported poor correlations ($r < 0.40$) between *in situ* nylon bag degradability parameters and *in vitro* gas production parameters (Table 3). In contrast, Lopez et al. (1998) reported a very strong correlation ($r = 0.90$) between DM degradation parameters measured using *in situ* nylon bag and *in vitro* gas production techniques (Table 3). Moreover Cone et al. (1998) reported a high correlation ($r = 0.91$) between kd_{NDF} measured using the *in situ* nylon bag and *in vitro* gas production technique (Table 3). They attributed the high correlation in their study to the fact that they fitted the gas production curves with a three-phase model and related the second phase to the degradation characteristics of NDF and OM measured *in situ*. They also reasoned the low correlation between *in situ* and *in vitro* in the studies of Khazaal et al. (1993) to the fact that they fitted the gas curves with a one-phase model. In our study although we fitted the gas curves with a two-phase model and related the second phase to the *in situ* degradation characteristics of NDF, a low correlation was observed. Moreover, when we fitted the gas curves to a three-phase logistic model and related the second phase to the *in situ* degradation characteristics of NDF the correlation did not improve.

Table 3. Published studies on the correlation between *in situ* (nylon bag) and *in vitro* (gas production) techniques in estimating degradation rate and characteristics of animal feed

Source	Sample preparation		Range in NDF	Range in kd	Correlations
	In situ	*In vitro*			
Khazaal et al. (1993)	Oven-dried, 2.5-mm screen	Oven-dried, 1-mm screen	373 to 625 g/kg DM	2.5 to 10.7 %/h	0.24 to 0.70
Ichinohe et al. (1998)	Oven-dried, 2-mm screen	Oven-dried, 1-mm screen	621 to 782 g/kg DM	3.7 to 5.8 %/h	0.23 to 0.37
Lopez et al. (1998)	Oven-dried, 1-mm screen	Oven-dried, 1-mm screen	420 to 680 g/kg DM	4.7 to 17.6 %/h	0.91 to 0.97
Cone et al. (1998)	Thawed fresh, cut to 1 cm	Freeze-dried, 1-mm screen	331 to 508 g/kg DM	2.9 to 7.9 %/h	0.86 to 0.91

We are unaware of studies that compared the *in situ* nylon bag with *in vivo* rumen evacuation technique and *in vitro* gas production for their estimates of kd_{NDF}. Most studies that compared *in situ*, *in vivo* and *in vitro* techniques focused on the ability of these techniques to estimate OM digestibility or degradability in order to estimate the amount of fermentable OM (FOM) (Gosselink, 2004). Unlike these studies, the present study focused on the ability of the three techniques to predict NDF degradation rate. Only the study of Cone et al. (1998) reported correlation between kd_{NDF} measured *in situ* and *in vitro* but it did not compare those with an *in vivo* estimate. In the present study, poor correlations were observed between the three techniques in kd_{NDF} and the ranking of the varieties according to their kd_{NDF} was different for the three techniques. The small range in kd_{NDF} and the absence of significant differences between the varieties might explain the poor correlation observed between techniques in this study.

4. Conclusions

In these experiments, six diploid varieties of perennial ryegrass were compared for their NDF fractional degradation rates (kd_{NDF}) using three techniques: *in situ* nylon bag, *in vivo* rumen evacuation and *in vitro* gas production. The three techniques showed that the different varieties did not differ significantly in their kd_{NDF}. Moreover the three techniques showed that the difference between the fastest degrading variety and the slowest one was less than 1.0 %/h. This suggests that there is a narrow range of less than 1.0 %/h of improvement for this trait. On average both the *in situ* and the *in vivo* estimates of kd_{NDF} were similar at around 2.5 %/h, whereas, the gas production estimates of degradation rates were much higher at around 6.0 %/h. The correlation between the three techniques in kd_{NDF} was very poor and the ranking of the varieties based on their kd_{NDF} was different for the three techniques.

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Chapter 4

The Effect of Feeding Perennial Ryegrass with Elevated Concentration of Water-Soluble Carbohydrates on Intake, Rumen Function and Performance of Dairy Cows

H. Z. Taweel,[1] B. M. Tas,[1] H. J. Smit,[2] A. Elgersma,[2] J. Dijkstra,[1] and S. Tamminga[1]

[1] Animal Nutrition Group (ANU), Department of Animal Science, Wageningen University, P. O. Box 338, 6700 AH, Wageningen, The Netherlands

[2] Crop and Weed Ecology Group (CWE), Department of Plant Science, Wageningen University, P. O. Box 430, 6700 AK, Wageningen, The Netherlands

Submitted to Journal of Dairy Science

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H. Z. Taweel, B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra, and S. Tamminga

Abstract

Twelve high-producing Holstein-Friesian dairy cows in mid lactation were used to investigate the effect of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates (WSC) on dry matter intake (DMI), rumen function, milk production and composition. The experiment was conducted in 2000 and repeated in 2001. In each year, six cows were stall-fed with six varieties of perennial ryegrass during three two-week periods, using two 3×3 Latin square designs. In each Latin square three varieties were fed, one of which was characterised by its high levels of WSC. Contrary to expectations, DMI was not positively influenced by the increased WSC content, as varieties with a high WSC tended to have similar DMI to the other varieties (16.2 vs. 16.6 kg/d). Ruminal pH, neutral detergent fibre fractional clearance (Kcl_{NDF}) and fractional degradation (kd_{NDF}) rates were not reduced as a result of feeding high WSC grasses. Total VFA were not changed, but the VFA composition was altered in favour of propionate at the expense of acetate when feeding high WSC grass. Ruminal ammonia concentration and milk urea concentration were reduced as a result of feeding high WSC grass. Milk yield and major constituents (fat, protein and lactose) were not influenced significantly. Feeding high sugar grass did not seem to be beneficial in improving DMI and milk production or in altering the composition of milk.

(**Key words**: rumen function, water-soluble carbohydrates, fibre degradation rate, perennial ryegrass)

1. Introduction

A profitable and environmentally friendly dairy production requires improvement in the utilisation of forages by maximizing intake and optimising microbial conversion in the rumen. Moreover, it also requires improvement in product quality and composition, and a reduction in excretion of pollutants to the environment. In temperate environments, perennial ryegrass (*Lolium perenne* L.) is the most widely used roughage for feeding dairy cows because of its high palatability and digestibility (Delagarde et al., 2000). However, perennial ryegrass on it is own cannot sustain a high milk production because of its limited intake. Besides, it results in high losses of N to the environment (Valk et al., 1990; Tamminga, 1992; Van Vuuren, 1993).

It is thought that grass quality in terms of intake and protein utilisation could be improved by selection and breeding. Breeding grasses for elevated concentrations of water-soluble carbohydrates (WSC) might increase palatability and therefore, increase intake and milk production (Miller et al., 2001; Lee et al., 2002). However, it is argued in the literature (Beerepoot et al., 1997) that more WSC in the grass might result in a lower pH in the rumen, affecting the activity of fibre degrading bacteria, and therefore, decreasing the degradation rate of the fibre. This will result in a longer residence time of the course material in the rumen and might negatively influence intake and milk production. Another advantage of breeding grasses for elevated concentration of WSC is thought to be the improvement in the synchrony of protein and energy availability for microbes in the rumen. This would improve microbial conversion, decrease ammonia production and concentration in the rumen, increase the efficiency of utilising dietary grass protein for milk production, and reduce N losses through the urine to the environment (Miller et al., 2001). Moreover, it is thought that more WSC in the grass would shift the fermentation towards a higher proportion of glucogenic VFA (i.e. propionate) in the rumen. This would improve the energy availability for the animal in the form of glucose and might result in higher protein content in the milk, as less plasma amino acids would be oxidised and more directed to protein production in the mammary glands in the udder tissue. Another advantage for the shift towards higher proportion of glucogenic VFA would be the reduction in energy loss in methane form, as the production of propionate is not coupled with hydrogen production, which is needed for methane formation. Furthermore, elevated levels of WSC in grass might negatively influence the lipogenic VFA (i.e. acetate) production and therefore result in a lower fat content in the milk. Therefore, the objectives of this work were to determine the effect of elevated levels of WSC in grass on dry matter intake, rumen fermentation kinetics and parameters, behaviour of the fibre in the rumen, and milk production and composition.

2. Materials and methods

2.1. Experiments

In two experiments performed in 2000 between 24th June and 1st September, and in 2001 between 29th June and 7th September, lactating dairy cows were stall-fed with six varieties of perennial ryegrass. Between the two years, the experiments differed slightly in that; different animals were used in each year, N fertilisation was 300 and 400 kg/ha per year in 2000 and 2001 and the animals received 4.6 and 2.8 kg of concentrates per day in 2000 and 2001, respectively. Moreover, the time points for rumen liquid sampling and faeces collection were different between years.

2.2. Animals and Feeding

In the year 2000, six high producing multiparous Holstein-Friesian dairy cows (625 ± 6 kg BW) in mid lactation (126 ± 21 DIM) were used in the experiment. The cows produced 28.8 ± 1.8 kg/d of milk at the beginning, and 21.9 ± 1.4 kg/d at the end of the experiment. In the year 2001, six high-producing multiparous Holstein-Friesian dairy cows (563 ± 5 kg BW) in early-mid lactation (77 ± 14 DIM) were used. These cows produced 28.4 ± 1.2 kg/d of milk at the beginning, and 24.2 ± 0.8 kg/d at the end of the experiment. For both years' experiments, cows were fitted with a large rumen cannula (Type 1C, 10 cm id., Bar Diamond, Inc., Parma, Idaho, U.S.A.). The surgery procedures and the experimental layout were approved by an ethical committee, and executed in accordance with Dutch legislation on the use of experimental animals. Two weeks before the experiment started, the cows were housed individually in a tie-stall and were fed on fresh grass. This was done to acclimatise the cows to the tie-stall and grass feeding. During the experiment, the animals were fed *ad libitum* with different varieties of fresh perennial ryegrass. Feeding took place daily at 6:00, 9:00, 12:00, 16:00, 18:00, and 22:00 h, taking utmost care that the animals always had an ample amount of grass in front of them. Grass was mown daily, starting at 13:00 h, and carried to the shed where it was sampled for DM determination, filled in boxes and weighed. Each box was filled with 10 to 12 kg of fresh grass. The boxes to be fed at 16:00 and 18:00 h were placed in the tie-stall next to the animals, whereas the other boxes to be fed at 22:00, 6:00, 9:00 and 12:00 h were placed in a cooling unit at (4 °C). In addition to the grass offered *ad libitum*, the cows received 4.6 kg in 2000 and 2.8 kg in 2001, of concentrates daily. The composition of the concentrates is shown in Table 1. Concentrates were fed twice daily, after the morning (6:00) and the evening (16:00) milking. The temperature in the tie-stall was maintained below 21 ± 2 °C, using cooling fans. Fresh water and mineral blocks were available all the time to the animals. Feed refusal (residue) was collected once daily at 16:00 h, weighed, and sampled for DM determination.

2.3. Diet and Experimental Periods

Six diploid varieties (cv1 to cv6) of perennial ryegrass (*Lolium perenne* L.) were sown in the autumn of 1999. They received the same management and N fertilisation level (300 kg/ha per year in 2000, and 400 kg/ha per year in 2001). These six varieties were selected based on their chemical composition, especially their WSC content, and on their heading date. Two of the varieties (cv1 and cv4) were expected to have a high WSC content, as cv1 was selectively bred for an elevated concentration of WSC and cv4 showed an elevated concentration of WSC in a previous experiment (Taweel et al. submitted). Of the six varieties, three (cv1, cv2 and cv6) had an intermediate heading date (25 to 28 May), and three (cv3, cv4 and cv5) had a late heading date (7 to 9

June). The varieties were cut daily at the same re-growth stage (4 weeks) and target yield (2000 kg DM/ha) and stall-fed to the six cows in three periods using two 3×3 Latin square designs. Varieties (cv1 to cv3) and varieties (cv4 to cv6) were used in the first and second Latin squares, respectively. In each Latin square, one variety with an elevated concentration of WSC was included (cv1 in the first and cv4 in the second Latin squares, respectively). Each period consisted of two weeks: one week for adaptation and the other for measurements.

Table 1. Chemical composition (g/kg as fed) and ingredients of the concentrates used in the experiments, as given by the feed company (Rijnvallei, Wageningen, The Netherlands)

Ingredients		Chemical composition	
Component	Percentage	DM g/kg	875
Beet pulp	21 %	Ash g/kg	46
Maize	21 %	Fat g/kg	27
Barley	21 %	Starch g/kg	261
Soya hulls	11.5 %	Sugars g/kg	106
Coconut peels	10 %	CP g/kg	126
Beet molasses	8 %	NDF g/kg	238
Argentinean Soya	7.5 %	ADL g/kg	20

2.4. Rumen measurements

In both years, NDF fractional clearance rate (Kcl_{NDF}) and fractional degradation rate (kd_{NDF}) were estimated using two consecutive rumen evacuations, separated by a 12-hour period of feed deprivation as described in detail by Taweel et al., (submitted) (Chapter 2). Briefly, two rumen evacuations were done for each animal at the end of the second week of each period. The first evacuation was done on Thursday evening at 19:30 h and the second evacuation on Friday morning at 9:00 h. The animals did not receive any feed between the two evacuations, but water was freely available. Fractional clearance rates of NDF and ADL were calculated based on the assumption of first order kinetics, using the logarithmic transformation of the exponential equation $R_{(t)} = R_{(0)} \times e^{-Kcl \times t}$, where $R_{(t)}$ is the amount present at the second evacuation, $R_{(0)}$ is the amount present at the first evacuation, and t is the time between the two evacuation in hours. To estimate kd_{NDF}, an estimate of the NDF fractional passage rate is needed. This was done by assuming that ADL is completely undegradable and its clearance (Kcl_{ADL}) from the rumen is only via passage. Moreover, it was assumed that NDF passes out of the rumen at the same fractional rate as ADL. Therefore, the Kcl_{ADL} was used to correct for passage.

In the measurement week of each period, rumen liquid samples were collected from each cow on Tuesday 22:00 h, Wednesday 7:00, 16:00, 23:00 h, Thursday 13:00 h, and Thursday immediately prior to the evacuation in 2000, and only on Thursday 8:00, 13:00, 18:00 h and immediately prior to the evacuation in 2001. In these samples, the pH was measured immediately, using a pH meter (pH electrode type 62, Testo 252, Testo GmbH & Co, Germany). Two sub-samples were taken, acidified with either phosphoric acid or Trichloroacitic acid (TCA) and stored in the freezer (-20 °C) pending VFA and ammonia-nitrogen (NH_3-N) analysis, respectively. The concentration of VFA was determined using gas chromatography (GC type Fisons HRGC MEGA2, Italy) and that of NH_3-N using the indophenol method or the Berthelot reaction, as described by Searle (1984).

2.5. Grass and milk sampling

Grass samples from the different varieties on offer were taken on Wednesday and Thursday of each measurement week and were stored in the freezer (-20 °C). At the end of the experiment, grass samples of Wednesday and Thursday of each variety in each period were pooled and freeze-dried, for further analysis.

Daily milk production by each cow was registered and milk was sampled every day during milking. Samples from the evening and the morning milk of each animal were pooled and stored in the refrigerator (4 °C) until they were analysed for fat, protein, and lactose. Another daily milk sample from each animal was taken and stored at (-20 °C) for urea analysis. Milk analyses (fat, protein, lactose and urea) were done in the milk control station in the Netherlands (Stichting Melkcontrolestation Nederland, Zutphen).

2.6. Samples and chemical analysis

Dry matter (DM) determination in the daily grass samples and the daily grass-residue samples that were used to calculate daily DMI was done by sampling approximately 300 g using a grass-core, and drying at (70 °C) for 24 h.

Pooled grass samples and rumen content samples from the evacuations were freeze-dried and ground to pass a 1-mm screen. Air-dry matter was determined after freeze-drying. The DM content of the samples (previously freeze-dried) was determined after oven drying (4 + 2 h at 104 °C). Organic matter (OM) content was determined by ignition (3 + 1 h at 550 °C). Neutral detergent fibre (NDF) was determined as described by Van Soest et al. (1991). Acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest (1973). Nitrogen was determined using the Kjeldahl method (ISO 5983). Pooled grass samples were also analysed for WSC content, as described by Thomas (1977).

2.7. Faeces Collection

For digestibility determination, quantitative 24-hour faeces collection was done for each cow in each measurement week of each period. Faeces were collected over 4 days, Monday till Thursday, six hours collection in each day. In 2000, faeces were collected on Monday from 9:00 to 12:00 and 21:00 to 24:00 h, Tuesday from 6:00 to 9:00 and 18:00 to 21:00 h, Wednesday from 3:00 to 6:00 and 12:00 to 15:00 h, and Thursday from 0:00 to 3:00 and 15:00 to 18:00 h. In 2001, faeces were collected on Monday from 18:00 to 24:00, Tuesday from 12:00 to 18:00, Wednesday from 6:00 to 12:00, and Thursday from 0:00 till 6:00 h. After each collection interval, collected faeces were weighed and a 5 % sample was taken, placed in a plastic bucket, and stored in a freezer (-20 °C). At the end of the experiment, faeces samples from each animal in each period were thawed, thoroughly mixed and sub-sampled fresh for DM and Kjeldahl N determination (ISO 5983). Another sub-sample was taken for freeze-drying, for the determination of ash, NDF and ADL.

2.8. Calculations

Daily grass DMI was calculated for each cow as the difference between the daily grass DM offered and the daily DM refused. To get an estimate of the DMI by each animal in each period, the daily DMI was averaged over the last eight days of each period, neglecting the last day when the evacuation was performed. The daily intake of the different components (OM, N, WSC, NDF and ADL) was estimated from the daily DMI and the percentages of these components in the grass varieties.

The NDF and ADL rumen pool sizes were estimated from the weight of the total fresh rumen contents in the first evacuation and the percentages of these components in the rumen contents. The VFA and NH_3-N rumen pool sizes were calculated from the liquid pool size and their respective concentrations in the rumen liquid using the average concentration of all the collections at the different time points. The non-glucogenic to glucogenic VFA ratio (NGR) was calculated as [(acetate + 2 × (butyrate + isobutyrate) + valerate)] / [propionate + valerate].

2.9. Statistical Analysis

The two Latin squares were analysed separately. Therefore, a comparison was possible between varieties (cv1 to cv3) in the first Latin square and between varieties (cv4 to cv6) in the second Latin square. Moreover, the two years' data were analysed separately. Animal data were subjected to a three-way ANOVA analysis, using general linear model procedures of SPSS (SPSS for Windows, Rel. 10.1.0. 2000. Chicago: SPSS Inc.). Sources of variation in the statistical analysis were the variety, the period, and the cow. Grass data were subjected to a two-way ANOVA, in which sources of variation were the variety and the period. When significant differences due to the variety were detected, the multiple comparison procedure Tukey was used to separate least square means.

3. Results and discussion

Table 2 shows the mean chemical composition of the different grass varieties in the two Latin squares in both years. In the first Latin square, varieties differed in their DM and WSC content in 2000 and only in their WSC content in 2001. As was expected, cv1 had a higher ($P < 0.05$) WSC than the other varieties in both years, as it was selectively bred for this trait. However, the magnitude of the difference was higher in 2001 than in 2000 (39 vs. 28 g/kg DM). In the second Latin square, the varieties only differed in their WSC content. Variety 4 had a higher WSC compared to the other varieties, but this was significant only in 2000, where the magnitude of the difference was almost 40 g/kg DM. In both years, the CP, NDF and ADL content did not differ significantly among varieties, however, varieties with a high WSC tended to have a lower CP and NDF content. A significant negative correlation was observed between WSC and CP content in the grass ($r = 0.73$, $P < 0.05$) in the present study. The fact that the varieties within a Latin square differed significantly only in their WSC content facilitated studying the effect of an elevated concentration of WSC in grass on intake, rumen parameters, milk production and composition. Lee et al. (2002) observed a significantly higher DMI by steers, when fed a high WSC grass variety compared to a control variety with normal WSC concentration. However, in their experiment the varieties also differed largely ($P < 0.01$) in NDF content (83 g/kg DM difference), which made it difficult to explain the cause of the observed difference in DMI (high WSC or low NDF). Moreover, in their experiment, the high WSC grass was cut late in the afternoon, whereas the other grass was cut early in the morning, to maximise WSC differential magnitude (82 g/kg DM). This also resulted in a high differential magnitude in DM content (35 g/kg DM). The negative influence of high moisture content (Lahr et al., 1983) and high NDF content (Allen, 2000) on daily DMI is well established. In the present experiment, the varieties were all cut at the same time (13:00 h) and the maximal difference in NDF content was 20 and 40 g/kg DM in 2000 and 2001, respectively, and was not statistically significant. Moreover, the maximum differential magnitude in DM content was less than 12 g/kg.

Table 3 shows the mean intake and apparent digestibility of different components of the six varieties in the two Latin squares in both years. In both Latin squares in the two years the DMI, DM digestibility and digestible DMI (DDMI) were not significantly different among varieties. In 2000, in both Latin squares; varieties with a high WSC (cv1 and cv4) had a slightly lower DMI compared to other varieties. In 2001, cv1 with the high WSC had a higher intake than cv3 with the low WSC content; however, cv2, which had a moderate WSC concentration had the highest intake. Despite the fact that no significant differences were observed in DM and OM digestibility, grasses with an increased WSC content had a numerically higher DM and OM digestibility than the other grasses (Table 3). This, however, did not lead to a significantly higher DDMI and digestible OMI (DOMI) by cows eating these grasses. Total WSC intake was significantly higher and total NDF intake was lower for the high WSC varieties (cv1 and cv4) in 2000 in the second Latin square, where a difference of 600 g/d in WSC and NDF intake was observed. Peyraud et al. (1997) fed mid lactating cows with perennial ryegrass fertilized at two levels of N, resulting in a significant difference in WSC content between the two grasses (64 g/kg DM differential magnitude). They reported a similar (P = 0.7) OMI and a lower NDF intake (P = 0.1) for the high WSC grass.

Table 2. Mean chemical composition of six perennial ryegrass varieties in two Latin squares in two years (2000 and 2001)

		Latin square								Total	
		1st Latin square				2nd Latin square					
		Variety				Variety					
Year		cv1	cv2	cv3	P	cv4	cv5	cv6	P	Mean	SEM
2000	DM[1]	167^{a}	168^{a}	177^{b}	0.02*	175	173	166	0.12	171	3
	OM	888	881	882	0.23	880	877	877	0.49	881	1
	CP	159	159	166	0.20	151	155	159	0.09	158	6
	WSC	181^{b}	160^{a}	153^{a}	0.00*	180^{f}	157^{e}	141^{d}	0.00*	162	8
	NDF	415	430	426	0.13	414	422	436	0.10	424	3
	ADL	17	16	16	0.20	15	17	17	0.14	16	0.3
2001	DM[1]	156	157	155	0.86	151	156	156	0.76	155	6
	OM	889	886	886	0.59	885	885	883	0.52	886	3
	CP	204	191	197	0.15	185	194	199	0.18	195	5
	WSC	125^{b}	111^{ab}	86^{a}	0.02*	108	97	97	0.17	104	4
	NDF	422	442	465	0.12	462	456	443	0.60	448	8
	ADL	21	20	22	0.43	20	22	20	0.44	21	1

^{a,b} Means in the same row within the 1st Latin square with different superscripts differ significantly ($P < 0.05$)

^{d,e,f} Means in the same row within the 2nd Latin square with different superscripts differ significantly ($P < 0.05$)

[1] DM is expressed in g/kg fresh grass whereas the other fractions are expressed as g/kg DM

*$P < 0.05$

Despite the fact that in the present study varieties had a slightly higher differential magnitude than 38 g/kg DM, no influence was observed on DMI. Moreover, the positive influence of the elevated concentration of WSC in grass on DMI and DDMI by steers and late lactating dairy cows as observed by Lee et al. (2002) and Miller et al. (2001), respectively, was not observed in our experiment with dairy cows in mid lactation. This indicated that the large difference in NDF concentration between the high and low WSC varieties (83 g/kg DM) in their experiments may have played a major role in reducing the DMI of the cows on the low WSC variety. The influence of NDF on DMI and DDMI might be more pronounced than that of the WSC, as in the present study in 2001, cv1 with the high WSC had a higher ($P = 0.05$) DDMI than cv3 with the low WSC. The magnitude of difference in WSC between those two varieties was more than 38 g/kg DM but also the magnitude of difference in NDF between them was more than 40 g/kg DM (Table 2). However, in 2000, cv4 and cv6 also differed in WSC by more than 38 g/kg DM (Table 2), but DMI and DDMI were similar (Table 3), as the magnitude of difference in NDF between those two varieties was less than 25 g/kg DM (Table 2). Moreover, Peyraud et al. (1997) fed cows with grasses that differed by 64 g/kg DM in their WSC and by less than 30g/kg DM in their NDF and observed no influence on OMI.

Table 4 shows the mean rumen pool sizes, pH, NDF fractional clearance (Kcl_{NDF}) and fractional degradation (kd_{NDF}) rates of the different varieties in the two Latin squares in both years. Water-soluble carbohydrates are expected to be rapidly fermented by rumen microbes. Therefore, if the rate of VFA production exceeds the rate of VFA clearance through absorption and outflow, high concentrations of VFA and low pH values may develop. Obara et al. (1991) observed a significant decrease in rumen pH on a sugar-supplemented diet compared with an un-supplemented control. In the present study, feeding high WSC grass (cv1 and cv4) increased the amount of WSC consumed by the animal up to 600 g/d (Table 3), but did not seem to have any influence on the pH in the rumen. The mean pH in the rumen ranged between 5.8 and 6.1 and was not lower ($P > 0.25$) in animals consuming the high WSC grass varieties. Moreover, the minimum pH and the standard error of the pH, which represents the fluctuation in pH during the day, were not different among varieties. Similarly, Lee et al. (2002) observed no negative effect on mean pH value when they fed steers on a high WSC grass compared to a control grass with a normal WSC content. It is known that pH in the rumen is related to VFA production, but it is also related to the rate of ingestion, rate of release of soluble contents from the cells and rate of salivation. In grass-fed animals, the rate of ingestion and release of soluble contents from the cell may limit the rapid fermentation of soluble sugars and therefore the drop in ruminal pH. Only rumen N pool was significantly different among varieties (Table 4, 2001, second Latin square). In general, lower N rumen pools were observed when grasses with an increased WSC were fed. This is probably related to the lower CP content of these varieties (cv1 and cv4) (Table 2).

Table 3. Mean intake and digestibility of different components of six varieties of perennial ryegrass (two of which have a high sugar content; cv1 and cv4) in two Latin squares in two years (2000 and 2001)

		Latin square								Total	
		1st Latin square				2nd Latin square					
		Variety				Variety					
Year		cv1	cv2	cv3	P	cv4	cv5	cv6	P	Mean	SEM
2000	DMI[1]	16.2	16.5	16.6	0.73	16.2	17.3	16.8	0.26	16.6	0.4
	TDMI[2]	20.2	20.5	20.7	0.73	20.3	21.4	20.9	0.26	20.7	0.4
	OMI	18.2	18.3	18.5	0.81	18.1	19.0	18.6	0.24	18.4	0.3
	NI, g/d	521	547	563	0.09	492^{d}	523^{e}	531^{e}	0.02*	529	14
	WSCI	3.3	3.1	3.0	0.11	3.4^{e}	3.1^{e}	2.8^{d}	0.01*	3.1	0.1
	NDFI	7.6	7.9	7.9	0.48	7.6^{d}	8.1^{e}	8.2^{e}	0.01*	7.9	0.2
	DMD[3]	82.6	79.8	80.7	0.48	81.8	81.6	80.4	0.90	81.2	0.8
	OMD[4]	84.1	81.6	82.7	0.53	83.8	83.4	82.5	0.90	83.0	0.7
	DDMI[3]	16.7	16.4	16.7	0.14	16.7	17.5	16.8	0.76	16.8	0.4
	DOMI[4]	15.3	15.0	15.3	0.07	15.2	15.8	15.3	0.76	15.3	0.4
2001	DMI[1]	15.7	16.0	15.0	0.45	15.2	15.2	15.7	0.66	15.5	0.2
	TDMI[2]	18.2	18.4	17.5	0.45	17.7	17.7	18.1	0.66	17.9	0.2
	OMI	16.3	16.5	15.6	0.44	15.8	15.8	16.2	0.65	16.0	0.2
	NI, g/d	534	507	508	0.10	455^{d}	489^{de}	518^{e}	0.01*	502	12
	WSCI	2.2	2.1	1.6	0.07	1.9	1.8	1.8	0.51	1.9	0.1
	NDFI	7.3	7.7	7.6	0.14	7.7	7.5	7.6	0.09	7.6	0.1
	DMD[3]	84.4	83.1	79.3	0.17	82.0	78.7	81.8	0.19	81.6	1.2
	OMD[4]	85.6	84.2	80.6	0.12	83.0	79.8	83.0	0.18	82.7	1.2
	DDMI[3]	15.3	15.3	13.9	0.05*	14.5	14.0	14.8	0.45	14.6	0.3
	DOMI[4]	13.9	13.8	12.6	0.08	13.1	12.6	13.4	0.42	13.3	0.3

$^{d,\ e}$ Means in the same row within the 2nd Latin square with different superscripts differ significantly ($P < 0.05$)

[1] DMI is grass dry matter intake in kg/d

[2] TDMI is total dry matter intake in kg/d (grass + concentrates), OMI is total organic matter intake in kg/d, NI is total N intake in g/d, WSCI and NDFI are total water-soluble carbohydrates intake and total NDF intake in kg/d respectively

[3] DMD is DM *in vivo* apparent digestibility (%), DDMI is digestible DM intake kg/d

[4] OMD is OM *in vivo* apparent digestibility (%), DOMI is digestible OM intake kg/d

*$P < 0.05$

Voluntary intake of forages by ruminants is believed to be largely dependent on the retention time of the course material in the rumen. Therefore, in this experiment we measured the Kcl_{NDF} and kd_{NDF}, using the rumen evacuation technique to study the effect of a high WSC content in the grass on fibre clearance and degradation rates. High WSC varieties (cv1 and cv4) did not consistently coincide with high or low Kcl_{NDF} and kd_{NDF} values (Table 4). The lack of influence of high WSC on rumen pH might explain this finding, as the activity of fibre-degrading bacteria is known to be reduced at lower pH values. Rook et al. (1987) observed that rumen pH and rumen fibre degradation of ensiled grass was reduced when additional sugars were infused into the rumen. In the present experiment, feeding high WSC grasses, which increased daily WSC intake, influenced neither rumen pH nor kd_{NDF}. This lack of effect of high WSC on both rumen pH and kd_{NDF} might be related to the fact that the additional sugars in the present study were provided as an integral part of the diet, which was ingested over the day rather than infusing at certain time points.

Table 5 shows the mean daily rumen VFA and NH_3-N concentrations, NGR, and rumen VFA and NH_3-N total pools. In the first Latin square in the year 2000, feeding high WSC grass (cv1) elevated ($P < 0.05$) propionate proportion by 2 % at the expense of acetate, which decreased by 2 % causing a significant decrease ($P < 0.05$) in NGR. In contrast, in the second Latin square in 2000, feeding high WSC grass (cv4) did not significantly increase the proportion of propionate. This variation in response to high WSC is probably due to the fact that different cows were used in the second Latin square. Teather et al. (1984) suggested that some of the animal to animal variation observed in dietary treatment responses is due to a tendency for individual animals to have a different bacteria to protozoa ratio in the rumen. Peyraud et al. (1997) and Lee et al. (2002) observed higher ($P < 0.05$) propionate and butyrate proportions and lower ($P < 0.05$) acetate proportion when fed a high WSC grass to mid lactating dairy cows and steers, respectively. In contrast, in the present study the proportion of butyrate was not increased significantly when feeding high sugar grass in both Latin squares in both years.

Feeding high WSC grass tended to decrease NH_3-N concentration (mg/L) and pool size (g) in the rumen. Although the effect was numerically visible (Table 5), it was not statistically significant ($P > 0.09$). This might be related to the lower ratio of digestible N intake to DOMI, due to the lower CP content of these grasses, or to the higher readily available energy provided by these high WSC grasses to rumen microbes. A lower ratio of digestible N intake to DOMI is known to decrease NH_3-N concentration in the rumen (Van Vuuren et al., 1993). Moreover, a higher supply of readily available energy improves rumen microbes' ability to utilise NH_3-N (Rooke et al., 1987) and reduces the utilisation of amino acids as energy source by these microbes (Nocek and Russell, 1988), reducing the NH_3-N concentration in the rumen.

Table 4. Mean rumen pool sizes, pH, and NDF fractional clearance (Kcl) and fractional degradation (kd) rates (% h^{-1}) of six perennial ryegrass varieties in two Latin squares in two years (2000 and 2001)

		Latin square								Total	
		1st Latin square				2nd Latin square					
		Variety				Variety					
Year		cv1	cv2	cv3	*P*	cv4	cv5	cv6	*P*	Mean	SEM
2000	pH_mean	5.8	5.8	5.7	0.33	5.8	5.8	5.9	0.88	5.8	0.1
	pH_min[1]	5.6	5.5	5.5	0.08	5.6	5.6	5.5	0.22	5.5	0.1
	pH_SE[2]	0.65	0.65	0.70	0.26	0.67	0.64	0.63	0.53	0.66	0.01
	RDM[3]	10.9	11.2	12.9	0.30	12.3	13.1	14.3	0.47	12.4	0.5
	R liquid	87.5	81.1	99.0	0.32	100.0	101.5	112.9	0.30	97.0	4.5
	RN, g	352	365	409	0.29	426	445	487	0.44	414	16
	RNDF	5.1	5.4	5.9	0.26	5.3	5.8	6.2	0.58	5.6	0.2
	Kcl_{NDF}	5.4	5.9	5.7	0.57	5.3	4.9	5.1	0.17	5.4	0.2
	Kcl_{ADL}	2.7	3.5	3.0	0.22	2.8	2.4	3.1	0.37	2.9	0.2
	kd_{NDF}	2.7	2.4	2.7	0.83	2.4	2.5	2.0	0.43	2.5	0.2
2001	pH_mean	5.8	5.9	6.1	0.24	5.9	5.8	5.9	0.41	5.9	0.1
	pH-min[1]	5.6	5.6	5.7	0.76	5.6	5.6	5.6	0.84	5.6	0.1
	pH-SE[2]	0.15	0.29	0.31	0.17	0.23	0.20	0.25	0.79	0.24	0.02
	RDM[3]	11.2	12.3	12.0	0.79	12.2	13.1	13.4	0.29	12.4	0.5
	R liquid	93.9	96.5	94.8	0.94	98.4	97.8	100.5	0.55	97.0	3.8
	RN, g	415	431	415	0.94	407^{d}	438^{de}	457^{e}	0.02*	427	21
	RNDF	4.8	5.5	5.5	0.46	5.9	6.3	6.3	0.64	5.7	0.3
	Kcl_{NDF}	5.5	5.6	5.4	0.93	5.4	5.2	5.4	0.81	5.4	0.1
	Kcl_{ADL}	3.6	3.1	3.4	0.11	3.0	2.9	3.4	0.59	3.2	0.2
	kd_{NDF}	1.9	2.5	2.0	0.41	2.4	2.3	1.9	0.79	2.2	0.2

$^{d, e}$ Means in the same row within the 2nd Latin square with different superscripts differ significantly ($P < 0.05$)

[1] pH_min is the minimum rumen pH value observed among all sampling times

[2] pH_SE is the standard error of rumen pH taking into account values at all sampling times

[3] RDM is rumen DM content in kg, R liquids is rumen liquid content in kg, RN, g is rumen N content in g, RNDF is rumen NDF content in kg, Kcl_{NDF} is NDF fractional clearance rate in (% h^{-1}), Kcl_{ADL} is ADL fractional clearance rate in (% h^{-1}), kd_{NDF} is the NDF fractional degradation rate in (% h^{-1})

*$P < 0.05$

Table 6 shows milk yield and composition of the different varieties used in both Latin squares in both years. The concentrations of the major milk constituents (fat, protein and lactose) were not influenced significantly by the increased WSC content of the grass. Miller et al. (2001) also observed no significant influence of an increased WSC content of the grass on the major milk constituents. Milk yield was not increased when the animals were offered the high WSC grasses (cv1 and cv4), contradicting the observations of Miller et al. (2001) who reported a significant increase in milk yield of

Table 5. Mean daily rumen VFA (mM) and NH_3-N (mg/L) concentrations, for cows fed perennial ryegrass varieties with an elevated levels of WSC (cv 1 and cv 4) and other varieties in two Latin squares in two years (2000 and 2001)

		Latin square								Total	
		1st Latin square				2nd Latin square					
		Variety				Variety					
Year		cv1	cv2	cv3	*P*	cv4	cv5	cv6	*P*	Mean	SEM
2000	VFAcon.[1]	125	123	130	0.32	125	116	127	0.08	124	2
	Acetate	63	65	65	0.13	65	65	67	0.10	65	0.4
	Prop.	23^{b}	21^{ab}	21^{a}	0.04*	20	20	19	0.08	21	0.4
	isoButy.	0.5	0.5	0.5	0.28	0.5	0.5	0.6	0.58	0.5	0.1
	Butyrate	12	12	12	0.94	13	13	12	0.17	12	0.2
	isovale	0.9	0.7	0.9	0.53	0.9	0.8	0.9	0.86	0.9	0.1
	Valerate	1.2	0.9	1.0	0.43	0.8	0.8	0.8	0.87	0.9	0.1
	NGR[2]	3.8^{a}	4.1^{ab}	4.3^{b}	0.04*	4.6	4.4	4.8	0.06	4.3	0.1
	NH_3-Nconc.[3]	76	102	116	0.13	108	131	119	0.62	109	16
	VFApool[4]	10.8	10.4	12.8	0.27	13.0	12.4	14.4	0.19	12.3	0.6
	NH_3-Npool[5]	6.9	7.1	10.2	0.61	11.7	14.1	11.2	0.82	10.2	1.7
2001	VFAcon.[1]	106	105	96	0.08	96	99	103	0.18	101	3
	Acetate	65	67	67	0.17	66	66	66	0.69	66	0.3
	Prop.	20	19	18	0.28	21	20	20	0.42	19.7	0.4
	isoButy.	0.8	0.8	0.9	0.24	0.7	0.8	0.8	0.40	0.8	0.0
	Butyrate	11	12	11	0.82	10	11	11	0.22	11	0.2
	isovale	1.2	1.2	1.2	0.88	1.1	1.2	1.2	0.72	1.2	0.1
	Valerate	1.2	1.1	1.1	0.07	1.1	1.1	1.1	0.91	1.1	0.1
	NGR	4.2	4.6	4.7	0.32	4.2	4.2	4.4	0.25	4.4	0.1
	NH_3-Nconc.[2]	233	213	226	0.76	170	219	234	0.09	216	15
	VFApool[3]	9.9	10.1	9.0	0.64	9.4	9.5	10.5	0.07	9.7	0.5
	NH_3-Npool[4]	22.2	21.1	21.8	0.94	16.9	23.3	23.3	0.25	21.4	2.1

[a, b] Means in the same row within the 1st Latin square with different superscripts differ significantly ($P < 0.05$)

[1] total VFA concentration in mmol/L, acetate, propionate, isobutyrate, butyrate, isovalerate and valerate are presented as % of the total VFA.

[2] NGR is the nonglucogenic to glucogenic VFA ratio calculated as [(acetate + 2(butyrate + isobutyrate) + valerate)] / [propionate + valerate]

[3] NH3-N is ammonia-N concentration expressed in mg/L

[4] VFA is the VFA rumen pool size in mole

[5] NH3-N is ammonia-N rumen pool size in g

$*P < 0.05$

around 2.5 kg/d when fed late lactating cows on a high WSC grass variety. They related the increase in milk yield to the increased DDMI when fed the high WSC grass, which had a significantly higher DM digestibility. In the present study with high producing cows in mid lactation, milk yields from animals offered the high and low WSC grasses were not significantly different (Table 6). This might be related to the fact that DMI and DDMI were not significantly different among varieties.

Table 6. Milk yield and composition of cows fed perennial ryegrass varieties with an elevated levels of WSC (cv 1 and cv 4) and other varieties in two Latin squares in two years (2000 and 2001)

		Latin square								Total	
		1st Latin square				2nd Latin square					
		Variety				Variety					
Year		cv1	cv2	cv3	*P*	cv4	cv5	cv6	*P*	Mean	SEM
2000	Milk yield	23.8	25.4	24.0	0.53	25.1	26.2	26.9	0.49	25.2	1.1
	Fat %	4.0	4.1	3.9	0.15	4.2	4.0	4.0	0.54	4.0	0.1
	Protein %	3.5	3.4	3.4	0.59	3.4	3.4	3.3	0.66	3.4	0.1
	Lactose %	4.4	4.5	4.4	0.30	4.6	4.6	4.5	0.46	4.5	0.0
	Urea[1]	18	20	21	0.10	17^d	20^e	20^e	0.00*	19.3	1
	FCM[2]	23.6	25.9	23.8	0.27	25.8	26.2	26.9	0.45	25.4	1.1
	FPCM[2]	23.7	25.8	23.9	0.29	25.8	26.3	26.9	0.38	25.4	1.1
2001	Milk yield	27.0	27.1	25.3	0.44	26.4	27.0	27.2	0.66	26.7	0.7
	Fat %	3.9	3.9	4.1	0.27	3.7	3.8	3.8	0.18	3.9	0.1
	Protein %	3.1	3.0	3.0	0.26	3.0	3.0	3.1	0.16	3.0	0.1
	Lactose %	4.4	4.4	4.4	0.14	4.4	4.4	4.4	0.24	4.4	0.0
	Urea[1]	41	39	36	0.21	35	36	37	0.56	37.4	1
	FCM[2]	26.4	26.6	25.7	0.57	25.3	26.4	26.4	0.47	26.1	0.6
	FPCM[2]	26.1	26.2	25.1	0.54	25.0	26.1	26.1	0.47	25.8	0.6

[d, e] Means in the same row within the 2nd Latin square with different superscripts differ significantly ($P < 0.05$)

[1] Urea is milk urea concentration in mg/dL

[2] FCM is fat corrected milk (4%) (kg/d), FPCM is fat and protein corrected milk (kg/d)

*$P < 0.05$

Milk urea concentration (mg/dL) was significantly influenced by the increased WSC content of the grass (Table 6, year 2000, first and second Latin squares). Milk urea concentrations from cows offered the high WSC grasses were lower ($P = 0.1$ and $P < 0.01$ for the first and second Latin squares in 2000, respectively) than those from cows offered grasses with low WSC content. The lower CP content and intake of the high WSC grass and the lower rumen NH_3-N concentration of the cows offered the high WSC varieties may explain this finding (Table 5).

In 2000 milk urea concentration was lower (19 vs. 37 mg/dL) and milk protein content was higher (3.4 vs. 3.0 %) than in 2001 (Table 6). The higher milk protein content in 2000 can be related to the advanced stage of lactation of the cows used in this year (126 vs. 77 DIM at the start of the experiment) and to the higher concentrates feeding level (4.6 vs. 2.8 kg). Cows in late lactation are in a better energy balance and are known to produce milk with higher protein content. High levels of concentrates feeding shift rumen fermentation towards propionate production, elevating its concentration, which is known to increase protein content in milk. In their review, Sutton and Morant (1989) reported that a higher propionate concentration has shown to decrease milk fat content and increase milk protein content. The lower milk urea concentration in 2000 can be related to the higher WSC content (162 vs. 104 g/kg), higher total WSC intake (3.1 vs. 1.9 kg/d), lower ratio of digestible N intake to DOMI (25.8 vs. 32.1), and the lower NH_3-N concentration in the rumen (109 vs. 216 mg/L) in this year.

4. Conclusions

Feeding high producing dairy cows on perennial ryegrass varieties with an elevated concentration of WSC: 1- tended to reduce NH_3-N concentration in the rumen. 2- shifted ruminal fermentation towards propionate at the expense of acetate. 3- did not influence ruminal pH, rate of fibre degradation or clearance. 4- decreased urea concentration in the milk. However, no effect was observed on DMI, milk yield and milk composition. An increased WSC content in the grass is usually coupled with decreased protein and fibre contents, making it difficult to unravel the cause of the observed difference in animal response and the mechanisms behind it.

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Chapter 5

Grazing behaviour, intake, rumen function and performance of dairy cows offered *Lolium perenne* containing different levels of water-soluble carbohydrates

H. Z. Taweel [1*], B. M. Tas [1], H. J. Smit [2], A. Elgersma [2], J. Dijkstra [1] and S. Tamminga [1]

[1] Animal Nutrition Group (ANU), Department of Animal Science, Wageningen Institute for Animal Sciences (WIAS), Wageningen University, P. O. Box 338, 6700 AH, Wageningen, The Netherlands

[2] Crop and Weed Ecology Group (CWE), Department of Plant Science, Wageningen University, P. O. Box 430, 6700 AK, Wageningen, The Netherlands

Grazing behaviour, intake, rumen function and performance of dairy cows offered *Lolium perenne* containing different levels of water-soluble carbohydrates

H. Z. Taweel, B. M. Tas, H. J. Smit, A. Elgersma, J. Dijkstra and S. Tamminga

Abstract

The objective of this study was to measure the grazing behaviour of dairy cows grazing different perennial ryegrass varieties, which differ in their chemical composition especially their water soluble carbohydrates (WSC) content and examine its effect on intake, rumen function and milk production and composition. For that purpose, four multiparous rumen cannulated dairy cows were offered four varieties of perennial ryegrass under a daily strip-grazing system using a 4 × 4 Latin square design. Grazing behaviour measurements were done using a solid-state behaviour recorder. Daily intake was estimated using the n-alkane technique. Rumen function was measured using rumen evacuations. The magnitude of difference in WSC content among varieties was around 35 g/kg DM. Although all varieties were subjected to the same management and environmental conditions, the growth rate of some varieties was lower than of others, leading to differences in herbage allowance and sward surface height (SSH). The variety with the lowest WSC content had also the lowest herbage allowance and the shortest SSH and the highest neutral detergent fibre (NDF) content ($P < 0.05$). Despite that, cows grazing the different varieties did not show a significantly different grazing behaviour in terms of total eating time (TET), bite rate, chewing rate, intake rate and bite mass. However, TET was numerically shorter (40 to 50 min less) for cows grazing the variety which had the lowest WSC content and herbage allowance and the shortest SSH. This tended to lower ($P = 0.07$) DMI intake and consequently lowered ($P = 0.04$) milk yield of cows grazing this variety, but milk composition was not affected. Ruminating behaviour and rumen pool sizes were not different between cows grazing the different varieties. The fractional clearance rate of NDF was also not different among varieties. The effect of WSC content in the grass in this study was confounded with SSH and herbage allowance as the low WSC variety had shorter sward (15.9 vs. 17.9 cm) and lower herbage allowance (34 vs. 40 kg DM/cow per day). Nevertheless, the level of difference in herbage allowance, SSH and WSC content reported in this study failed to influence grazing behaviour, rumen pool sizes and clearance rate significantly.

Key words: grazing behaviour; water-soluble carbohydrate; rumen function; tensile strength

1. Introduction

Grazed pasture is by far the cheapest source of nutrients for dairy cows. Consequently, milk produced during the grazing season is much less costly than that produced during the winter months. The rapid increase in genetic merit and hence production potential of the dairy herd in recent years has placed an increased demand on pasture as a source of nutrients. Grass pasture suffers from the major disadvantage that dairy cows are unable to consume enough DM to achieve high levels of milk production (Gibb et at., 1999). Moreover, grass also suffers from the disadvantage of having an unbalanced nutrient composition. The high concentration of rapidly rumen degradable protein and slowly fermentable fibre in grass DM is thought to be the cause of these major limitations and disadvantages (Van Vuuren et al., 1993). Slowly fermentable fibre resides in the rumen for a long time, occupying space and limiting the capacity of the rumen to receive additional feed and as a consequence limiting dry matter intake (DMI). High levels of rapidly rumen degradable protein result in higher ammonia concentration in the rumen, which requires sufficient energy to be efficiently utilised by the rumen microflora. However, due to a lack of synchrony in the release of protein and energy of ingested grass, a large proportion of rumen ammonia will be absorbed through the rumen wall to the blood and consequently excreted in the urine as urea leading to high N losses.

Grass chemical composition can be manipulated by selection and breeding to improve its DMI and protein utilisation. Recently, breeders succeeded in producing grass with high sugar and low fibre content (Miller et al., 2001; Lee et al., 2002). Efforts are on the way to produce grass with rapidly degradable fibre (Taweel et al., 2002) and slowly degradable protein. The effect of elevated concentrations of sugars in the grass on DMI, rumen function, and milk production and composition was studied extensively (Miller et al., 2001; Lee et al., 2002). However, these experiments were conducted under stall-feeding conditions, which cannot be extrapolated to the grazing situation. Moreover, these experiments lacked data on eating time and behaviour of the animals on the different varieties.

During grazing, DMI and rumen function are largely determined by bite mass (BM), bite rate, total eating time (TET) and ruminating time, which in turn are largely influenced by sward state variables, such as sward surface height (SSH) (Gibb et al., 1997) and herbage bulk density (Laca et al., 1992). Animal variables, such as lactation status and nutritional demand have also shown to influence grazing behaviour parameters and hence herbage intake (Gibb et al., 1999) and rumen function. Grass chemical and mechanical characteristics, such as sugar and fibre content and tensile strength might also influence grazing behaviour parameters and hence herbage intake and rumen function. Therefore, the objective of this study was to measure the grazing behaviour of dairy cows grazing different perennial ryegrass varieties, which differ in their chemical composition especially their sugar content and examine its effect on intake, rumen function and milk production and composition.

2. Materials and methods

2.1. Pasture, Cows and Experimental Design

Four varieties of perennial ryegrass (*Lolium perenne* L.) (cv1 to cv4) were selected based on their difference in water-soluble carbohydrate (WSC) content in a stall-feeding experiment a year earlier. Two varieties (cv1 and cv4) were expected to have a high WSC content, as cv1 was selectively bred for high WSC content and cv4 showed a high WSC content in a previous stall-feeding experiment (Taweel et al., 2002). The other two varieties were expected to have a moderate (cv2) and a low (cv3) WSC content based on the results of that experiment. The four varieties were sown in the autumn of 2001 on two adjacent pastures (A and B) and they received the same N fertilisation level and management all over the experimental period. Each pasture was divided into four fields, each of 22-m wide and 110-m long. On each field one variety was randomly sown. Each field was divided into 18 plots, which represent days, therefore, each plot was 22-m wide and 6-m long (132 m^2). A step-cutting regime was employed to allow the grass on each plot to grow for 23 days (yielding approximately 2000 kg of DM/ha) before the cows were moved onto it.

Four high-producing Holstein-Friesian dairy cows (65 ± 8 DIM), previously fitted with a large rumen cannula in the dorsal rumen sac (10 cm id., Bar-Diamond Inc., Parma, Idaho, U.S.A.), were used. The cows (532 ± 9 kg BW) were in their second and third lactation and produced 29.3 ± 1.3 kg of milk at the beginning and 23.1 ± 0.9 kg of milk at the end of the experiment. The experiment was approved by an ethical committee, and executed in accordance with Dutch legislation on the use of experimental animals.

The experiment was conducted as a 4 × 4 Latin square design, with four treatments (ryegrass variety), four periods and four cows. Each period lasted for two weeks: one week for adaptation and the other for measurements. The experiment started on July 4 and ended September 1, 2002. During the first and third period and during the second and fourth period the cows grazed on pastures A and B, respectively. The experimental day started at 12:00 h, when the animal was moved to a new plot and stayed in that plot until 12:00 h the next day. The cows were housed individually by separating the plots with a double electrical wire at 40 and 100 cm height. Therefore, the cows could see and touch the adjacent cows but could not eat the grass variety allocated to the other cows. The cows were milked twice daily at 6:00 h and 16:00 h using a mobile milking parlour. Daily milk yield from each cow was registered, and milk was sampled at each milking and stored at 4 °C pending analysis for fat, protein and lactose. When weather conditions were not favourable for grass growth and DM yields were lower than anticipated the plot size was enlarged to 1.5 or 2 times the original size of 132 m^2.

2.2. Dry Matter Intake Measurements

As cows were housed individually on the different plots, individual DMI (kg/d) was measured using the n-alkanes technique. The cows were dosed twice daily with approximately 500 mg of C32 alkane, starting the first day of each period. To avoid daily oral dosing, a special concentrate diet was prepared to which C32 alkane dissolved over cellulose powder (1:10), was added before pelleting. The cows received 1.5 kg of these concentrates at every milking in each day in the milking parlour. On days 8 to 13 of each period, grass and faeces were sampled and stored (-20 °C) pending analysis. Grass was sampled three times a day at 14:00, 20:00 and 7:00 h to get a representative sample of what the cows ate. Faeces were sampled after the animal was removed from the plot to the next day's plot by taking two full tablespoons from each dung patch. Grass samples were oven-dried (at 60 °C for 48 h), ground to pass a 1-mm screen, pooled over the three daily collection times and then pooled over the six days of collection per animal, on equal dry matter bases. Faeces samples were freeze-dried, ground to pass a 1-mm screen and then pooled over the six days of collection per animal, on equal dry matter bases. Samples from the concentrate diet were also collected at day 12 of each period, pooled, oven-dried and ground to pass a 1-mm screen. Grass, faeces and concentrates samples were analysed for ash, crude protein (CP) (N × 6.25), NDF, ADL and WSC as described by Goelema et al. (1998), and for n-alkanes (C27 to C36) using GC as described by Mayes et al. (1986) and modified using a capillary column as described by Laredo et al. (1991). The DMI for each animal in each period was calculated using C32 as a dosed alkane and C33 as a natural herbage alkane as described by Mayes and Dove (2000) and shown in the equation below:

$$\text{Herbage DMI} = Dj / ((Hi * Fj / Fi) - Hj).$$

Where Hi and Fi are the herbage and faecal concentrations (mg/kg DM) of the odd-chain alkane (C33), Hj and Fj are the herbage and faecal concentrations of the even-chain alkane (C32) and Dj is the daily dose of the even-chain alkane (C32) in mg.

2.3. Grazing Behaviour Measurements

Grazing behaviour measurements were done in two different days for each cow in each period. On Monday (day 10) and Wednesday (day 12) of the second week of each period, 24-h grazing behaviour measurements were done using a solid-state behaviour recorder (Rutter et al., 1997). The jaw recorders were fitted to the cows at 12:00 h immediately before moving to the new plots and remained there until 12:00 h the next day. Recordings were analysed using peak recognition algorithm software with a noise threshold capable of identifying periods of grazing and ruminating activity and biting and non-biting total grazing jaw-movements (TGJM) (Graze Version 0.8, Institute of Grassland and Environmental Research 1994 - 1999) (Rutter, 2000). Total eating time (TET), number of biting and non-biting jaw-movements during eating and the number of mastications during ruminating were determined as described by Gibb et al. (1999). In calculating the intake rate (IR), bite rate and TGJM rate, the time base employed was the TET. Average daily BM was calculated as DMI divided by the number of bites the animal made during that day. The average values of grazing behaviour measurements of the two days for each cow were used in the statistical analysis.

2.4. Rumen Function Data

Two rumen evacuations were done for each animal at the end of the second week of each period. The first evacuation was done on Thursday evening (day 13) at 19:00 h and the second evacuation on Friday morning (day 14) at 8:00h. The animals did not receive any feed between the two evacuations, but water was freely available. Rumen pool sizes of the different components (DM, OM, N, NDF, ADL) were determined from the total fresh weight of rumen content in the first evacuation and the percentage of these components in the rumen contents. Fractional clearance rates of NDF (Kcl_{NDF}) and ADL (Kcl_{ADL}) were calculated using the logarithmic transformation of the exponential equation $R_{(t)} = R_{(0)} \times e^{-Kcl \times t}$, where $R_{(t)}$ is the amount present at the second evacuation, $R_{(0)}$ is the amount present at the first evacuation, and t is the time between the two evacuations in hours.

2.5. Sward Data

To estimate dry matter yield (DMY) and hence herbage allowance, 7 % of the area of each plot was mown (at 4 cm height) before the introduction of the animal to the plot, on days 10, 11, 12 and 13, of each period. The mown grass was collected, weighed fresh and sampled using a grass-core for DM determination (oven drying at 70 °C for 24 h). Sward surface height was measured daily by taking 10 measurements in each plot using a falling plate meter as described by Elgersma and Schlepers (1997).

Because grazing behaviour was measured on days 10 and 12 of each period, the average DMY, SSH and herbage allowance of these two days was used for the analysis.

Tensile strength was measured using the method described by Henry et al. (1996) using the conventional clamps instead of the cylindrical clamps. Briefly, on Thursday (day 13) of each period, 10 leaves from each plot were cut at ground level with a pair of scissors and stored in water to prevent dehydration. In the lab the width (mm) and length (cm) of the leaves were measured using millimetre paper and a ruler, respectively. Then the maximum force in Newton (N or $kg \cdot m/s^2$) needed to break the leaf was measured.

2.6. Statistical Analysis

Plant data (chemical composition, SSH and tensile strength) were subjected to a two-way ANOVA analysis, in which the sources of variation were the variety and the period, with three degrees of freedom each, leaving nine degrees of freedom for the error. Animal data (grazing behaviour, rumen function, DMI and milk production) were subjected to a three-way ANOVA analysis, in which the sources of variation were the variety, period and the cow, with three degrees of freedom each, leaving six degrees of freedom for the error. All the analyses were done using general linear model procedures of SPSS (SPSS for Windows, Rel. 10.1.0. 2000. Chicago: SPSS Inc.). When significant differences due to the treatment (ryegrass variety) were detected, the multiple comparison procedure Tukey was used to separate least square means. Unless stated otherwise, differences of $P < 0.05$ were considered to be significant.

3. Results and Discussion

Table 1 shows the mean chemical composition of the different varieties. The varieties differed ($P < 0.05$) in their OM, CP, NDF and WSC content. The maximal differential magnitude in OM, CP and NDF between the varieties did not exceed 25 g/kg DM, whereas the maximal differential magnitude in WSC between the varieties was almost 35 g/kg DM. As was expected, cv1 and cv4 had a higher ($P < 0.05$) WSC content than cv3, which had the lowest WSC content. Moreover, cv1 and cv4 had a lower ($P < 0.05$) CP and NDF content compared to cv3. In this study, a negative correlation was observed between WSC and CP (r = 0.72, $P < 0.01$) and NDF (r = 0.75, $P < 0.01$) content of the grass. Many previous studies have shown that the content of WSC in perennial ryegrass is inversely related to CP content (Wilman and Altimimi, 1982; Humphreys, 1989). However, McGrath (1992) observed a poor relationship when data of different experiments were pooled, but within each experiment the relationship was evident.

Table 1. Mean chemical composition (g/kg DM) of four varieties of perennial ryegrass

	Variety					
	cv1	cv2	cv3	cv4	SEM[1]	*P* value
DM[2]	183.3	187	195.7	183.1	4.0	0.16
OM	897.7^{c}	893.4^{bc}	888.1^{a}	890.7^{ab}	1.7	0.01
CP	180.2^{ab}	173.9^{a}	186.8^{b}	172.2^{a}	6.5	0.03
NDF	434.8^{a}	457.4^{b}	459.5^{b}	441.9^{ab}	10.7	0.01
ADL	16.6	16.8	18.8	14.8	1.2	0.08
WSC	136.6^{b}	123.9^{b}	104.4^{a}	137.6^{b}	13.5	0.01

$^{a, b, c}$ Means in the same row with different superscripts differ significantly ($P < 0.05$)

[1] SEM is the standard error of the mean (n = 16)

[2] DM is in g/kg fresh material

Table 2 shows herbage allowance and sward physical and mechanical characteristics of the different grass varieties. Despite the similar re-growth period used for all varieties to obtain a similar DMY and therefore a similar herbage allowance, the growth of some varieties (cv2 and cv3) was slower than of others (cv1 and cv4), as illustrated by their lower (P = 0.01) DMY. This caused differences in herbage allowance (Table 2), as the area of the plots offered to the cows was similar for all varieties. However, on average herbage allowance for all varieties was above the anticipated value of 25 kg/cow/day (Table 2). Herbage allowance is one of the most important sward characteristics that influence herbage intake (Hodgson, 1977). It was shown that as herbage allowance increases, DMI increases but at a progressively slower rate (Meijs and Hoekstra, 1984; Peyraud et al., 1996; Dalley et al., 1999). Summarising results from these studies showed that the marginal response in herbage intake to additional herbage declines as herbage allowance increases, indicating that the relation between herbage allowance and DMI is curvilinear asymptotic. However, research results are very variable with regard to the plateau value of herbage allowance at which no extra increase in DMI is expected. Peyraud et al. (1996) reported an increased DMI as herbage allowance increased from 20 to 54 kg DM/cow/day, with a plateau occurring at an allowance of 33 kg DM/cow/day. However, Dalley et al. (1999) reported an increased DMI as herbage allowance increased from 20 to 70 kg DM/cow/day, with a plateau occurring at an allowance of 55 kg DM/cow/day. In the present study, average herbage allowance of all varieties was above 33 kg DM/cow/day, the plateau value reported by Peyraud et al. (1996), but was way below the plateau value reported by Dalley et al. (1999). Therefore, herbage allowance may have had an influence on grazing behaviour and DMI in this study.

The SSH differed ($P < 0.05$) among varieties. Although the difference was at maximum 2 cm only, it was statistically significant (Table 2). The lowest yielding variety (cv3) had also a shorter SSH compared to the high yielding varieties (cv1 and cv4). Grazing behaviour of dairy cows on temperate pastures, mainly TET and BM have been shown to be influenced significantly by SSH (Gibb et al., 1997; McGilloway et al., 1999). The fact that the variety with the low WSC content (cv3) also had the shortest sward (Table 2) makes it difficult to draw solid conclusions concerning the effect of an elevated content of WSC in the grass on grazing behaviour and DMI in this study.

Table 2. Mean DM yield (kg/ha), herbage allowance (kg DM/cow/day), sward surface height (SSH) (cm) and tensile strength of four varieties of perennial ryegrass

	Variety					
	cv1	cv2	cv3	cv4	SEM[1]	*P* value
DM yield (kg/ha)	2251^{b}	2007^{a}	1977^{a}	2277^{b}	115	0.01
Herbage allowance	38.9^{ab}	34.4^{a}	34.0^{a}	39.6^{b}	2.8	0.01
SSH (cm)	17.7^{b}	17.1^{ab}	15.9^{a}	17.9^{b}	0.63	0.01
Leaf width (mm)	3.2	3.2	3.1	3.1	0.05	0.87
Tensile strength[2] (N)	8.5	8.6	8.4	8.7	0.27	0.95
Tensile strength[3]	2.7	2.7	2.7	2.8	0.07	0.62

[a, b] Means in the same row with different superscripts differ significantly ($P < 0.05$)

[1] SEM is the standard error of the mean (n = 16)

[2] is the maximum force needed to break the leaf in N (kg.m/s^2)

[3] is the maximum force needed to break the leaf per mm leaf width in N/mm

It was hypothesised that stronger leaves might require more energy and time from the grazing animals to harvest them, therefore reducing bite rate, and hence reducing herbage intake, unless the animal compensates by increasing TET or BM. In the present study, tensile strength of the different varieties was measured to gain more insight on its effect on grazing behaviour and herbage intake, and to have an extra variable that might help in explaining the observed differences between the varieties. No differences in tensile strength and leaf width among the varieties were observed (Table 2). Therefore, any observed difference in grazing behaviour parameters, and hence DMI, of the different varieties in the present study cannot be attributed or related to differences in tensile strength.

Table 3 shows grazing behaviour parameters and DMI of dairy cows grazing different varieties of perennial ryegrass. Despite the differences in WSC content (Table 1), herbage allowance and SSH (Table 2) among varieties, dairy cows grazing the different varieties showed a similar grazing behaviour ($P > 0.2$). None of the grazing behaviour parameters (TET, TGJM rate, bite rate, chewing rate, BM and IR) was significantly different among varieties. We are unaware of any studies that evaluated the effect of perennial ryegrass variety or high sugar grass on grazing behaviour of dairy cows. Many other studies, however, evaluated the effect of SSH and herbage allowance on grazing behaviour and showed that TET increases as SSH decreases (Gibb et al., 1999; McGilloway et al., 1999), indicating that dairy cows try to compensate the low herbage availability by increasing TET. In contrast, in our study, dairy cows on the variety with the shortest sward (cv3) grazed shorter ($P = 0.2$) time than cows grazing the other varieties, which had a taller SSH. This may be related to the fact that cv3 was more infected with crown rust than the other varieties (Tas et al., 2003). Bite mass was not significantly different ($P = 0.7$) between cows grazing the different varieties. Gibb et al. (1999) and Patterson et al. (1998) reported a significant increase in BM with increasing SSH from 5 to 9 cm and from 8 to 18 cm, respectively. However, Christie et al. (2000) observed a non-significant effect of SSH on BM when SSH increased from 25 to 40 cm and reported a constant BM of 640 mg DM/bite. Patterson et al. (1998) also observed that BM appears to level off at SSH greater than 18 cm, which may reflect either a maximum physical limit to BM in very tall swards, or a reduction in bulk density of the grazed horizon in tall swards (Stobbs, 1975). The absence of significant effects of SSH and herbage allowance on TET and BM in this study may be due to the small, although significant, difference in SSH (2 cm) and herbage allowance (5.6 kg/d) among varieties. Bite rate (bites/min) and TGJM rate (TGJM/min) were similar ($P > 0.6$) for the cows grazing the different varieties. Bite rate was around 57 min^{-1} and TGJM rate was around 71 min^{-1} for the cows grazing the different varieties. Cows on the shorter sward with the lower WSC content (cv3) did not compensate by increasing bite rate or TGJM rate. Similarly, Gibb et al. (1999) and Chilibroste et al. (2000) observed a non-significant effect of SSH on bite rate.

Daily DMI tended to be different ($P = 0.07$) between cows grazing the different varieties. Cows grazing cv3 (which had the lowest herbage allowance and WSC content and the shortest SSH), had a lower ($P = 0.07$) DMI than cows grazing cv4 (Table 3). The combination of shorter ($P = 0.2$) TET, the lower ($P > 0.6$) number of bites during a grazing day of cows grazing cv3 (Table 3) and the crown rust infection of this variety might explain the observed lower DMI. Cows grazing cv3 grazed 50 min less and took 3000 bites less than cows grazing cv4. In addition, the low herbage allowance and the shorter SSH of cv3 might also have contributed to the lower DMI of cows grazing this variety. Many studies have shown that pasture DMI decreases as herbage allowance and SSH decrease (Peyraud et al., 1996; Dalley et al., 1999). Moreover, the lower ($P < 0.05$) WSC and higher ($P <0.05$) NDF content of cv3 may also have contributed to the lower DMI of cows grazing this variety. Miller et al. (2001) and Lee et al. (2002) reported a lower DMI of late lactating dairy cows and steers, respectively when stall-fed a grass variety that is characterised by its low WSC and high NDF content compared to a variety with high WSC content. Therefore, the observed trend in DMI among the different varieties in this study cannot be attributed to only one factor (i.e. WSC content), but other factors such as herbage allowance, SSH, crown rust infection and NDF content may have played a role too.

The slightly longer ($P = 0.2$) TET of cows grazing the high WSC varieties (cv1 and cv4) could not be related to sward mechanical characteristics such as tensile strength, as it was shown that in this study tensile strength was similar ($P > 0.6$) for all varieties (Table 2).

Table 3. Mean DMI, total eating time (TET), intake rate (IR) and bite mass (BM) of dairy cows grazing four different varieties of perennial ryegrass

	Variety					
	cv1	cv2	cv3	cv4	SEM[1]	*P* value
DMI [2] (kg/d)	16.5^{ab}	15.5^{ab}	15.0^{a}	17.7^{b}	0.5	0.07
TET (min)	530	502	490	540	13	0.2
Total GJM	37043	35154	35422	37836	1185	0.7
Bites in GJM	29563	28624	28059	31088	993	0.6
Bites (%)	79	82	80	82	1	0.5
TET (% of day)	37	36	35	38	0.9	0.3
Bite rate (min^{-1})	56	57	58	58	1.5	0.8
Chewing rate (min^{-1})	14	13	15	12	1.0	0.3
GJM rate (min^{-1})	70	70	73	70	1.3	0.6
IR [2] (kg DM/h)	1.86	1.86	1.85	1.97	0.04	0.7
IR [2] (g DM/min)	31	31	31	33	0.6	0.7
BM [2] (mg DM)	568	544	533	573	13.3	0.7

[1] SEM is the standard error of the mean (n = 16)

[2] calculated using the n-alkane technique intake estimates

[a,b] means in the same row with different superscripts tend to differ significantly ($P < 0.1$)

Table 4 shows mean rumen pools, NDF clearance rate (Kcl_{NDF}) and rumination behaviour of dairy cows grazing different varieties of perennial ryegrass. Despite differences in chemical composition, herbage allowance and SSH among varieties, rumen pools, Kcl_{NDF} and ruminating behaviour were not significantly different among varieties. Cows grazing the different varieties had similar rumen DM, OM, N, and NDF pools. Taweel et al. (2002) in a stall-feeding experiment showed that rumen pools were not different among perennial ryegrass varieties. Varieties with the high WSC content did not posses the lowest Kcl_{NDF} and the maximum difference in the Kcl_{NDF} between the different varieties was less than 1.0 %/h in agreement with what was observed about the same varieties in an earlier stall-feeding experiment (Taweel et al., 2002). Cows on all varieties seemed to spend 34 % of the day ruminating, without a clear relation with NDF or ADL content of the grazed grass, or with rumen NDF pools. It was hypothesised that cows grazing on varieties with higher NDF content (cv3) might spend more time ruminating than cows grazing on varieties with a lower NDF content (cv1 and cv4). The small difference in NDF content among varieties (25 g/kg) (Table 1) and the fact that cows grazing the high NDF variety (cv3) consumed less DM (Table 3), may have masked the relation between NDF content and rumination time. Rumination time is believed to be positively related to DMI; lactating cows that consume more herbage daily ruminate longer (+ 30 min) than dry cows that consume less herbage (Gibb et al., 1999). Moreover, lactating cows that were offered a taller sward, consumed more herbage and ruminated longer (+ 30 min daily) than lactating cows that were offered a shorter sward and consumed less herbage (Gibb et al., 1999). Although in the present study cows grazing cv3 consumed 1.5 and 2.7 kg DM less than cows grazing cv1 and cv4 respectively, no significant differences were observed in rumination time.

Table 4. Mean rumen pools, NDF fractional clearance rate (Kcl_{NDF}) and ruminating behaviour of dairy cows grazing four varieties of perennial ryegrass

	Variety					
	cv1	cv2	cv3	cv4	SEM[1]	*P* value
Rumen liquid pool (kg)	100	94.7	101.7	98.5	3.8	0.7
Rumen DM pool (kg)	12.7	11.6	12.8	12.8	0.5	0.2
Rumen OM pool (kg)	11.3	10.2	11.1	11.2	0.4	0.2
Rumen N pool (kg)	0.47	0.42	0.47	0.46	0.02	0.1
Rumen NDF pool (kg)	5.3	4.9	5.3	5.4	0.2	0.3
Rumen ADL pool (kg)	0.49	0.47	0.53	0.48	0.03	0.4
Kcl_{NDF} (%/h)	5.8	6.2	5.8	5.9	0.2	0.8
Total ruminating time (min)	482	482	481	505	8	0.4
Ruminative mastications	30255	28606	30070	32489	913	0.2
Boli processed (d^{-1})	601	714	632	640	35	0.3
Rumination rate[2] (min^{-1})	63	59	63	64	1	0.4
Rumination[3] (min/kg DMI)	30	31	33	29	1	0.4
Rumination[4] (min/kgNDF)	68	69	71	65	2	0.7
Boli processing rate (min^{-1})	1.3	1.5	1.3	1.3	0.1	0.2
Ruminating time (% of day)	34	34	34	36	0.6	0.3

[1] SEM is the standard error of the mean (n = 16)
[2] Rumination rate is calculated as: number of ruminative mastications / min rumination time
[3] Calculated as min spent rumination per kg dry matter ingested
[4] Calculated as min spent rumination per kg NDF ingested

Table 5 shows the milk yield and composition of cows grazing different perennial ryegrass varieties. Cows grazing cv3, which had the lowest WSC content and herbage allowance and the shortest SSH, produced less ($P = 0.04$) milk than those grazing cv1 and cv4. This might be related to the fact that cows grazing cv3 consumed less herbage than cows on the other varieties (Table 3). The maximum observed difference in milk yield between the varieties was 2.6 kg/d. This range is in agreement with Miller et al. (2001), who observed a 2.5 kg/d increase in milk yield when stall-fed late lactating dairy cows with perennial ryegrass varieties that differed mainly in their WSC and NDF content. They attributed this increase in milk production to the increased digestible DMI of cows fed the high WSC variety. In contrast, Taweel et al. (submitted) did not observe any influence on milk yield when stall-fed the same varieties to mid lactating dairy cows in an earlier experiment. In that experiment, no difference was observed in DMI, which might explain the lack of influence on milk yield. However, in the present study under grazing, SSH, herbage allowance and WSC content were all lower for cv3, leading to a lower ($P = 0.07$) DMI of cows grazing this variety and consequently to a lower milk yield (Table 6). Milk yield is largely controlled by the quantity of nutrients supplied to the udder (Sutton and Morant, 1989; DePeters and Cant, 1992), which in turn is influenced by digestible DMI.

Table 5. Mean milk yield and composition of dairy cows grazing four varieties of perennial ryegrass

	Variety					
	cv1	cv2	cv3	cv4	SEM[1]	*P* value
Milk yield (kg)	27.8^{b}	26.5^{ab}	25.1^{a}	27.7^{b}	0.76	0.04
Milk fat (%)	3.87	3.94	4.03	3.85	0.05	0.1
Milk protein (%)	3.13	3.12	3.10	3.10	0.02	0.8
Milk lactose (%)	4.49	4.49	4.45	4.54	0.04	0.3
FPCM [2] (kg)	27.0^{b}	26.0^{ab}	24.9^{a}	26.9^{b}	0.74	0.04

[1] SEM is the standard error of the mean (n = 16)

[2] FPCM is fat and protein corrected milk calculated as (0.337 + (0.116 × % Fat) + (0.06 × % Protein)) × milk yield

[a, b] Means in the same row with different superscripts differ significantly ($P < 0.05$)

The major milk constituents (fat, protein and lactose) concentrations were not significantly affected by the grazed grass variety, although milk fat tended to be highest for cv3 and lowest for cv1 and cv4 ($P = 0.10$). This trend in milk fat content might be related to the higher NDF content of cv3 than cv1 and cv4 (Table 1). The positive relationship between NDF content in the ration of dairy cows and increased milk fat content is well-established (Sutton et al., 1988). Similarly, Miller et al. (2001) observed no significant influence of grass variety on the concentration of the major milk constituents when fed two grass varieties differing mainly in their WSC and NDF content to late lactating dairy cows. Milk composition is largely influenced by the type of nutrients supplied to the udder tissue (Sutton and Morant, 1989). In the present study, the supply of nutrients to the duodenum and the blood was not measured or estimated. However, the insignificant effect of variety on milk composition might indicate that the type of nutrients supplied to the udder tissue by the different varieties was not significantly different.

4. Conclusions

In this experiment, caution should be taken when interpreting the results, as many factors were confounded such as herbage allowance, SSH and WSC content of the grass. The variety with the lowest WSC content had the lowest herbage allowance and the shortest SSH, both of which are known to influence grazing behaviour and DMI. Cows grazing this variety had a lower DMI and milk yield. However, milk composition, grazing behaviour, rumination behaviour, rumen pool sizes and NDF clearance rates were all not significantly different among varieties. Therefore, the results suggest that the level of difference in herbage allowance, SSH and WSC content among the varieties examined in this study failed to influence grazing behaviour, rumen pool sizes and clearance rate significantly.

Acknowledgements

The authors wish to extend their gratitude to Barenbrug Holland BV and The Ministry of Economic Affairs (Economisch Zaken) in The Netherlands, for financing this work.

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Chapter 6

Intake Regulation and Grazing Behaviour of Dairy Cows under Continuous Stocking

H. Z. Taweel,[1] B. M. Tas,[1] J. Dijkstra,[1] and S. Tamminga[1]

[1] Animal Nutrition Group (ANU), Department of Animal Science, Wageningen Institute for Animal Sciences (WIAS), Wageningen University, P. O. Box 338, 6700 AH, Wageningen, The Netherlands

Accepted for Publication by Journal of Dairy Science, 26/05/2004
In Press (2004)

Intake Regulation and Grazing Behaviour of Dairy Cows under Continuous Stocking

H. Z. Taweel, B. M. Tas, J. Dijkstra, and S. Tamminga

Abstract

This experiment aimed at studying the behavioural strategies grazing dairy cows employ to satisfy their nutritional needs as the day progresses and the possible role of rumen fill in controlling these behavioural strategies. The day was divided into three main periods (0600 to 1200 h, 1200 to 1800 h and 1800 to 2400 h) where the three main grazing bouts (dawn, afternoon and dusk) of dairy cows usually occur. Four late lactating rumen-cannulated dairy cows were used in a repeated measures design, with the grazing bout as the within subjects factor. The cows had access to a 1-ha grass sward under a continuous stocking system. To estimate dry matter intake, bite rate, bite mass (BM) and intake rate at the three grazing bouts, cows were rumen-evacuated at 0600, 1200, 1800 and 2330 h and the jaw recorders were fitted to the cows between these time points. Time spent eating by dairy cows at the dusk grazing bout was much longer than that at the other two grazing bouts and made about 40 % of the daily total eating time. Total grazing jaw movements (TGJM) rate was constant during the day at around 75/min. Bite rate, BM and hence intake rate increased, but chewing rate decreased as the day progressed. The increase in BM was mainly due to the increase in dry matter content of the grass at dusk time rather than increased bite dimensions. Therefore, it could be concluded that the main behavioural strategies dairy cows employ to satisfy their nutritional needs under continuous stocking include manipulating their eating time, biting rate and chewing rate, with little control over TGJM rate and BM. Dairy cows interrupted the first two grazing bouts (dawn and afternoon) long before reaching their maximal rumen capacity, indicating that rumen fill is less likely to play a significant role in signalling the termination of these two grazing bouts. However, rumen pool sizes were always maximal at the time when the dusk grazing bout ceased indicating that rumen fill is more likely to play a major role in signalling the termination of the dusk grazing bout.

(**Key words**: rumen fill, grazing behaviour, intake rate, bite mass)

1. Introduction

In grazing animals, accurately estimating herbage intake (kg DM/day) is considered one of the most important tools in improving management and production. An accurate estimation of daily herbage intake can be achieved as the product of total eating time (TET) and intake rate (Gibb et al., 1998). The latter is known to be the product of bite rate (bites/min) and bite mass (BM) (mg DM/bite) (Allden and Whittaker, 1970). Factors of plant (sward), animal and environmental origin have been shown to influence TET, bite rate and BM, and hence herbage intake (Pulido and Leaver, 1995). Sward surface height, herbage allowance, tiller density, tensile strength and fibre content are among the most important factors of plant origin (Penning et al., 1991; Gibb et al., 1997). Physiological status, lactation stage, body weight, nutritional demand and productive capacity are among the most important factors of animal origin (Gibb et al., 1999). Ambient temperature, relative humidity and rainfall are important factors of environmental origin (Champion et al., 1994). Despite the above mentioned factors (plant, animal and environmental) and the grazing system, dairy cows have shown to exercise three main grazing events (bouts) during the day (dawn, afternoon and dusk) (Rook and Huckle, 1997). However, plant, animal and environmental factors and the grazing system may influence the duration of these three main grazing bouts. During a hot day, cows appear to be reluctant to graze before 1800 h reducing the duration of the second (afternoon) and increasing the duration of the third (dusk) main grazing bouts (Gibb et al., 1998). When offered the new pasture at 1200 h mid-day in a strip grazing system, dairy cows tend to increase the duration of the second (afternoon) and reduce the duration of the first (dawn) main grazing bouts (personal observation).

To satisfy its nutritional needs under the circumstances imposed by the sward, management and the environment, the grazing dairy cow manoeuvres by adjusting its eating behaviour in terms of eating time, bite rate, chewing rate, BM and intake rate. Therefore, not only the duration of eating at the three main grazing bouts during the day will be different, but also bite rate, chewing rate, BM and intake rate may differ as well. Daily herbage intake is the sum of the amount eaten at the different grazing bouts, which in turn is determined by the duration of the bout and the intake rate during that bout. Hence, estimating bite rate, BM and intake rate at the three main grazing bouts as influenced by plant, animal, and environmental factors, presents an opportunity to improve the modelling of herbage intake and hence its prediction in grazing ruminants. Gibb et al. (1998) observed a significant effect of time of the day on bite rate, BM and intake rate. However, in their experiment intake rate was measured using the short-term weight gain method in which the animals were deprived from food and water for one hour to measure insensible weight loss rate before the one hour intake rate measurement period commenced. This food deprivation period that proceeded the short measurement period might have made the animals hungry and therefore influenced their eating behaviour especially during the first hour after release.

Many theories to what initiates and terminates a grazing bout and to what controls the short-term daily herbage intake exist. Rumen fill and distension, rate of disappearance of feed from the rumen, fermentation end-products concentrations (total VFA, acetate, propionate, butyrate and NH_3) in the rumen and/or blood and rumen osmolality are among the most plausible ones. Although, Mbanya et al. (1993), Van Soest (1994), Forbes (1995) and Chilibroste (1999) are in favour of the multi-factorial control theory, which states that the initiation and termination of a meal seems more likely to be controlled by a combination of signals rather than a single signal. Chilibroste et al. (1997) studied the first grazing session (bout) extensively and concluded that cows interrupted it before their maximum rumen capacity was reached, indicating that rumen fill does not play a major role in controlling the termination of the first grazing bout. However, this cannot be extrapolated to the other two grazing bouts especially the third grazing bout at dusk time. An experiment was therefore conducted to estimate eating duration, bite rate, BM and intake rate at the three main grazing bouts during the day. Moreover, rumen pool sizes and fermentation end-products concentrations at the start and the end of each grazing bout were measured to investigate their role in controlling the initiation and termination of a grazing bout.

2. Materials and methods

2.1. Animals and Pasture

Four rumen-cannulated (10 cm id., Bar-Diamond Inc., Parma, Idaho, USA) Holstein-Friesian dairy cows (600 ± 20 kg BW), in late lactation (310 ± 21 DIM) were used in the experiment. Two cows were in their third lactation and the other two cows were in their fourth and ninth lactation. All the cows were pregnant at the onset of the experiment and produced 18.0 ± 0.4 and 19.1 ± 1.5 kg of milk at the start and the end of the experiment, respectively.

The pasture (1 ha) was sown in the autumn of 1999 with two varieties of perennial ryegrass. In 2003, the pasture was cut on the 25th of May for silage making and was allowed undisturbed growth till June 15th when the experiment actually started. The pasture had approximately 1700 kg of DM on it at the start of the experiment. The cows were allowed access to the whole pasture under a continuous stocking system with *ad libitum* herbage allowance and no compound feed from the start (15th June) till the end (4th July) of the experiment.

2.2. Experimental Design

The experiment lasted 20 d, of which the first 10 d were for adaptation. On day 11, the cows were fitted with jaw recorders (Rutter et al., 1997) for 24 h starting at 0630 h just after the morning milking and ending at 0600 h just before the morning milking the next day. This was done to adapt the cows to the equipment and to determine the time of commencement and end of the three main grazing bouts during the day. On day 12, hourly rumen liquid samples were collected from the four cows, starting at 0630 h just after the morning milking and ending at 2330 h. This was repeated on day 13, but this time the jaw recorders were also fitted to the cows during the collection period (0630 till 2330 h).

On the basis of the temporal patterns of grazing activities found in the 24-h recordings of day 11, which showed that the first, second and third main grazing bouts occur between 0600 to 1200, 1200 to 1800 and 1800 to 2330 h, respectively, it was decided to rumen-evacuate the animals at these time points. Therefore, on days 17 and 19 the jaw recorders were fitted to the four cows, starting at 0530 h before the morning milking and two cows on day 17 and the other two cows on day 19 were rumen-evacuated simultaneously at 0600, 1200, 1800, 2330 and 0800 h the next morning. After each rumen-evacuation the cows were allowed to graze freely, except after the evacuation at 2330 h when the jaw recorders were removed and the two cows were tethered (in a small fenced area on the side of the pasture) and deprived from food and water till 0800 h the next morning. This was done to allow an estimation of the clearance rate, which is needed to calculate the intake during each grazing bout.

Moreover, on days 17 and 19 grass samples were collected from the pasture at three time points: 0800, 1400 and 2000 h. These time points were chosen as they closely represent the quality of the grass the animal consumed during the three main grazing bouts. These samples (approximately 500 g fresh herbage) were weighed immediately and stored in the freezer (-20 °C). At the end of the experiment these samples were freeze-dried, ground to pass 1-mm screen and analysed for DM, OM, CP (N × 6.25), water-soluble carbohydrates (WSC) and NDF as described by Goelema et al. (1998). Non-structural carbohydrates (NSC) content was calculated as OM – CP – NDF – fat, assuming that fat content in grass was constant at all time points at 40 g/kg DM.

2.3. Rumen Liquid Sampling

To take the hourly rumen liquid samples on days 12 and 13, the cows were brought to a small fenced area on the side of the pasture and samples were collected from the rumen ventral sac using a solid plastic tube (85-cm long and 2.5-cm in diameter). In these samples the pH was measured immediately, using an electronic pH meter (pH electrode type 62, Testo 252, Testo GmbH & Co, Lenzkirch, Germany). Then two sub-samples were taken, acidified with either phosphoric acid or TCA and stored in the freezer (-20 °C) pending VFA and NH_3 analyses, respectively, as described by Chilibroste (1999).

2.4. Rumen-Evacuations

The cows to be rumen-evacuated (two on day 17 and two on day 19) were brought to a small fenced area on the side of the pasture and were rumen-evacuated simultaneously. All rumen contents that could be removed by hand (called mat) were emptied into a big rectangular insulated container. During removal of rumen contents a 10 % sample was taken by putting each 10th hand-full in another small plastic container (20 L). Material not removable by hand (bailable liquid) was bailed into another 20-L plastic container using a 1-L plastic bottle. Rumen contents in the big and small containers were weighed and the bailable liquid was proportionally added to the big and the small containers. The contents of the small container were mixed thoroughly and two samples of 400 g were taken and stored in the freezer (-20 °C) pending analysis for DM, ash, N and NDF. After the rumen contents samples had been taken, all rumen contents were put back in the rumen and the time when the evacuation ended was noted.

2.5. Calculations

The basic idea of this experiment was to determine the intake and the grazing behaviour (eating time, number of bites and number of chews) at the three main grazing bouts during the day. This will allow the calculation of bite rate, BM and intake rate at these three main grazing bouts. The intake during each of these grazing bouts was estimated from the changes in rumen pools and clearance of material during the grazing bout obtained from the rumen evacuation data on days 17 and 19. Firstly, rumen pool sizes of the different components (DM, OM and NDF) at each evacuation were estimated from the weight of the total fresh rumen contents and the percentages of these components in the rumen contents at each evacuation. Secondly, the fractional clearance rate (Kcl) was calculated from the last two evacuations at 2330 h and 0800 h next morning based on the assumption of a first order kinetics using the logarithmic transformation of the exponential equation: $RP_{(0800)} = RP_{(2330)} \times EXP^{-Kcl \times t}$. Where RP is rumen pool size (kg) and t is the time elapsed between the end of the evacuation at 2330 and the start of the evacuation at 0800 h next morning. Thirdly, herbage intake at each grazing bout was calculated as shown in equation 1:

$$\text{Herbage intake} = [RP(t2) - (RP(t1) \times EXP^{-Kcl \times t})] / (EXP^{-Kcl \times (0.5 \times t)}) \ldots\ldots (\text{Eq } 1)$$

Where
RP = rumen pool size (kg)

t2 = the ending time of a grazing bout, which was 1200, 1800 and 2330 h for the dawn, afternoon and dusk, respectively
t1 = the starting time of a grazing bout, which was 0600, 1200 and 1800 h for dawn, afternoon and dusk, respectively
Kcl = the fractional rate of clearance of material from the rumen by degradation and passage
t = the time elapsed between the start and the end of a grazing bout.

The denominator in the equation was used to correct for the clearance of the freshly ingested material assuming that on average half of it is subject to disappearance, which may not be necessarily the case. Clearance by degradation is restricted to the potentially degradable pool and passage comprises the entire pool. Nevertheless, the clearance of freshly ingested material generally represents a small portion of herbage intake, and is therefore considered not to be of a major quantitative importance (Chilibroste et al., 1997).

Jaw recordings made on days 11, 13, 17 and 19 were analysed using peak recognition algorithm software with a noise threshold capable of identifying periods of grazing and ruminating activity and biting and non-biting grazing jaw movements (Graze Version 0.8, Institute of Grassland and Environmental Research 1994-1999) (Rutter 2000). TET, number of biting and non-biting jaw movements during eating were determined for each grazing bout. In calculating bite rate, total grazing jaw movements (TGJM) rate and chewing rate at each grazing bout, the time base employed was the TET during that grazing bout. BM at each grazing bout was calculated as the herbage intake at the grazing bout divided by the number of bites the animal made during that grazing bout. Intake rate in g/min at each grazing bout was calculated as the product of bite rate (bites/min) and BM (g DM consumed/bite) during that grazing bout.

2.6. Statistical Analysis

Grazing behaviour, intake rate and BM data were analysed as repeated measures using general linear models of SPSS (SPSS for Windows, Rel. 10.1.0. 2000. Chicago: SPSS Inc.). The within-subjects factor was the grazing bout, which had three levels: dawn grazing bout (0600 to 1200 h), afternoon grazing bout (1200 to 1800 h) and dusk grazing bout (1800 to 2400 h). Grass chemical composition data were analysed as repeated measures with time as the within-subjects factor with three levels (0800 h, 1400 h and 2000 h). Rumen pools (fresh, DM, OM, N and NDF) were analysed as repeated measures with time as the within-subjects factor with four levels: 0600 h, 1200 h, 1800 h and 2330 h. Rumen fermentation end-products concentrations (acetate, propionate, butyrate and NH_3) were statistically analysed in two ways: (a) as a repeated measures with time of the day as the within-subjects factor and (b) fermentation end products at the beginning and the end of each grazing bout for each cow were noted and analysed using a paired t-test procedure, to compare if fermentation end-products concentrations differed between the start and the end of each grazing bout. This was done by studying closely the grazing behaviour recordings on the day the rumen liquid samples were collected and considering periods of continuous grazing for more than 40 min as grazing bouts.

3. Results and Discussion

Table 1 shows the mean chemical composition of the grass in the two days at the three main grazing bouts. Although DM content of the grass numerically and seemingly linearly increased, as the day progressed from 190 to 250 g/kg fresh matter, this increase was not statistically significant ($P = 0.34$). The OM content did not change as the day progressed and stayed constant at around 915 g/kg DM. The NSC content of the grass was influenced ($P = 0.04$) by time of day and tended to increase linearly ($P = 0.09$) as the day progressed from 257 to 293 g/kg DM. Many authors (Delagarde et al., 2000; Lee et al., 2002) reported an increased DM and NSC content at evening compared to morning grass. In these studies the increase in DM and NSC in the evening was attributed to the loss of surface water and to the accumulation of photosynthesis end products especially simple sugars, respectively. The CP content was not influenced significantly ($P = 0.10$) by time of the day and tended to decrease linearly ($P = 0.17$) as the day progressed from 170 to 155 g/kg DM. The NDF content was influenced ($P = 0.02$) by time of day and showed a quadratic relation ($P = 0.04$) being highest at mid-day and lowest in the evening. In contrast, Delagarde et al. (2000) reported a non-significant change in both CP and NDF content of perennial ryegrass between the morning and the evening.

Table 1. Mean chemical composition (g/kg DM) of perennial ryegrass (*Lolium perenne L.*) at different times during the day

	Time of day				Significance of effect of		
Fraction	0800 h	1400 h	2000 h	SEM	Time	Linear	Quadratic
DM, g/kg [1]	191.6	225.0	250.6	13.9	0.40	0.34	0.91
OM	913.3	914.6	917.0	0.7	0.20	0.19	0.26
CP	170.3	153.4	155.3	3.8	0.10	0.17	0.26
NSC [2]	256.8 ^{a}	271.8 ^{ab}	293.3 ^{b}	6.8	0.04	0.09	0.53
WSC	134.6	168.3	190.3	10.6	0.06	0.16	0.07
NDF	446.1 ^{b}	449.5 ^{b}	428.4 ^{a}	4.4	0.02	0.10	0.04

[1] DM is expressed in g/kg fresh material

[2] NSC is calculated as OM – CP – NDF – fat, assuming a constant fat content of 40 g/kg DM

$^{a, b}$ Means in the same row with different superscripts differ significantly ($P < 0.05$)

Table 2 shows grazing behaviour parameters, dry matter intake (DMI), BM and intake rate of dairy cows at the three main grazing bouts of the day; dawn (0600 to 1200 h), afternoon (1200 to 1800 h) and dusk (1800 to 2400 h). TET was different (*P* < 0.05) between the three main grazing bouts and it increased linearly (*P* < 0.05) as the day progressed. Dairy cows grazed longer at dusk (on average 71 min more eating time than during the dawn grazing bout and 28 min more than during the afternoon grazing bout). Moreover, the time spent eating during the dusk grazing bout made about 40 % of the daily TET. Rook and Huckle (1997) also reported a longer eating time by grazing ruminants during the dusk meal. The number of TGJM, bites and chews increased linearly as the day progressed, being low during dawn and high during dusk grazing bouts. However, when TGJM rate, bite rate and chewing rate were calculated as the number of TGJM, bites and chews, respectively, divided by eating time in minutes, it appeared that TGJM rate was almost constant during the day at around 75/min. Whereas, bite rate increased linearly (*P* = 0.08) from 54 to 61/min and chewing rate decreased linearly (*P* = 0.08) from 20 to 16/min as the day progressed. Moreover, the proportion of bites in TGJM increased linearly (*P* = 0.06) from 73 % at dawn to 79 % at dusk. This indicates that dairy cows tried to maximise their intake during the dusk grazing bout by increasing eating time and bite rate and decreasing chewing rate in preparation for the dark hours, when little grazing and much ruminating usually occur. Gibb et al. (1998) also observed a non-significant change in TGJM rate, which appeared to fluctuate around 78/min, and a significant increase in bite rate and the proportion of bites in TGJM as the day progressed. Moreover, Pillips and Leaver (1986) and Phillips and Hecheimi (1989) observed higher bite rates of cows and heifers, respectively, at the dusk meal compared to other meals.

DMI was different (*P* < 0.01) between the three grazing bouts and increased linearly (*P* < 0.01) as the day progressed from 3 kg during the dawn grazing bout to 7 kg during the dusk grazing bout (Table 2). This was probably due to the increased eating time and bite rate during the dusk grazing bout. Assuming a constant BM at all grazing bouts, the increased eating time and bite rate accounted only for 63 % of the difference in DMI between dawn and dusk. This indicated that BM was not constant during the day and that differences in BM between the three grazing bouts must have occurred. When BM was calculated for the three grazing bouts it appeared that the average BM was different (*P* = 0.03) between the grazing bouts and it increased linearly (*P* = 0.03) from 400 mg DM during the dawn grazing bout to 563 mg DM during the dusk grazing bout. Therefore, the much higher DMI during the dusk grazing bout can be related to the increased eating time, bite rate and BM during this grazing bout. Other authors (Orr et al., 1997; Gibb et al., 1998) also observed a higher BM during the dusk meal by sheep and dairy cows, respectively.

Table 2. Grazing behaviour parameters, dry matter intake (DMI), intake rate and bite mass of late lactating dairy cows at the three main grazing bouts during the day

	Grazing bout				Significance of effect of		
	Dawn	Afternoon	Dusk				
Behaviour [1]	6-12 h	12-18 h	18-24 h	SEM	Bout	Linear	Quadratic
TET, min	132 ^{a}	175 ^{ab}	203 ^{b}	10	0.01	0.005	0.59
No. bites	7135 ^{a}	10235 ^{a}	12272 ^{b}	710	0.00	0.008	0.53
No. chews	2661	2862	3210	142	0.17	0.154	0.71
No. TGJM	9796 ^{a}	13097 ^{ab}	15481 ^{b}	805	0.01	0.008	0.64
TGJM_rate	74	75	76	1	0.30	0.251	0.54
Bite_rate	54 ^{a}	58 ^{ab}	61 ^{b}	1	0.03	0.082	0.20
Chew_rate	20 ^{b}	16 ^{a}	16 ^{a}	1	0.04	0.078	0.18
Bite/GJM, %	73 ^{a}	78 ^{b}	79 ^{b}	1	0.02	0.063	0.15
DMI, kg	2.9 ^{a}	4.2 ^{a}	6.9 ^{b}	0.6	0.00	0.006	0.27
BM, mg/bite	401 ^{a}	411 ^{a}	563 ^{b}	28	0.03	0.026	0.25
IR, g/min	22 ^{a}	24 ^{a}	34 ^{b}	2	0.02	0.028	0.25

[1] TET is total eating time in min, No. TGJM is total number of grazing jaw movements (bites plus chews), TGJM_rate, Bite_rate and Chew_rate are all expressed per min, Bite/GJM is the percentage of bites in the total grazing jaw movement, DMI is dry matter intake in kg, BM is the bite mass in mg DM/bite and IR is intake rate in g DM/min.

[a, b] Means in the same row with different superscripts differ significantly ($P < 0.05$)

The increased BM (mg DM) during dusk time in this study can be related to the increased DM content of the grass at this time of the day (Table 1). The 40 % difference in BM (mg DM) between the dawn and the dusk grazing bouts was reduced to 6 % when BM was expressed in mg fresh matter. This indicates that the change in DM content of the grass as the day progressed is the main factor causing the difference in BM between the grazing bouts under continuous stocking system. Similarly Gibb et al. (1998) observed a non-significant change in BM as the day progressed when it was expressed as mg fresh matter and a significant increase when it was expressed in mg DM or mg OM. In their experiment they related the lower BM at the dawn grazing bout to the lower DM content and to the higher surface moisture of the grass at dawn time, which might have increased slippage between the incisors and dental pad. Intake rate (g DM/min) was different (P = 0.02) between the three grazing bouts and increased linearly (P = 0.03) as the day progressed from 22 to 34 (Table 2). The increased intake rate at dusk is probably related to the increased bite rate and BM at this time, as the intake rate is the product of bite rate and BM. These observations are in line with Gibb et al. (1998) who reported that intake rate increased linearly as the day progressed from 17 to 23 g DM/min.

Table 3. Mean rumen pool sizes of grazing late lactating dairy cows at different times during the day

	Time of day					Significance of effect of		
Pool [1]	0600 h	1200 h	1800 h	2330 h	SEM	Time	Linear	Quadratic
	kg							
Rfresh	80.7 ^{a}	83.0 ^{a}	86.7 ^{a}	106.5 ^{b}	5.6	0.001	0.007	0.001
RDM	9.5 ^{a}	8.9 ^{a}	9.9 ^{a}	13.1 ^{b}	0.7	0.001	0.006	0.005
ROM	8.5 ^{a}	8.0 ^{a}	8.9 ^{a}	11.8 ^{b}	0.6	0.001	0.005	0.005
RN	0.30 ^{a}	0.29 ^{a}	0.31 ^{a}	0.40 ^{b}	0.02	0.001	0.011	0.004
RNDF	4.7 ^{a}	4.4 ^{a}	5.0 ^{a}	6.6 ^{b}	0.4	0.001	0.006	0.011
	g/kg MBW[2]							
RDM	76 ^{a}	71 ^{a}	79 ^{a}	104 ^{b}	7.3	0.001	0.006	0.006
ROM	67 ^{a}	63 ^{a}	71 ^{a}	94 ^{b}	6.4	0.001	0.006	0.006
RNDF	37 ^{a}	35 ^{a}	39 ^{a}	52 ^{b}	3.8	0.001	0.008	0.013

[1] Rfresh is total fresh rumen pool, RDM is rumen DM pool, ROM is rumen OM pool, RN is rumen N pool, RNDF is rumen NDF pool expressed in kg and in g/kg MBW.

[2] MBW is metabolic body weight calculated as body weight to the power 0.75

[a, b] Means in the same row with different superscripts differ significantly ($P < 0.05$)

Table 3 shows mean observed rumen pool sizes (fresh, DM, OM, N and NDF) at 0600, 1200, 1800 and 2330 h. Rumen pool sizes were significantly ($P < 0.01$) larger at 2330 h compared to the other times during the day. This was probably related to the longer eating time, higher bite rate and BM during dusk time (Table 2), which resulted in a higher herbage intake at that time of the day (Table 2). Because rumen evacuations in the present study were performed at fixed time points during the day and not immediately when grazing ceased, it was important to estimate the fluctuation in rumen fill between the measured points if we want to draw valid conclusions concerning its role in regulating the cessation of grazing. To estimate rumen fill fluctuation during the day, a small dynamic mechanistic model was constructed. This model was based on the measured TET, the exact timing of each individual grazing event, bite rate and BM, clearance rate and NDF content in grass and in rumen material. The model consisted of one state variable, which was the rumen NDF pool (Q_{NDFr}). In this model Q_{NDFr} receives input from feed intake (NDF flow into the rumen) and its output is disappearance from the rumen through clearance (the sum of degradation and passage). The NDF flow into the rumen was calculated as the intake rate (kg DM/h) multiplied by NDF content in the grass (g/g DM). The intake rate was calculated from BM (kg DM/bite) and bite rate (bites/h) using the exact measured values. Both BM and bite rate, were allowed to change as day progressed as shown in Table 2. Moreover, intake rate, BM and bite rate were all set to zero when the animal was not grazing based on the observed grazing behaviour patterns of each individual cow. The equations that constitute the model and the variables and parameters describing the properties of the model are listed in Table 4.

Table 4. Variables, constants and equations constituting rumen NDF pool simulation model

Variable [1]	Type	Unit	Equation/ Value	If condition
Q_{NDFr}	State	kg	$dQ_{NDFr}/dt = FNDF_{in} - FNDF_{out}$	No
$FNDF_{in}$	Auxiliary	kg/h	$= IR \times (NDF\_{grass}/1000)$	No
$FNDF_{out}$	Auxiliary	kg/h	$= Q_{NDFr} \times Kcl$	No
IR	Auxiliary	kg/h	= Bite_rate × Bite_mass × 0.06	= 0, when animal was not eating
Bite_mass	Pseudo-constant	g	= 0.40, 0.41 and 0.56 between 6-12 h, 12-18 h and 18-24 h, respectively	= 0, when animal was not eating
Bite_rate	Pseudo-constant	/min	= 54, 58 and 61 between 6-12 h, 12-18 h and 18-24 h, respectively	= 0, when animal was not eating
NDF_{grass}	Pseudo-constant	g/kg DM	= 446, 450 and 428 between 6-12 h, 12-18 h and 18-24 h, respectively	No
Kcl	Constant	/h	= 0.056	No

[1] Q_{NDFr} is rumen NDF pool, $FNDF_{in}$ is the flow of NDF into the rumen through intake, $FNDF_{out}$ is the flow of NDF out of rumen NDF pool through both degradation and passage (clearance), IR is the intake rate, NDF_{grass} is the NDF content in the grass in g/kg DM and Kcl is the clearance rate of NDF.

The relationship between observed and model-estimated Q_{NDFr}, and the grazing behaviour patterns during the day are shown in Figure 1. Model estimates of Q_{NDFr} correlated strongly ($r^2 = 0.91$) with the observed values and the coefficient was not different from unity (Y = 1.02 X). The figure shows that despite the fact that cows grazed on average 132 min and 175 min during the morning and afternoon grazing bouts, respectively, Q_{NDFr} did not reach the value it reached at 2330 h. This indicates that dairy cows interrupted the first two grazing bouts (dawn and afternoon) before reaching their maximal rumen fill capacity, indicating that rumen fill is less likely to play a significant role in signalling the termination of these two grazing bouts. Rumen DM fill at 2330 h exceeded 100 g/kg MBW[1] (Table 3) and equalled that observed with silage based winter diets, an event usually not seen under grazing, where the maximum rumen DM fill has been observed to be between 75 and 80 g/kg MBW[1] (Van Vuuren, 1993). Observed and model-estimated rumen pool size values indicate that rumen fill is more likely to play a major role in signalling the termination of the dusk grazing bout, as cows seemed to graze at dusk till their maximal rumen capacity. This was evidenced by the difficulties we experienced in re-filling the rumens of the cows at 2330 h due to limited space, to such a degree that some gentle force had to be used to re-introduce the last kilograms of material back into the rumens of these cows. The fact that dairy cows tend to graze longer time with a higher bite rate and tend to

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[1] MBW is the metabolic body weight calculated as body weight to the power 0.75

fill their rumen to its maximal capacity at dusk time might be related to climatic or adaptive reasons. The climatic reasons are related to the fact that the grazing season is mostly during summer when temperatures may be very high during the day, therefore, it is easier and less energy consuming for the cows, to lose extra body heat if they are active at dusk, when temperatures are moderate. The adaptive reasons could be that the ruminants are adopting an optimal foraging strategy by taking advantage of the higher concentration of DM and photosynthetic products (sugars) in the leaves late in the day (Orr et al., 1997). This means that cows, might have learned that grass quality in terms of DM and sugar improves at dusk time, therefore, they try to maximise intake at that time of the day, to achieve the maximum benefit in terms of nutrients input, with the minimum effort in terms of TGJM. It is also possible that grazing ruminants aim to finish their dusk meal with a full rumen therefore they start that meal at a time that ensures that their rumen is full by sunset. This relates to the fact that ruminants in temperate climates avoid (for whatever reason) grazing at night. Similarly, other authors (Penning et al., 1991) suggested that the ruminants adopt such a strategy to ensure a high rumen fill and hence a sufficient substrate for rumen flora during the night when there is little grazing.

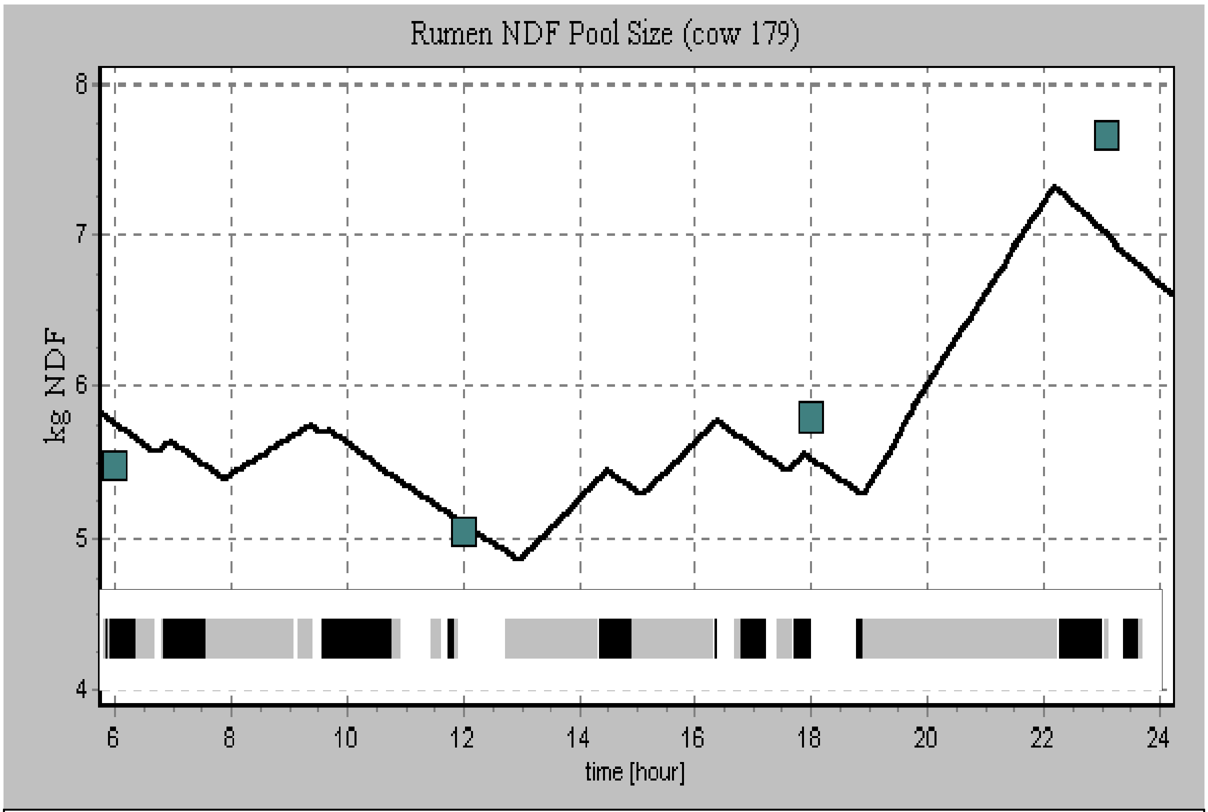

Figure 1. Observed (squares) and model-estimated (black line) rumen NDF pool fluctuation of a grazing dairy cow during the day coupled with the observed grazing behaviour pattern during that day (on the x-axis); eating (gray), ruminating (black) and idling (white).

Table 5 shows mean rumen fermentation end products concentrations at the start and the end of a grazing bout. This was done to try to understand their role in controlling the initiation and termination of eating behaviour and hence feed intake in grazing dairy cows. It was proposed in many studies (Faverdin et al., 1992; Mbanya et al., 1993) that dairy cows' feed intake might be regulated by fermentation end-products concentrations through receptors in the rumen. If that were to be true, dairy cows should have a different rumen concentration of a certain end product at the end of the grazing bout (when eating ceased) as compared to when the grazing bout starts. The results showed that concentrations of all fermentation end products at the start of a grazing bout were not significantly different from those at the end of a grazing bout. This may indicate that rumen fermentation end products may not play a major role in initiating or terminating a grazing bout. However, NH_3 concentration had a low *P* value (0.13) and numerically differed between the start and the end of a grazing bout (Table 5) indicating that it might be more important than the other fermentation end products in regulating eating behaviour under grazing.

Table 5. Mean fermentation end-products concentrations (mM) and pH at the start and the end of a grazing bout in the rumen of dairy cows

	Grazing bout			Significance level		
	Start	End	SEM	*P* (2-tailed)	Correlation[1]	P-cor[2]
Acetate	69.7	70.4	1.3	0.70	0.49	0.02
Propionate	22.0	22.7	0.7	0.29	0.72	0.00
C2/C3	3.3	3.2	0.1	0.08	0.86	0.00
Butyrate	13.7	14.1	0.3	0.31	0.45	0.04
TVFA	108.7	110.8	2.0	0.49	0.46	0.04
NH_3, mg/L	146.8	161.3	7.9	0.13	0.68	0.00
NH_3/TVFA[3]	0.078	0.084	0.003	0.12	0.70	0.00
pH	6.18	6.13	0.05	0.28	0.74	0.00

[1] Correlation between the start and the end values

[2] *P* value of the correlation between the start and the end values

[3] NH_3/TVFA is NH_3 in mM divided by TVFA in mM

Figure 2 shows rumen concentration of fermentation end products and pH during the day coupled with the observed grazing behaviour pattern. The concentration of all fermentation end products fluctuated during the day in response to eating and rumination. This diurnal fluctuation was minor for total VFA, acetate, propionate, butyrate and pH, whereas, the observed fluctuation in NH_3 concentration was major. Rumen NH_3 concentration fluctuated ($P < 0.05$) from 80 mg/L to more than 200 mg/L and showed two peaks during the day; at 1230 h mid-day and at 2230 h in the evening. For a fermentation end product to play a significant role in signalling termination or initiation of a grazing bout, its concentration should fluctuate significantly rather than remain within a small range. In the present study a huge significant fluctuation was only observed in NH_3 concentration (Figure 2, A) rendering it as a main candidate for a possible role in signalling the termination of a grazing bout. Other authors (Chilibroste, 1999), observed a sharp increase in NH_3 concentration with time while VFA concentration exhibited a plateau during one hour of grazing and concluded that NH_3 concentration might be more important than VFA in regulating meals under grazing. In general, NH_3 concentration seemed to increase after grazing probably due to the ingestion of grass, which contains a high proportion of non-protein nitrogen (NPN) and rapidly degradable protein. The fact that NH_3 peak after dawn grazing bout occurring at 1230 h mid-day was sharper and higher than that after dusk grazing bout occurring at 2230 h is probably related to the lower protein and higher WSC content of the grass at dusk time (Table 1). Higher WSC makes more energy available for the microbial population improving its ability to utilise NH_3-N available in the rumen for growth purposes, and reduces the need of microbes to ferment protein to obtain energy, a process known to be the main contributor to NH_3 concentration in the rumen.

The effect of VFA (acetate, propionate and butyrate) on intake was previously illustrated in intraruminal infusion studies (Montgomery et al., 1963; Faverdin et al., 1992; Anil et al., 1993), in which the infusion was done for a short term (3 to 5 h) under indoors situation. In these studies, the concentration of acetate, propionate and butyrate were almost doubled due to the infusion of 5 to 9 moles of the acids as compared to the control treatment. In the present study, under grazing, where VFA enter the rumen only through the fermentation of the ingested herbage, the concentration of all VFA fluctuated during the day in response to eating and rumination, but was never doubled (Figure 2). Total VFA and acetate concentration fluctuated slightly ($P > 0.05$) between 90 and 120 mmol/L and between 60 and 72 mmol/L, respectively, and showed only one peak during the day at 2330 h just before mid-night (Figure 2, B and C). Moreover, time of day did not significantly influence the total VFA and acetate concentration ruling them out as candidates for controlling meals under grazing. Other VFA (propionate and butyrate) seemed to increase ($P < 0.05$) gradually as the day progressed, showing a sharp rise at dusk time and reaching a peak at 2330 h just before mid-night (Figure 2, D and E). In contrast, rumen pH decreased ($P < 0.05$) gradually as the day progressed from 6.5 to 6.0 (Figure 2, F) probably as a result of the increased VFA concentration during the day. The increased concentration of VFA at dusk time could be related to the higher bite rate, BM and hence higher intake rate and DMI at that time of the day (Table 2). The increased concentration of propionate and butyrate as day progressed follows the pattern of WSC content of the grass and DMI, which increased linearly as the day progressed (Table 1 and 2) resulting in higher WSC intake at dusk. The relation between higher WSC intake, and increased propionate and butyrate concentration in the rumen is well established (Lee et al., 2002). The fact that propionate and butyrate were significantly influenced by time of day only due to a difference between the last time point at 2330 h and the other time points undermines their possible role in meal control under grazing.

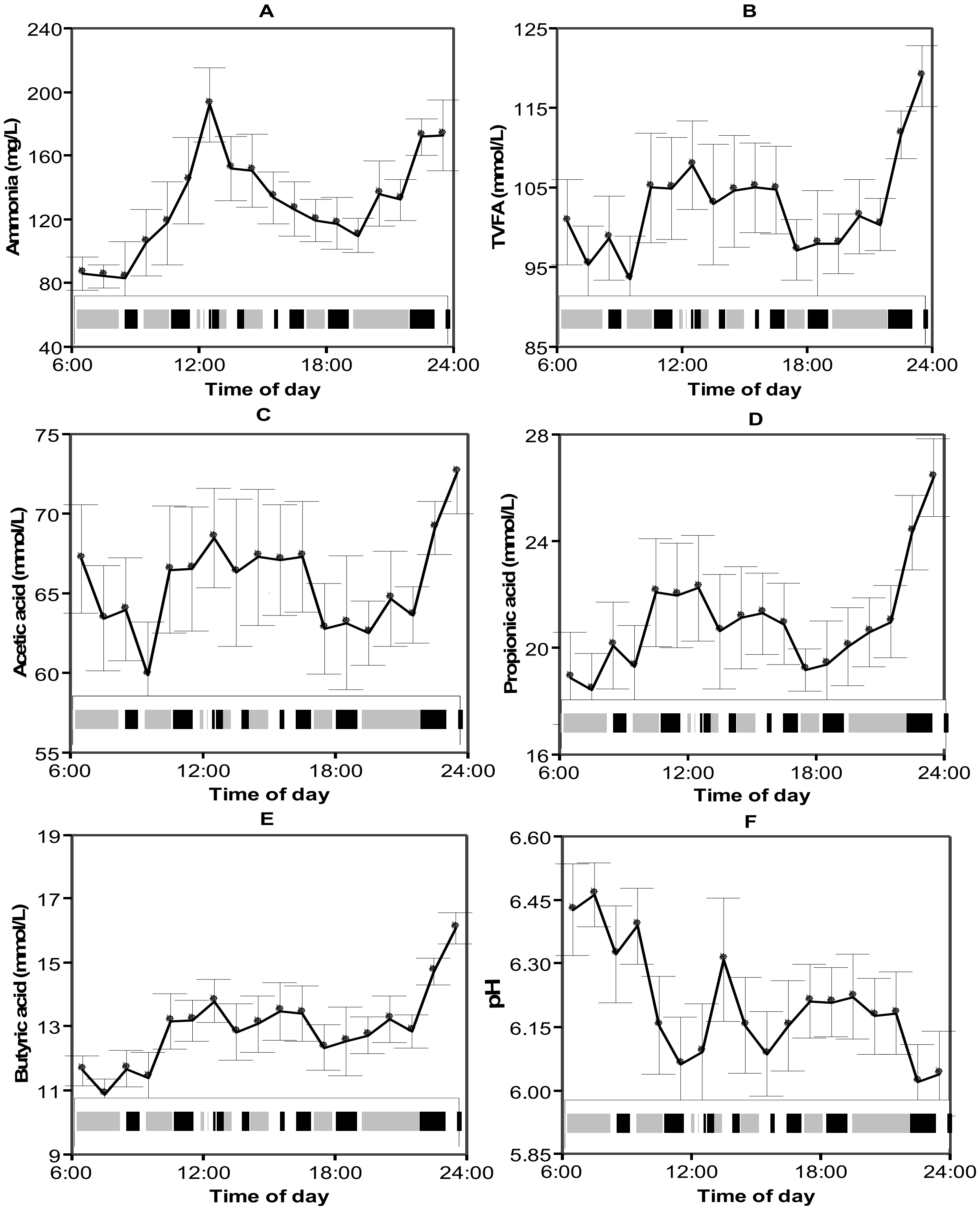

Figure 2. Diurnal fluctuation in rumen NH_3, VFA and pH of grazing dairy cows coupled with grazing behaviour pattern (on the x-axis); eating (gray), ruminating (black) and idling (white)

Except for NH_3 concentration that showed two peaks during the day, other fermentation end-products concentrations and rumen pools were maximal at, or shortly after dusk grazing bout ceased. Therefore the multi-factorial theory of meal control seems to be adequate to explain why animals interrupted the last grazing bout at dusk time but fails to adequately explain why animals interrupted the first two grazing bouts at dawn and afternoon. This was evidenced by the fact that, at these two grazing bouts, cows ceased grazing long before their rumens were full or the concentration of a certain fermentation end product or a combination of fermentation end products was maximal.

4. Conclusions

Under continuous stocking system, where grass availability is not limited, late lactating dairy cows tried to maximise their DMI at dusk by increasing eating time and bite rate and decreasing chewing rate. TGJM rate consisting of total number of bites and total number of chews a cow can make per minute was constant during the day at around 75/min. This indicated that cows couldn't manoeuvre much by increasing their TGJM rate. However, cows could manoeuvre largely by changing the composition of TGJM between bites and chews and seemed to maximise bite rate and minimise chewing rate at dusk time. BM was constant during the day, as the cows seemed to ingest a similar amount of fresh herbage per bite at different times during the day. However, due to the increase in DM content of the grass at dusk, BM in mg DM was much higher at that time of the day. Dairy cows interrupted the first two grazing bouts (dawn and afternoon) long before reaching their maximal rumen capacity indicating that rumen fill is less likely to play a significant role in signalling the termination of these two grazing bouts. However, rumen pool sizes were always maximal at the time when the dusk grazing bout ceased indicating that rumen fill is more likely to play a major role in signalling the termination of the dusk grazing bout. The concentrations of all fermentation end products at the start of a grazing bout were not significantly different from those at the end of a grazing bout, indicating that rumen fermentation end products may not play a major role in initiating or terminating a grazing bout.

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General Discussion

Grazing and grass feeding dairy production systems are gaining importance in the western world due to public concern and demand. Product quality, animal wellbeing, and landscape improvements form the bases of these concerns. In addition to the fact that keeping dairy cows year round indoors may compromise their health and welfare, it also contributes to the impoverishment of the landscape in the countryside. Moreover, it has been reported that milk produced by cows fed indoors on silage and concentrates contains lower concentrations of conjugated linoleic acids (CLA) than milk produced by cows grazing grass pasture, or fed on freshly cut grass (Elgersma et al., 2003). Unsaturated fatty acids in general are favourable against coronary heart disease and polyunsaturated fatty acids in particular are thought to have beneficial effects on human health (Banni et al., 2001; Turpeinen et al., 2002).

Dairy cows fed mainly or only on grass pasture produce less milk compared to dairy cows under other systems. Research during the last decade has shown that nutrient supply of grazing and grass-fed dairy cows is insufficient to sustain milk production levels higher than 28 kg/day (Van Vuuren, 1993). For dairy cows to produce sufficient milk to keep farmers in business, they require mainly, but not only, a sufficient amount of energy, which should be provided through their feed. Under grazing or grass-feeding dairy systems, this energy is mainly in the offered grass. Many grass species, which differ in their morphological, chemical and structural characteristics and their nutritive value, exist. Nevertheless, in Western Europe perennial ryegrass (*Lolium perenne* L.) is the most widely used species for feeding dairy cows. At an early stage of maturity, perennial ryegrass is highly digestible and has a high nutritive value in terms of energy and protein and thus was considered an ideal feed for dairy cows (Van Vuuren, 1993).

Despite its high nutritive value, perennial ryegrass suffers from two main disadvantages: firstly, its unbalanced nutrient content in terms of crude protein (CP) and energy availability at rumen level (Van Vuuren, 1993) and secondly, its low dry matter intake (DMI) by high producing dairy cows (Meijs, 1981; Bargo et al., 2003). The low DMI of perennial ryegrass is thought to be due to physical constraints, rate of removal from the rumen through degradation and passage, and water consumption associated with pasture (Beever and Thorp, 1997). Daily energy intake for maintenance and milk production is the product of daily DMI and energy density of the feed. Energy content of young and leafy perennial ryegrass has been shown to be high and fluctuates between 6.3 and 7.1 MJ NE_L/ kg DM depending on grass genotype, season and management conditions (i.e. fertilisation rate). However, Bruinenberg et al. (2002) pointed out a possible overestimation of grass energy density in intensively managed grasslands, which was suggested to be related to the unbalanced CP to energy ratio of the grass. This unbalanced ratio causes high N losses in the form of urea through urine and results in a low protein utilisation efficiency by dairy cows. Based on that, Bruinenberg (2003) performed some calculations and proposed a correction to be applied, either to the grass energy value, or to the maintenance requirements of grass-fed dairy cows. After applying such a correction, however, young and leafy perennial ryegrass is still characterised by a good energy density. Therefore, the observed low milk yield of cows grazing or fed freshly cut perennial ryegrass is believed to be mainly related to its low DMI rather than to its energy density. Kolver and Muller (1998) reported that cows grazing high quality pasture in the spring consumed 4.5 kg DM less than cows fed a nutritionally balanced total mixed ration (TMR). Furthermore, many other authors (Leaver, 1985; McGilloway and Mayne, 1996; Beever and Thorp, 1997) identified the low grass pasture DMI as a major factor limiting energy intake and hence milk production of high producing cows under grazing systems.

Maximizing DMI and optimising rumen fermentation of perennial ryegrass to maximise energy intake, reduce losses and achieve high milk production from grass-based dairy systems are desirable to assure low production costs and environmental protection. In ruminant animals, DMI is a function of the balance between eating motivation, which is strongly related to palatability, on one hand and rumen capacity on the other. Therefore, increasing DMI could be achieved by either improving palatability or increasing rumen capacity or both. Rumen capacity is related to the rate of clearance (kcl) of material from the rumen, which is the summation of both rates of degradation (kd) and passage (kp). Palatability is mainly a function of flavour and taste, which arise from certain compounds in the grass, especially water-soluble carbohydrates (WSC). This thesis investigated the possibility of improving perennial ryegrass DMI by dairy cows through manipulating both palatability and rumen capacity.

The main hypothesis in this thesis was that DMI and hence milk production would be improved by feeding ryegrass varieties that contain a high WSC content or have a faster rate of removal from the rumen. A high WSC content in the grass improves its sweetness and thus palatability, encouraging the animals to consume more and hence have a higher DMI. A fast rate of removal of grass fibre from the rumen increases its capacity to hold more material and delays rumen fill signals, encouraging the animals to consume more and thus have a higher DMI. These two concepts were tested with indoor-feeding experiments as well as with grazing experiments, therefore in this discussion the results were dealt with separately. The first section of this discussion deals with perennial ryegrass quality and nutritive value in general, the second section deals with the concept of fast rate of clearance and degradation, and the third section deals with the concept of a high WSC content in the grass. The last chapter of this thesis (Chapter 6) dealt with short-term intake regulation under grazing and specifically investigated the roles that rumen fill, pH, and fermentation end products may play in signalling the termination of a grazing event. It also investigated the behavioural strategies dairy cows employ to satisfy their nutritional needs under grazing as the day progresses. Because this chapter deviated from the main idea of this thesis, concerning the manipulation of palatability and clearance characteristics of perennial ryegrass through the choice of variety as means to improve its DMI, it will be discussed separately in the final section of this general discussion.

1. Perennial ryegrass composition and nutritive value

It should be kept in mind that this thesis deals with young and leafy perennial ryegrass, as the experiments and measurements were mostly performed in vegetative stage. The experiments were conducted in 2000 and 2001 and stretched over most of the grazing season in The Netherlands (June to September). The grass was managed intensively and was fertilized at a moderate to high level (300 to 400 kg of N/ha/year). In the first year, 300 kg of N/ha was applied, whereas in the second year, 400 kg of N/ha was applied. The average re-growth age of the grass varieties in both years was 25 days post harvest, with a range between 21 and 29 days. In both experiments grass samples were taken once a day at cutting time between 13:00 and 15:00 h, therefore, the fluctuation in grass nutritive value during the day was not measured. Table 1 shows the ranges in chemical composition and nutritive value of grass as observed in the stall-feeding experiments described in this thesis (Chapters 2 and 4). From the pooled data of the two years a range of 13 to 25 % DM, 87 to 92 % OM, 14 to 23 % CP, 8 to 22 % WSC, 40 to 52 % NDF, and 5.7 to 7.0 MJ NE_L/kg DM was observed. The ranges observed here agree with what was reported about young and leafy temperate pastures by Clark and Kanneganti (1998) and Muller and Fales (1998), except that they all reported a higher range in CP of about 18 to 25 %. The largest ranges in grass chemical composition in our experiments were observed for WSC, DM and CP contents. However, despite the large variation in WSC and CP contents, digestible OM (DOM) and NE_L of the grass were not substantially influenced and had the smallest ranges (Table 1).

The year, the season and the variety explained a substantial proportion (84 %) of the large variation in WSC content. The year explained 54 % of this variation, whereas the season and the variety explained 20 % and 10 %, respectively. However, these three factors (year, season and variety) could only explain 35 % of the large variation observed in DM content. In relation to CP content, the year explained 43 % of the variation, maybe mainly due to the different N fertilisation rate applied in the second year, whereas the season and variety only explained 8 % and 3 % of the variation, respectively. For NE_L, the season was more important than the other factors as it explained 30 % of the variation observed in the NE_L value, whereas the year and the variety explained 10 % and 7 %, respectively. The variety had the least influence on chemical composition and nutritive value when compared to year (which includes effects of management and weather), and season.

Table 1. The range of grass chemical composition and nutritive value observed in two stall-feeding experiments conducted during the summers of 2000 and 2001

Component	Minimum	Maximum	Mean	SD[1]
DM, g/kg fresh	129	249	169	29
OM, g/kg DM	871	924	888	13
CP, g/kg DM	134	228	177	22
WSC, g/kg DM	81	217	126	28
NDF, g/kg DM	404	523	443	31
ADL, g/kg DM	14	30	19	4
DOM[3], %	70	83	78	3
NE_L[4], MJ/kg DM	5.71	7.02	6.55	0.29

[1] SD is the standard deviation

[3] DOM is the digestible organic matter % predicted by NIRS

[4] NE_L is net energy lactation calculated as described by Van Es (1978)

The correlation coefficients between the different components of the grass and its digestibility and nutritive value are shown in Table 2. The strongest correlation between grass chemical components was observed for WSC and CP, which were inversely related ($r = 0.73$; $P < 0.01$). The WSC content was also negatively correlated with NDF and positively correlated with DOM, however, these correlations were weaker (Table 2). Many previous studies have shown that the content of WSC in perennial ryegrass is inversely related to CP content (Wilman and Altimimi, 1982; Humphreys, 1989). A strong correlation was also observed between DOM and NE_L ($r = 0.90$; $P < 0.001$). This correlation is not surprising as the NE_L is calculated based on DOM (Van Es, 1978). The grass NDF content was inversely related to WSC, CP, DOM and NE_L, indicating a negative influence of NDF content on the nutritive value of perennial ryegrass.

Under grazing, chemical composition and nutritive value of the grass fluctuate as the day progresses in response to weather, plant physiological processes such as photosynthesis and transpiration, and animal activity such as grazing (defoliation). Photosynthesis leads to the accumulation of sugars in the leaves, increasing the sugar content of the grass, whereas transpiration leads to the loss of water from the leaves, increasing the DM content. The high temperature at mid day and in the afternoon leads to the evaporation of surface water from the leaves, increasing the DM content of the grass at that time of the day. Animal activity leads to the defoliation of the grazed sward and the loss of green leaf mass, increasing the proportion of stem and dead material, and hence the NDF content of the grass. In the grazing experiments reported in this thesis (Chapter 5), grass samples were collected daily at three time points (7:00, 14:00 and 20:00 h) during the measurement weeks. These time points were selected to coincide with the three main grazing events dairy cows are known to have during the day (morning, afternoon, and dusk) (Rook and Huckle, 1997). Thus the fluctuation in grass chemical composition and nutritive value was followed closely in these experiments. It should be kept in mind that in our experiments a daily strip grazing system was employed and the allocation of the new pasture was done at 12:00 h, therefore, samples taken at 7:00 h next morning represent grass from a depleted sward. Furthermore, the grass swards were also managed intensively, fertilized at a moderate rate (300 kg of N/ha/year) and allowed a re-growth age of between 21 and 28 days post harvest. The experiments were conducted in 2002 and 2003.

Table 2. The correlation coefficients between grass chemical components and nutritive value in the stall-feeding experiments

Component	WSC	CP	NDF	DOM[1]	NE_L[2]
WSC	1	-0.73*	-0.29*	0.38*	0.15
CP		1	-0.28*	-0.04	0.21*
NDF			1	-0.53*	-0.34*
DOM[1]				1	0.90*
NE_L[2]					1

[1] DOM is the digestible organic matter % predicted by NIRS

[2] NE_L is net energy lactation MJ/kg DM calculated as described by Van Es (1978)

* The 2-tailed *P* value of the correlation is less than 0.05

Table 3 shows the chemical composition of grass at three time points during the day and the ranges in grass composition observed in the two years' grazing studies. The ranges observed under grazing were similar to those observed under stall-feeding (Table 1), except for the higher range in DM content, which might have been caused by the hotter summers of 2002 and 2003. The composition of the grass in terms of DM, WSC and NDF fluctuated largely during the day. Both DM and WSC contents were higher in the evening (20:00 h) than in the morning and the afternoon. Similarly, many authors (Delagarde et al., 2000; Lee et al., 2002; Taweel et al., 2004) observed a higher DM and WSC content of perennial ryegrass at the evening time. The fluctuation in NDF was different, as the NDF content was higher in the morning (7:00h) than at other times of the day. This was most likely the result of the daily strip grazing design employed in our studies, where the new pasture was offered at 12:00 h, therefore, by 7:00 in the morning the plot was depleted and the cows were grazing the lower horizon close to the pseudo-stem. Delagarde et al. (2000) reported a significantly higher NDF content in the lower horizons (0-5 cm) and (5-10 cm), compared to the upper horizons (10-15 cm) and (> 15 cm). Although a constant ADL content was observed during the day suggesting a constant NDF quality, both DOM and NE_L followed closely the fluctuation in NDF content and were highest when NDF content was lowest.

Table 3. The fluctuation in grass chemical composition and nutritive value during the day observed in two grazing experiments performed in the summers of 2002 and 2003.

	Time of day			Statistics			
Component	7:00 h	14:00 h	20:00 h	Min	Max	Mean	SD[1]
DM, g/kg fresh	170	218	220	170	288	199	36
OM, g/kg DM	893	889	891	873	909	890	11
CP, g/kg DM	182	196	178	142	234	185	25
WSC, g/kg DM	99	118	132	69	209	117	44
NDF, g/kg DM	517	463	476	445	533	485	23
ADL, g/kg DM	28	30	28	19	38	28	5
DOM[2], %	76	80	78	69	85	78	4
NE_L[3], MJ/kg DM	6.43	6.89	6.64	5.75	7.56	6.68	0.50

[1] SD is the standard deviation

[2] DOM is the digestible organic matter % predicted by NIRS

[3] NE_L is net energy lactation calculated as described by Van Es (1978)

In the grazing experiments, the largest ranges were observed for WSC, DM and CP content and the smallest ranges were observed for the DOM and NE_L, in agreement with what was observed in the stall-feeding experiments. The year, season, time of the day and variety explained 62 % of the variation in WSC content, with the season explaining 43 %, the variety and time of day 8 % each, leaving 3 % for the year. These four factors (year, season, variety and time of the day) could only explain 43 % and 72 % of the variation in DM and CP contents, respectively. Once again, the season was the most important factor and could explain 47 % of the variation in CP content leaving 17 %, 6 % and 2 % for the year, time of the day and the variety, respectively. These observations are not in line with the stall-feeding experiments, where the year (not the season) explained the major share of the variation in both WSC and CP contents. That was most likely due to the different N fertilisation rate between years in the stall-feeding experiments, in contrast to a similar fertilisation rate between years in the grazing experiments. However, data from both stall-feeding and grazing experiments showed that in this study the variety had a small effect on chemical composition and could only explain up to 10 % of the observed variation at maximum.

The correlation coefficients between grass chemical components, digestibility and nutritive value in the grazing experiments are shown in Table 4. Similar to what was observed in the stall-feeding experiments, a significant but slightly weaker negative correlation ($r = 0.57$; $P < 0.05$) was observed between WSC and CP content in the grazing experiments. Moreover, a similar negative correlation was observed between NDF content and WSC, CP, DOM and NE_L.

Table 4. The correlation coefficients between grass chemical components and nutritive value in the grazing experiments

Component	WSC	CP	NDF	DOM[1]	NE_L[2]
WSC	1	-0.57*	-0.56*	0.27*	0.11
CP		1	-0.15	0.49*	0.64*
NDF			1	-0.66*	-0.57*
DOM[1]				1	0.97*
NE_L[2]					1

[1] DOM is the digestible organic matter % predicted by NIRS

[2] NE_L is net energy lactation MJ/kg DM calculated as described by Van Es (1978)

* The 2-tailed *P* value of the correlation is less than 0.05

2. Perennial ryegrass with fast clearing and degrading fibre

This section deals only with stall-feeding experiments, as some of the rumen measurements were not conducted in the grazing experiments. Moreover, this and the latter sections will not deal with varietal differences as it was shown in the earlier section that the variety did not explain more than 10 % of the observed differences in chemical composition and nutritive value. Therefore, the emphasis will be placed on discussing the concepts rather than varietal differences. The first concept in this thesis states that DMI would be improved substantially by increasing rumen capacity through selecting or choosing grasses with fast clearing and/or degrading fibre. To be able to select for fast clearing or degrading fibre, varieties should differ in their fibre clearance or degradation rates. Therefore, the first step was to evaluate or screen different varieties of perennial ryegrass for these characteristics (fibre clearance and degradation rates) and define the available ranges for selection. This was done using an *in vitro* gas production test (Chapter 1), an *in vivo* rumen evacuation experiment (Chapter 2) and an *in situ* nylon bag test (Chapter 3). The *in vivo* rumen evacuation experiment showed that perennial ryegrass varieties used in this study did not differ in their fractional NDF clearance rate (kcl_{NDF}). In addition, the three tests showed that varieties of perennial ryegrass did not differ in their NDF degradation characteristics and fractional rates (kd_{NDF}). Moreover, the three tests also showed that the difference in fractional degradation rate between the variety with the fastest degrading fibre and the one with the slowest degrading fibre was less than 1 %/h (Chapters 2 and 3). Nevertheless, because the experiments stretched over most of the grazing season in The Netherlands (June to September) and the measurements were performed once every two weeks, a data set with a good range in DMI, rumen parameters, fractional clearance and degradation rates and milk production was generated (Table 5).

Table 5. The range in animal, intake, rumen and milk data observed in the two stall-feeding experiments performed in 2000 and 2001.

Variable	Minimum	Maximum	Mean	SD[1]
	Animal Data			
Days in milk	49	287	142	55
Body weight, kg	515	715	594	47
Grass DMI, kg	12.3	19.0	15.7	1.4
Total DMI, kg	14.8	23.1	19.0	1.8
ER[2], MJ/d	103.5	156.2	124.3	11.6
EI-grass[3], MJ/d	70.1	128.4	99.7	10.8
EI-total[3], MJ/d	87.7	157.7	123.2	14.6
EB[4], MJ/d	-47.0	37.5	-1.0	16.6
	Rumen Data			
DM pool, kg	7.9	16.3	12.3	2.1
OM pool, kg	7.1	14.3	10.9	1.8
NDF pool, kg	3.4	8.8	5.6	1.1
ADL pool, kg	0.29	0.79	0.49	0.10
kcl_{NDF}, %/h	3.77	7.87	5.60	0.90
kcl_{ADL}, %/h	0.74	5.75	3.09	0.99
kd_{NDF}[5], %/h	0.48	4.69	2.51	0.85
VFA,mM	80	144	116	18
Acetate, %	62	69	66	2
Propionate, %	16	23	20	2
Butyrate, %	9	14	12	1
NGR[6]	3.5	5.8	4.4	0.5
NH_3-N, mg/L	37	346	179	68
pH	5.47	6.47	5.94	0.24
	Milk Data			
Milk yield, kg/d	16.5	33.6	25.9	3.8
Fat, %	3.37	5.28	4.03	0.35
Protein, %	2.71	3.98	3.19	0.27
Lactose, %	4.13	4.76	4.44	0.14
Urea, mg/dL	15.1	46.7	31.6	8.8
FPCM[7], kg/d	17.3	35.7	25.8	3.7

[1] SD is the standard deviation

[2] ER is the daily energy requirements in MJ, calculated as described by Van Es (1978)

[3] EI is energy intake MJ/day

[4] EB is energy balance calculated as EI-total minus ER

[5] kd_{NDF} is the fractional degradation rate of NDF calculated using kcl_{ADL} to estimate kp_{NDF}

[6] NGR is the non-glucogenic to glucogenic VFA ratio

[7] FPCM is fat and protein corrected milk calculated as (0.337 + (0.116 × % Fat) + (0.06 × % Protein)) × milk yield

This range allowed us to evaluate the effect of varying NDF fractional clearance and degradation rates on DMI, rumen parameters and milk production. The data set was divided based on NDF fractional clearance rate (kcl_{NDF}), which was transformed into a categorical variable with three levels as follows: slow clearing (kcl_{NDF} from 3.77 to 5.14), moderate clearing (kcl_{NDF} from 5.15 to 6.51) and fast clearing (kcl_{NDF} from 6.52 to 7.87). These three levels were not chosen arbitrary but were calculated based on the total range of kcl_{NDF} (7.87 - 3.77 = 4.10) and the number of categories (3). Then the data set was analysed using a one-way ANOVA with the kcl_{NDF} category as a fixed factor with three levels. The effect of varying the kcl_{NDF} in perennial ryegrass on DMI, rumen pools and milk yield and composition is shown in Table 6. On average the kcl_{NDF} of the slow, moderate and fast clearing categories were 4.7, 5.8 and 7.1 %/h, respectively, and the kd_{NDF} were 2.4, 2.4 and 3.3 %/h, respectively. Moreover, the data set was balanced with regard to lactation status, which is known to largely influence DMI, as cows in all categories (slow, moderate and fast) were in a similar lactation status (140 to 154 days in milk) (Table 6).

Contrary to expectations, grass DMI and total DMI were not different among the three categories and averaged around 15.7 and 19.0 kg/d, respectively (Table 6). Animals in the slow kcl_{NDF} category seemed to compensate by having a larger rumen size as illustrated by the higher ($P < 0.05$) rumen DM, OM, NDF pools of these animals. Rumen parameters in terms of VFA and VFA molar proportions were similar for the three categories. However, rumen pH was significantly lower for the animals in the slow kcl_{NDF} category (5.80 vs. 6.01) (Table 6). Interestingly, milk yield and composition in terms of fat and protein were all numerically higher for the animals in the slow kcl_{NDF} category (Table 6). As mentioned earlier, fibre clearance rate is the summation of both rates of degradation and passage. Therefore, a high fibre clearance rate does not necessarily mean a higher degradation rate and higher utilisation. It may also mean a higher passage rate, leading to more escape of potentially degradable fibre and hence less utilisation. This might explain the higher milk yield, milk fat and milk protein observed for the animals in the slow kcl_{NDF} category, which seemed to have a much slower passage rate (2.44 vs. 3.81 %/h) and possibly a higher utilisation of the ingested fibre. The passage rates of the liquid and solid phases were not directly measured in our experiments. Nevertheless, an estimate of the passage rate was obtained by assuming ADL to be completely undegradable in the rumen and considering its clearance to be only due to passage. Thus, ADL clearance rate (kcl_{ADL}) was assumed to be equal to NDF passage rate (kp_{NDF}). This allowed us to calculate kd_{NDF} by subtracting the kcl_{ADL} from the kcl_{NDF}. In other experiments (Van Vuuren et al., 1992 and 1993) it has been shown that kcl_{ADL} and ADL fractional passage rate (kp_{ADL}) are higher than the NDF fractional passage rate (kp_{NDF}). Thus, using the Kcl_{ADL} or kp_{ADL} to estimate kp_{NDF} will underestimate $kd_{NDF.}$ Furthermore, many authors (Van Soest, 1982; Tamminga et al., 1989) showed that ADL is a poor marker for passage and digestion studies and they outlined a number of factors that might influence its recovery. Based on that, the estimates of kd_{NDF} from the rumen evacuation data were considered biased and were not used for further analysis in this discussion.

Table 6. The effect of varying NDF clearance rate (kcl_{NDF}) on DMI, rumen function and milk production and composition observed in two stall-feeding experiments

	NDF clearance rate (kcl) category			
Variable	Slow	Moderate	Fast	SD[1]
		Grass Data		
Re-growth, days	24	24	24	2
Height, cm	20	20	20	2
DM yield, kg/ha	2093	2254	2158	384
WSC, g/kg DM	131	123	124	29
CP, g/kg DM	173	179	182	23
NDF, g/kg DM	441	443	438	32
		Intake Data		
Days in milk	142	140	154	55
Grass DMI, kg	15.7	15.7	15.6	1.4
Total DMI, kg	19.1	18.8	18.5	1.8
		Rumen Data		
DM pool, kg	13.7 ^{a}	11.8 ^{b}	11.1 ^{b}	2.1
OM pool, kg	12.0 ^{a}	10.4 ^{b}	9.8 ^{b}	1.8
NDF pool, kg	6.2 ^{a}	5.4 ^{b}	5.0 ^{b}	1.1
ADL pool, kg	0.54 ^{a}	0.48 ^{b}	0.42 ^{b}	0.10
kcl_{NDF}, %/h	**4.69 ^{a}**	**5.79 ^{b}**	**7.06 ^{c}**	0.90
kcl_{ADL}, %/h	2.33 ^{a}	3.40 ^{b}	3.81 ^{b}	0.99
kd_{NDF}, %/h	2.36 ^{a}	2.39 ^{a}	3.26 ^{b}	0.85
VFA,mM	119	112	110	17
Acetate, %	66	66	65	2
Propionate, %	20	20	21	2
Butyrate, %	12	12	12	1
NGR[2]	4.4	4.5	4.3	0.5
NH_3-N, mg/L	172	180	184	69
pH	5.80 ^{a}	5.98 ^{b}	6.01 ^{b}	0.24
		Milk Data		
Milk yield, kg/d	26.0	26.0	24.0	3.8
Fat, %	4.06	4.05	3.87	0.34
Protein, %	3.28	3.16	3.15	0.27
Lactose, %	4.46 ^{a}	4.44 ^{a}	4.31 ^{b}	0.14
Urea, mg/dL	28.0	32.5	34.3	8.9

[1] SD is the standard deviation

[2] NGR is the non-glucogenic to glucogenic VFA ratio

The *in situ* nylon-bag technique estimates NDF degradation characteristics and rates more accurately, as it measures the disappearance of NDF from the nylon bag and does not require an estimate of the kp_{NDF}. In the stall-feeding experiment conducted in 2000, samples from all grass varieties in all experimental periods were taken and subjected to an *in situ* test (Chapter 3). The *in situ* data was merged with the animal data of that year, in order to evaluate the influence of kd_{NDF} on DMI, rumen function and milk production and composition. The data set was divided based on kd_{NDF}, which was transformed into a categorical variable with two levels as follows: slow degrading ($kd_{NDF} < 2.5$) and fast degrading ($kd_{NDF} \geq 2.5$). The point of division was set at the mean value of kd_{NDF} observed in the experiment (2.5 %/h). The reason for using only two levels this time compared to three levels when the data was divided based on kcl_{NDF} is mainly due to the much smaller range in kd_{NDF} observed *in situ* (2.11 to 2.98). The means of the two categories were compared using an independent samples t-test with the kd_{NDF} category as the grouping variable. The effect of varying the kd_{NDF} in perennial ryegrass on DMI, rumen pools and milk yield and composition is shown in Table 7.

The data set was balanced with regard to lactation status, as cows in the slow and fast degrading categories were in a similar lactation status (141 vs. 150 days in milk) (Table 7). On average the kd_{NDF} of the slow degrading category was 2.36 and that of the fast degrading category was 2.68 %/h. Although the difference in kd_{NDF} between the slow and fast categories was small (0.32 %/h), due to the small range observed in this experiment, it was statistically significant. Grass DMI and total DMI were not significantly different between the two categories and averaged around 17 and 20 kg/d, respectively (Table 7). Moreover, rumen pools, VFA concentration and molar proportions, and rumen pH were all not different between the two categories. However, milk yield was numerically higher and milk fat content was slightly lower for the fast degrading category. The reason for the marginal effect of kd_{NDF} on DMI, rumen parameters, VFA concentration and milk yield in this study might be the small range observed in kd_{NDF}. Lykos et al. (1997) varied the degradation rate of the non-structural carbohydrates (not the fibre) from 6.0 to 7.9 %/h in the ration of dairy cows and observed no effect on DMI, rumen pools and pH.

From the above two paragraphs, it could be concluded that selecting for a fast clearing fibre might not be necessarily beneficial in improving DMI and milk production, and it might reduce the utilisation of the ingested fibre leading to the opposite expected results. Selecting for fast degrading fibre seemed to be beneficial in improving DMI, fibre utilisation and milk yield, but it appeared to be very limited for perennial ryegrass as a very small range was observed.

Table 7. The effect of varying the degradation rate of NDF (kd_{NDF} *in situ*) in perennial ryegrass on dry matter intake (DMI), rumen function and milk production and composition

	NDF degradation rate (kd) category		
Variable	Slow	Fast	SD[1]
in situ kd_{NDF}, %/h	2.36^{a}	2.68^{b}	0.21
Days in milk	150	141	52
	Intake data		
Grass DMI, kg	16.5	17.1	1.4
Total DMI, kg	20.5	21.1	1.4
	Rumen data		
DM pool, kg	12.8	11.8	1.9
OM pool, kg	11.2	10.4	1.6
NDF pool, kg	5.7	5.3	0.9
ADL pool, kg	0.48	0.44	0.09
kcl_{NDF}, %/h	5.27	5.49	0.81
kcl_{ADL}, %/h	2.70	2.93	0.83
VFA,mM	126	125	9
Acetate, %	66	65	2
Propionate, %	20	20	2
Butyrate, %	12	12	1
NGR[2]	4.4	4.3	0.4
NH_3-N, mg/L	112	161	71
pH	5.84	5.88	0.21
	Milk data		
Milk yield, kg/d	26.8	28.2	3.1
Fat, %	4.02	3.86	0.29
Protein, %	3.33	3.32	0.18
Lactose, %	4.52	4.52	0.10
Urea, mg/dL	21.4	24.5	7.5

[1] SD is the standard deviation

[2] NGR is the non-glucogenic to glucogenic VFA ratio

3. Perennial ryegrass with a high sugar content

One of the main objectives of this thesis was to investigate the effect of feeding high sugar grass on intake, rumen function and pH, milk production and composition under stall-feeding (Chapter 4) and grazing (Chapter 5) conditions. The discussion of this section was divided into two parts: the first part dealt with the two stall-feeding experiments carried out in 2000 and 2001, and the second part dealt with the two grazing experiments carried out in 2002 and 2003. We adopted such an approach because of two reasons: firstly, intake under grazing is known to be controlled by many factors that are absent under stall-feeding, such as herbage allowance, sward surface height and tiller density. Secondly, eating and rumination behaviour of the cows was only measured under grazing and will be discussed in the second part of this section that deals with the grazing experiments.

3.1. Stall-feeding high sugar grass to dairy cows

The argument that a high sugar content in the grass improves its sweetness and thus palatability, encouraging the animals to have a higher DMI, is counterbalanced by the argument that high sugar intake increases the acid load on the rumen, decreasing fibre degradation and hence reducing intake. Measuring and quantifying palatability is difficult, but measuring intake can be done accurately under stall-feeding conditions. The ranges in chemical composition, DMI, rumen parameters, milk yield and composition are presented in Tables 1 and 5. To evaluate the effect of increasing WSC content in the grass on DMI, rumen function and milk production, the data set was divided, based on the WSC content of the grass on offer, into two categories: low sugar content and high sugar content. Due to the different N fertilisation rates applied in the two years, the ranges and means in WSC and CP contents were largely different between years (see Chapters 2 and 4). Therefore, to assure that the two categories are represented equally in each year, the division was done within year and the point of division was set at the mean value observed for each year. In the first year the division was made at 138 g/kg DM, considering values < 138 to be in the low sugar category and values ≥ 138 to be in the high sugar category. In the second year the division was made at 114 g/kg DM, considering values < 114 to be in the low sugar category and values ≥ 114 to be in the high sugar category. The means of the two categories within year were compared using an independent samples t-test with the WSC category as the grouping variable.

Table 8 presents the effect of varying grass WSC content on DMI, rumen pH, pools, parameters and function, and milk production and composition. It is important to realise that the stage of lactation of cows in the low and high sugar categories should be similar, in order to eliminate any discrepancy that might arise from that on DMI and milk production. The results showed that in the first year (2000) cows in the low and high sugar categories were 167 and 174 days in milk and cows in both categories in the second year (2001) were exactly in the same stage of lactation (114 days in milk). Contrary to expectations, feeding high sugars grass did not show any positive effect on either DMI or milk yield in both years. This contradicts what was reported by other authors who fed high sugar grass to late lactating dairy cows (Miller et al., 2001) and steers (Lee et al., 2002) and observed a significant increase in milk yield and DMI, respectively. In Miller et al. (2001), the observed high milk yield of cows on the high sugar grass was attributed to the higher intake of digestible DM, as the two grasses differed significantly in their NDF content and digestibility. In our

Table 8. The effect of varying WSC content in perennial ryegrass on DMI, rumen pH and function, and milk production and composition under stall-feeding conditions

	Year					
	2000			2001		
	WSC category			WSC category		
Variable	Low	High	SD[1]	Low	High	SD[1]
			Grass data			
WSC, g/kg DM	119 ^{a}	177 ^{b}	30	98 ^{a}	129 ^{b}	18
CP, g/kg DM	183 ^{a}	149 ^{b}	23	195 ^{a}	173 ^{b}	19
NDF, g/kg DM	437 ^{a}	420 ^{b}	17	450	458	38
OM digestibility	79.1	80.5	1.5	76.8	75.9	2.8
			Animal & intake data			
Days in milk	167	174	52	114	114	42
Grass DMI, kg	16.0	16.4	1.5	15.2	15.3	1.1
			Rumen data			
DM pool, kg	12.1	12.6	2.3	12.6	12.2	1.9
OM pool, kg	10.6	11.0	2.0	11.1	10.9	1.7
NDF pool, kg	5.3	5.7	1.1	5.7	5.9	1.0
ADL pool, kg	0.46	0.46	0.11	0.51	0.53	0.09
kcl_{NDF}, %/h	5.49	5.17	0.92	5.63	5.97	0.84
kcl_{ADL}, %/h	2.73	2.66	0.91	3.30	3.53	0.95
kd_{NDF} %/h	2.76	2.51	0.72	2.33	2.44	0.93
VFA,Mm	128	124	10	98 ^{a}	110 ^{b}	17
Acetate, %	66	65	2	66	66	1
Propionate, %	20 ^{a}	21 ^{b}	2	20	20	2
Butyrate, %	12	12	1	11	11	1
NGR [2]	4.6 ^{a}	4.3 ^{b}	0.5	4.5	4.3	0.5
NH_3-N, mg/L	189 ^{a}	82 ^{b}	69	236 ^{a}	174 ^{b}	56
pH	5.98 ^{a}	5.76 ^{b}	0.23	5.98	5.98	0.25
			Milk data			
Milk yield, kg/d	25.6	24.9	4.5	25.6	26.3	3.0
Fat, %	4.11	4.08	0.41	3.94	3.99	.27
Protein, %	3.32	3.43	0.24	3.05	3.01	.18
Lactose, %	4.51	4.48	0.15	4.38	4.38	.11
Urea, mg/dL	29.3 ^{a}	18.3 ^{b}	8.1	38.1	37.0	4.2

[1] SD is the standard deviation

[2] NGR is the non-glucogenic to glucogenic ratio

experiments, the OM digestibility was similar for the high and low sugar content categories in both years (Table 8). Moreover, rumen DM, OM, NDF and ADL pools, and the fractional clearance and degradation rates were not different between the two categories in the two years. However, in line with expectations, feeding high sugar grass increased propionate proportion, and reduced pH and NH_3-N concentration in the rumen leading to a lower urea concentration in milk. This was more pronounced in 2000 than in 2001, most likely due to the larger differential magnitude in WSC and CP content in 2000 than 2001 (Table 8). The CP content of the diet is believed to be more important than WSC content in influencing rumen NH_3-N and milk urea concentration, but this was hard to test in our study due to the strong negative correlation between CP and WSC content ($r = 0.73$) (Table 2). Nonetheless, a bivariate correlation analysis revealed that milk urea is strongly positively related to rumen NH_3-N concentration ($r = 0.64$; $P < 0.05$), CP content in grass ($r = 0.53$; $P < 0.05$) and rumen pH ($r = 0.44$; $P < 0.05$), and negatively related to WSC ($r = 0.57$; $P < 0.05$). Surprisingly, the correlation coefficient between milk urea and grass WSC content was stronger than that between milk urea and grass CP content. A similar correlation analysis revealed that rumen NH_3-N concentration is positively related to grass CP content ($r = 0.84$; $P < 0.001$) and negatively related to grass WSC content ($r = 0.74$; $P < 0.001$). The higher correlation coefficient for CP compared to that for WSC, indicates that grass CP content has more influence on rumen NH_3-N concentration than WSC content. A stepwise linear regression analysis resulted in a good predictive equation for rumen NH_3-N concentration from CP and WSC contents in the grass: $NH_3\text{-N (mg/L)} = -91 + 19.7 \times \text{CP (\%)} - 6.4 \times \text{WSC (\%)}$ ($R^2 = 0.74$).

3.2. Pasture with high sugar grass for grazing dairy cows

Under grazing, DMI is largely influenced by herbage allowance and animal grazing behaviour, in addition to nutritional demand and lactation status of the animal. These factors in turn are largely influenced by pasture state variables, such as sward surface height (SSH) (Gibb et al., 1997) and herbage bulk density (Laca et al., 1992). The main objective of this section was to evaluate the effect of varying sugar content in pasture grass on DMI, grazing behaviour, and milk production and composition under grazing and compare it with the observed effect under stall-feeding. Therefore, the data set of the two grazing experiments conducted in 2002 and 2003 was divided into two categories: low sugar content and high sugar content, based on the WSC content of the grass pasture on offer. Once again, to assure that the two categories are represented equally in each year, the division was done within year and the point of division was set at the mean value observed for each year. In the first year the division was made at 126 g/kg DM and in the second year the division was made at 110 g/kg DM. The means of the two categories within year were compared using an independent samples t-test with the WSC category as the grouping variable. In addition to DMI and milk production data, in these experiments the grazing behaviour was measured allowing us to evaluate the effect of varying sugar content in grass pasture on grazing behaviour parameters. We are unaware of any experiments that studied the effect of high sugar grass on grazing behaviour and DMI of grazing dairy cows on pasture.

Table 9. The effect of varying WSC content in perennial ryegrass pasture on DMI, grazing behaviour and milk production and composition under grazing conditions

	Year					
	2002			2003		
	WSC category			WSC category		
Variables[1]	Low	High	SD[2]	Low	High	SD[2]
Grass data						
WSC, g/kg DM	78^{a}	174^{b}	54	85^{a}	132^{b}	30
CP, g/kg DM	192^{a}	153^{b}	21	207	187	23
NDF, g/kg DM	510^{a}	463^{b}	29	483	486	16
OM dig, %	73.1^{a}	77.4^{b}	3.1	80.4	80.5	2.9
DM allowance	43^{a}	31^{b}	11	30	28	5
SSH, cm	19^{a}	16^{b}	3	13^{a}	16^{b}	3
Animal & intake data						
Days in milk	100^{a}	72^{b}	22	107	103	19
Grass DMI, kg	16.7	18.1	2.4	19.1	18.0	2.0
Grazing behaviour data						
TET, min	506	525	51	527	544	63
TGJM rate, /min	72	69	5	70	72	4
Bite rate, /min	59	55	6	56	57	4
Chew rate, /min	13	14	4	15	15	2
Bites/TGJM	0.82	0.80	0.05	0.79	0.79	0.03
Bite mass, g	0.56	0.63	0.08	0.67	0.59	0.12
TRT, min	487	488	33	441	460	71
Rumination rate	63	61	5	63	65	3
Boli processed	645	648	139	503	459	93
Milk data						
Milk yield, kg/d	24.8^{a}	28.8^{b}	3.0	26.9	28.3	2.8
Fat, %	3.90	3.94	0.21	3.66	3.83	0.20
Protein, %	3.14	3.08	0.09	3.15	3.09	0.18
Lactose, %	4.39^{a}	4.60^{b}	0.17	4.38	4.40	0.10

[1] DM allowance is expressed in kg/cow/day, SSH is sward surface height, TET is total daily (24 h) eating time, TGJM rate is total grazing jaw movements rate, Bites/TGJM is the proportion of bites in TGJM, TRT is total daily (24 h) ruminating time, rumination rate is calculated as the number of ruminative mastications expressed per min rumination time

[2] SD is standard deviation

The data division procedure described above resulted in a good data set with a good differential magnitude in WSC content between the two categories (see Table 9). However, the data set of 2002 was not balanced with respect to the lactation status, as cows in the low sugar category were in an advanced stage of lactation compared to cows in the high sugar category (100 vs. 72 days in milk) (Table 9). This was mainly due to the fact that in this year, particularly, grass sugar content decreased sharply as season progressed (Figure 1). Therefore, when the data was divided based on sugar content, the low category data came only from the late season observations, whereas the high sugar category data came only from the early season observations. Thus, the observed differences between the two categories in this year can be mainly related to lactation status, NDF content and OM digestibility of the grass rather than to grass sugar content (Table 9). In 2003, the sugar content of the grass fluctuated slightly during the season, but was as high at late season as at early season (Figure 1), leading to the inclusion of observations from early and late season in both categories. Therefore, the data set of 2003 was balanced with respect to lactation stadium, herbage allowance and OM digestibility, which are known to largely influence DMI. In 2003, cows in the low and high sugar categories were in a similar average lactation stadium (107 vs. 103 days in milk), had a similar average herbage allowance (30 vs. 28 kg DM/cow/day) and were offered grass with a similar OM digestibility (Table 9). Because of that, only the data set of 2003 will be discussed in this section. In agreement with the stall-feeding experiments, the grazing experiment showed no positive effect of high sugar content in pasture grass on DMI and milk production (Table 9). Moreover, no effect was observed on the grazing behaviour as cows in the low sugar category had a similar grazing time, bite rate, chew rate, rumination time and ruminative mastication rate to that of cows in the high sugar category.

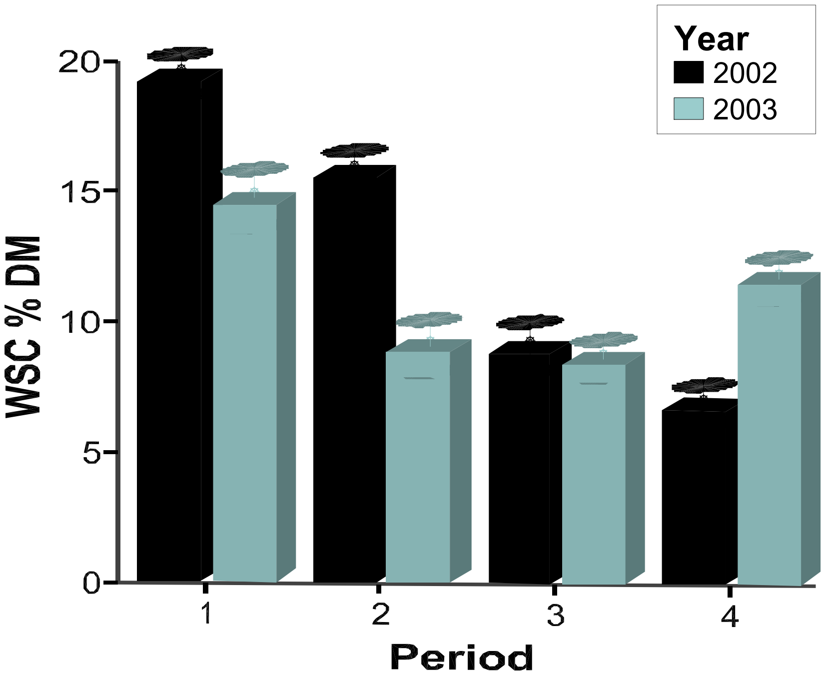

Figure 1. The fluctuation in pasture grass WSC content during the season in two years (2002 and 2003), periods 1, 2, 3 and 4 represent mid July, late July, mid August and late August, respectively.

From both stall-feeding and grazing experiments, one can conclude that a high sugar content in the grass did not improve grass DMI and milk yield by dairy cows. However, feeding high sugar grass reduced NH_3-N concentration in the rumen, shifted rumen fermentation towards propionate, did not slow down fibre degradation and clearance and decreased urea concentration in the milk. The observed effect on both rumen NH_3-N and milk urea concentrations was also related to the lower CP content of the high sugar grass, as a strong negative correlation was observed between CP and WSC content in the grass. These findings may hint to a better protein utilisation by microbes in the rumen and a higher microbial protein synthesis when feeding high sugar grass, but this cannot be confirmed, as in our studies microbial protein synthesis and duodenal flows were not measured. Moreover, milk protein content was not significantly influenced (Table 9).

4. Grazing behaviour of dairy cows on perennial ryegrass pasture

In general, grazing behaviour constitutes all activities the animal gets involved in during a day, such as, eating, rumination, idling and drinking. The interest of a nutritionist mainly lies in three aspects: firstly, the duration of each activity, secondly, the timing of each activity and thirdly the number and type of jaw movements the animal makes within each activity. Jaw movements are divided into two types, those related to chewing activities and those related to biting activities. During rumination, the animal makes jaw movements of the chewing type only, whereas during eating (grazing), the animal alternates biting and chewing jaw movements and vice versa. Therefore, the total grazing jaw movements (GJM) are made of both the chews and bites the animal makes during grazing. When eating time, number of bites and chews are known, bite rate, chew rate and GJM rate can be easily calculated as the number of bites, chews and GJM (bites + chews), respectively, divided by eating time. Obviously, eating behaviour exerts a large influence on DMI; therefore, any factor that influences eating behaviour will directly influence DMI. Factors of plant (sward), animal, environmental and managerial origin have been shown to influence eating behaviour and hence pasture DMI (Pulido and Leaver, 1995). Recently, a large amount of research was directed towards understanding and quantifying the effect of both plant and animal factors on eating behaviour by dairy cattle (Penning et al., 1991; Gibb et al., 1997; Gibb et al., 1999). In contrast, little was done to study the effect of management, in terms of the grazing system applied, and weather conditions on eating behaviour of dairy cow. Moreover, most of the studies focused on the daily eating behaviour and neglected the fact that dairy cows have three main meals during the day (Rook and Huckle, 1997), during which they may largely alter their eating behaviour. Therefore, in this last section of the discussion the focus will be placed on the influence of the grazing system (continuous stocking vs. one-day strip grazing) and the weather conditions (temperature) on eating behaviour at the three main meals during the day. This will allow us to identify the behavioural strategies dairy cows' employ to satisfy their nutritional needs as the day progresses, under different grazing systems. Moreover, the effect of sward surface height and lactation status will be investigated and compared to previous studies.

In 2003, two experiments were performed: in the first experiment, four cows were given access to 1-ha pasture under a continuous stocking system, where grass availability was not limiting at any time of the day or the experiment (Chapter 6). In the second experiment four cows were given access to 528 m^2 (132 m^2 each) of a pasture daily, under a one-day strip grazing system, in which the new pasture was allocated at 12:00 h mid day (Chapter 5). In this experiment, herbage allowance was above 25 kg DM/cow/day. In both experiments eating behaviour, in terms of eating duration, number of meals, meal duration, number of bites and chews, was measured using IGER jaw recorders (Rutter et al., 1997). In the first experiment, eating behaviour was measured four times for each cow in two weeks (twice/week), whereas, in the second experiment, eating behaviour was measured eight times for each cow in eight weeks. The day was divided into three main periods (6:00 to 12:00 h, 12:00 to 18:00 h and 18:00 to 24:00 h), where the three main grazing bouts (dawn, afternoon and dusk) of dairy cows usually occur. The recordings were analysed for each bout and eating behaviour in each bout was quantified. The data was analysed as a repeated measures design with the bout as the within subjects factor and the grazing system as the between subjects factor.

4.1. The effect of the grazing system on eating behaviour

Figure 2 shows eating behaviour (total eating time (TET), bite rate, chew rate, GJM rate) at the three main grazing bouts during the day for cows grazing under continuous stocking and strip grazing systems. Dairy cows under continuous stocking grazed more time ($P < 0.05$) during the dusk grazing bout compared to the other grazing bouts during dawn and afternoon, whereas under strip grazing TET was longer ($P < 0.05$) during the afternoon bout. This was most likely related to the fact that in the strip grazing experiment the new pasture was offered at 12:00 h, making fresh and tall sward available to the animals during the afternoon bout and probably encouraging the cows to prolong their TET during this bout. Moreover, under strip grazing, TET during the dawn grazing bout was extremely shortened by the cows compared to that under continuous stocking. This is also believed to be related to the fact that the new pasture was offered at 12:00 h mid day, meaning that the pasture was depleted by next morning and the cows were grazing the lowest horizon during that time, which probably increased searching time and reduced eating time.

The GJM rate, which represents the number of both bites and chews the animal makes during a grazing minute, was constant during the day at around 72/min for cows under continuous stocking, but it increased ($P < 0.05$) linearly for cows under the strip grazing as the day progressed. Under strip grazing system, GJM rate was lowest in the morning bout at around 65/min. This was thought to be related to the low availability of herbage during this bout under this system due to the allocation of the new pasture at 12:00 h. In both grazing systems, bite rate was always higher ($P < 0.05$) and chewing rate was lower ($P < 0.05$) during the dusk grazing bout. This indicated that dairy cows were trying to maximise their intake at dusk time before darkness falls by changing the composition of their GJM towards more bites and less chews, regardless of the grazing system they are exposed to.

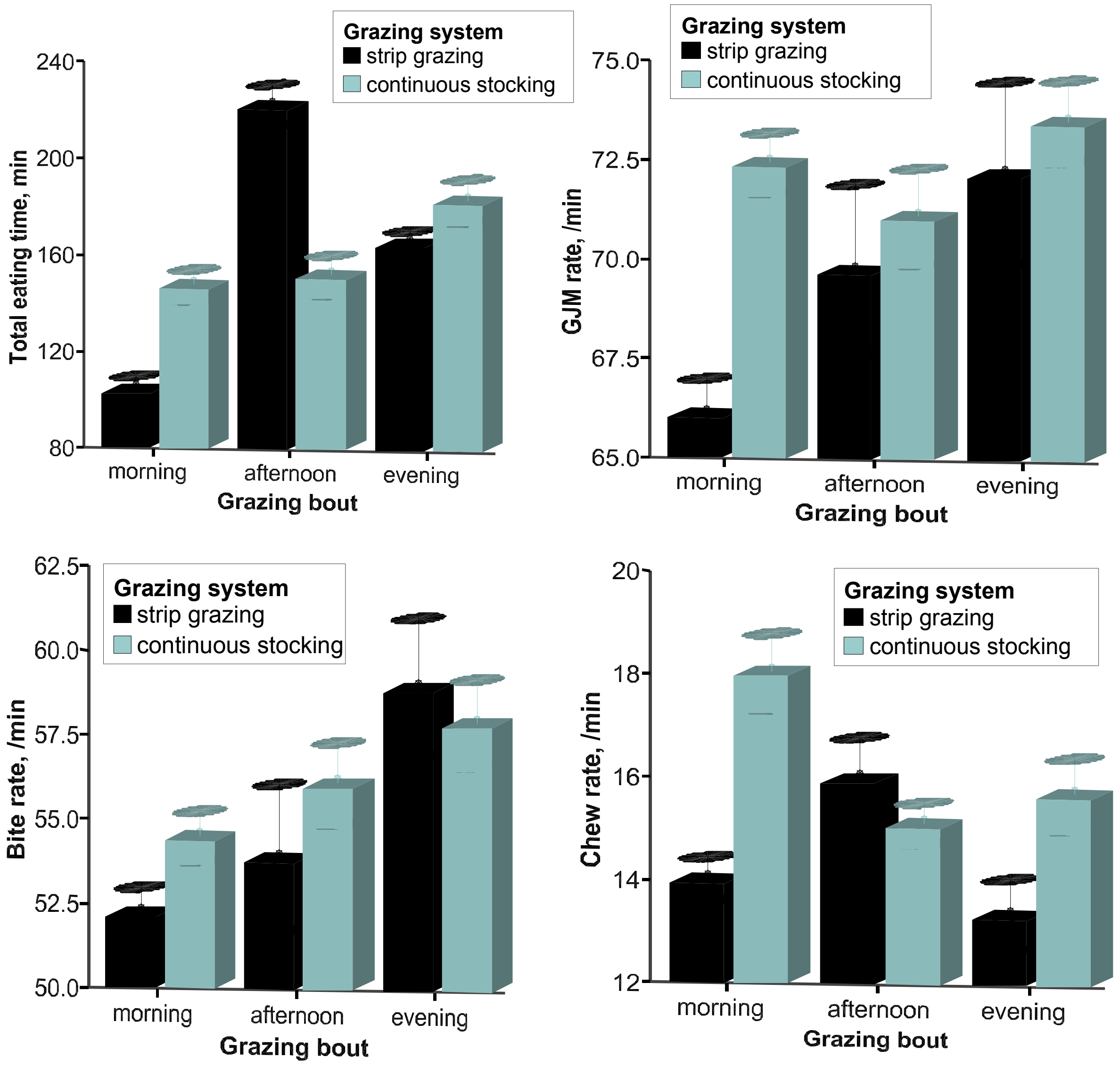

Figure 2. The effect of grazing system (continuous stocking vs. strip grazing) on eating time, GJM rate, bite rate and chewing rate at the three main grazing bouts of the day

4.2. *The effect of weather (temperature) on eating behaviour*

In both experiments (continuous stocking and strip grazing), grazing behaviour measurements were taken for many days where weather conditions were very variable. Weather conditions during the days of grazing behaviour measurements of both experiments were obtained from the weather station that belongs to the department of meteorology of Wageningen University. This station is located at Haarweg (Longitude: 05 40 E, Latitude: 51 58 N, Elevation: 7 m), which is less than 100 m and 1000 m from the pastures where the continuous stocking and strip grazing experiments were performed, respectively. To study the effect of temperature on eating behaviour parameters, the data set was divided within each grazing system based on daily average temperature into two categories: hot days and normal days. The division was made at an average daily temperature value of 20 °C, considering days with an average temperature of > 20 °C to be hot and days with an average temperature of ≤ 20 °C to be normal days. Firstly, the effect of temperature on the total eating time, average GJM rate, bite rate and chew rate during the day (6:00 to 24:00 h; night grazing not included) was examined by analysing the data using a full factorial two-way ANOVA analysis with the grazing system and temperature category as fixed factors. Secondly, the effect of temperature on eating time, GJM rate, bite rate and chew rate at the three main grazing bouts during the day was investigated by analysing the data as repeated measures with the grazing bout as the within subjects factor and the temperature category and grazing system as the between subjects factors. The results of both analyses are shown in Table 10 and Figure 3, respectively.

The average and maximum daily temperature of the hot days' category was higher under strip grazing compared to that under continuous stocking (25 vs. 21 and 33 vs. 27 for daily average and maximum temperature, respectively). This was illustrated by the significant interaction between system and temperature category for those variables (Table 10). Therefore, the effect of temperature on grazing behaviour was expected to be more pronounced under strip grazing than under continuous stocking. However, under both systems dairy cows tended to reduce their daytime grazing when the temperature was high from around 510 to 400 minutes. This reduction in daytime grazing during the hot days seemed to be compensated for by night grazing, as the cows grazed more than 70 min during the night in hot days compared to less than 10 min of night grazing in normal days (data not shown). Moreover, under both systems, temperature did not significantly influence the other grazing behaviour parameters such as GJM rate, bite rate and chewing rate. However, under strip grazing system there appeared to be a tendency for reduced daily average GJM rate, bite rate and chewing rate when the temperature was high. This indicates that dairy cows reduce their activity during hot days mainly by reducing their daytime grazing and GJM rate. The fact that this reduction in daily average GJM rate in response to the high temperature was only observed under strip grazing may be related to the much higher average daily temperature the occurred under this system (Table 10).

Table 10. The effect of temperature (T°) and grazing system on total eating time, average GJM rate, bite rate and chew rate during the day (night grazing not included).

	Grazing system				Significance of the effect [2]		
	Strip grazing		Continuous stocking				
	Temperature		Temperature				
Variable [1]	≤ 20 °C	> 20 °C	≤ 20 °C	> 20 °C	System	T°	System × T°
AV T°	16.6	24.9	16.0	21.0	**	**	*
Max T°	22.6	32.8	20.9	26.8	**	**	*
TET, min	513	403	507	393	NS	**	NS
GJM rate [3]	71	65	72	72	**	NS	NS
Bite rate [3]	56	51	56	57	NS	NS	NS
Chew rate [3]	15	14	16	16	*	NS	NS
Bites %	79	79	77	78	NS	NS	NS

[1] AV T° is the average daily temperature; Max T° is the maximum daily temperature; TET is total eating time (night grazing not included); GJM rate is total grazing jaw movements rate; and bites % is the percentage of bites in total grazing jaw movements.

[2] NS means the effects are not statistically significant ($P > 0.05$); ** refers to highly significant effects ($P \leq 0.01$); and * refers to significant effects ($0.05 \geq P > 0.01$)

[3] rates are presented in min $^{-1}$

Figure 3 shows the effect of temperature and grazing system on eating behaviour at the three main grazing bouts. In both systems, TET in the afternoon bout was significantly reduced when temperature was high. This was expected as temperature peaks during the afternoon bout. Other authors (Gibb et al., 1998) reported that during a hot day, cows appear to be reluctant to graze before 18:00 h reducing the duration of the afternoon and increasing the duration of the dusk grazing bouts. Dairy cows tend to reduce their activities when temperature is high in order to reduce body heat production, which is hard to get rid of when the temperature is high. The temperature did not significantly influence bite rate at the three main grazing bouts. Bite rate increased linearly as the day progressed from dawn to dusk, regardless of the daily temperature and the grazing system (Figure 3). However, under strip grazing bite rate was lower when daily temperature was high than when daily temperature was low. The chewing rate at the three bouts was influenced by temperature, only under strip grazing, where the chewing rate was largely reduced in the afternoon bout when temperature was high. Whereas, under continuous stocking, the chewing rate decreased linearly as the day progressed regardless of daily temperature.

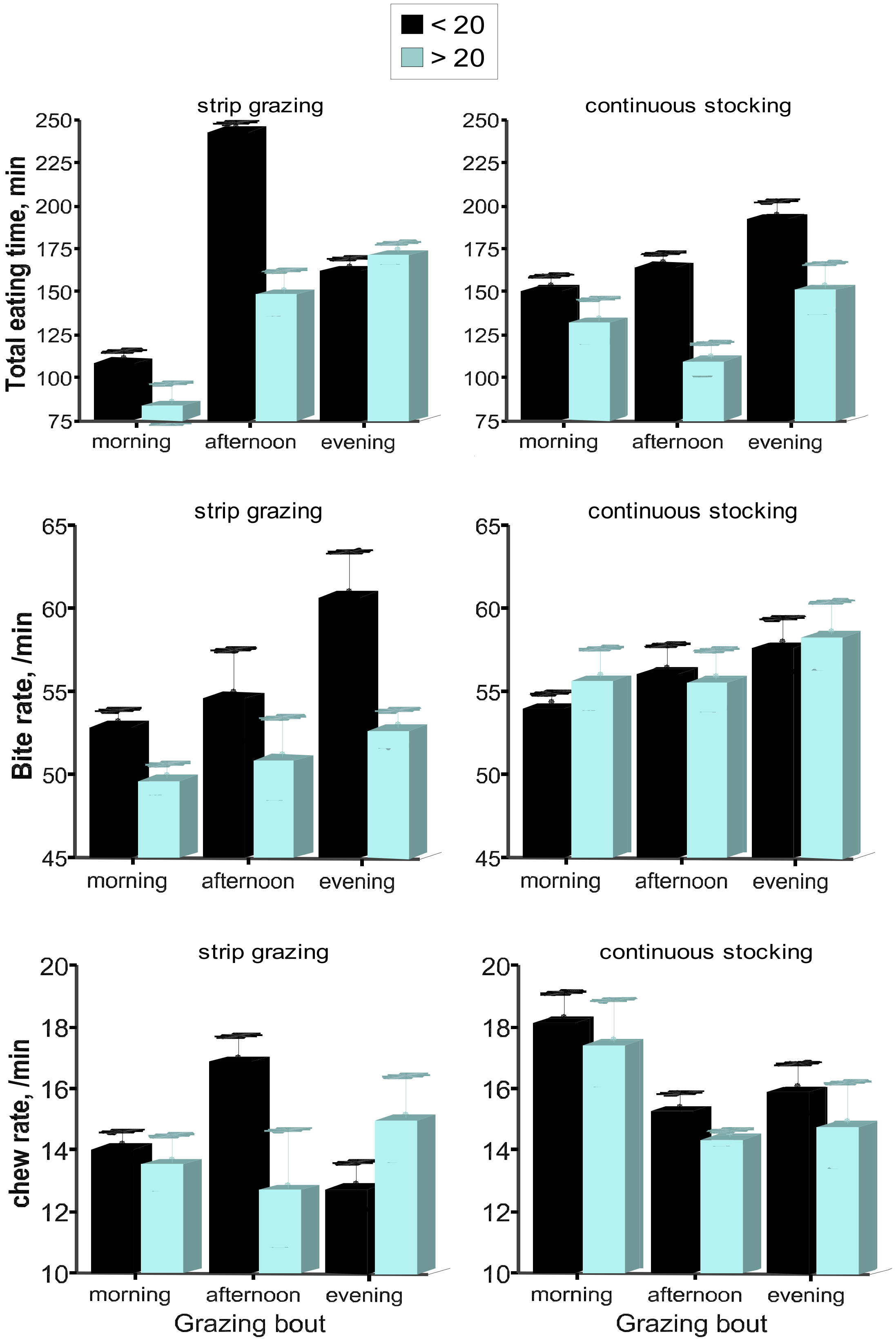

Figure 3. The effect of grazing system and the weather (temperature) on eating behaviour (eating time, bite rate and chewing rate) of dairy cows at the three main grazing bouts of the day. Temperature is expressed as < 20 ºC and > 20 ºC.

4.3. The effect of sward surface height (SSH) on grazing behaviour

To study the effect of varying SSH on grazing behaviour of dairy cows, only the data set of the two years' strip grazing experiments were used. That was because the measurements in the continuous stocking experiment were conducted over the duration of two weeks in a 1-ha sward, allowing only a limited fluctuation in SSH during the measurement period. On the other hand, the measurements of the strip grazing experiments were conducted over 8 weeks time, allowing a good variation in SSH. The data of the strip grazing experiments was divided based on average SSH, into two categories: short SSH and tall SSH. The division was made at an average SSH value of 15 cm, considering swards with an average SSH > 15 cm to be in the tall sward category and swards with an average SSH ≤ 15 cm to be in the short sward category. The data was analysed using a two independent samples t-test with the SSH category as the grouping variable.

Table 11. The effect of varying sward surface height (SSH) on grazing behaviour of lactating dairy cows

Variable	SSH category Short (≤ 15 cm)	 Tall (> 15 cm)	SD[1]	*P* (2-tailed)
	Plant data			
SSH, cm	12.4 ^{a}	17.7 ^{b}	3.0	0.001
Herbage allowance[2]	27.2 ^{a}	35.9 ^{b}	9.5	0.01
	Animal data			
Days in milk	88	100	21	0.13
	Grazing behaviour data			
TET[3], min	561 ^{a}	507 ^{b}	57	0.008
GJM rate, min^{-1}	72	70	5	0.27
Bite rate, min^{-1}	57	56	5	0.46
Chew rate, min^{-1}	14	14	3	0.63
Bites in GJM,%	80	80	4	0.86
Bite mass, g DM	0.62	0.61	0.1	0.86
TRT[4], min	450	479	58	0.19
Rumination rate, min^{-1}	63	63	4	0.85
Boli processing rate[5]	1.2	1.2	0.3	0.77

[1] SD is standard deviation

[2] Herbage allowance is expressed in kg DM/cow/day

[3] TET is total daily eating time

[4] TRT is total daily ruminating time

[5] Boli processing rate is expressed in boli processed/ min of rumination time

The effect of varying SSH on grazing behaviour is shown in Table 11. The data division procedure described above resulted in a good data set with a good differential magnitude in SSH between the two categories (> 5 cm; $P < 0.01$) (see Table 11). Moreover the data set was sufficiently balanced with regard to lactation stage, as animals on the short swards were in a similar lactation stage as those on the tall swards (88 vs. 100 days in milk). Among the grazing behaviour parameters, SSH significantly influenced only daily total eating time. Cows on shorter swards appeared to compensate by spending more ($P < 0.01$) time eating during the day (+ 55 min) (Table 11). Other grazing behaviour parameters were not significantly different between cows grazing swards with different SSH. Many other studies investigated the effect of SSH and other sward characteristics on grazing behaviour of dairy cows (Penning et al., 1991; Gibb et al., 1997). In agreement with our findings, they all reported that dairy cows on short swards compensated the low grass availability by significantly increasing their daily eating time. Moreover, they also reported that other grazing behaviour parameters were not significantly influenced by SSH, except for bite mass which was significantly higher in the taller swards. In contrast, our data show that daily average bite mass was similar for the short and tall swards. This might be related to the fact that the short swards in our experiments had an average SSH of 12.5 cm, whereas in Gibb et al. (1997) the short sward had a SSH of 5 to7 cm.

4.4. The effect of lactation stage on grazing behaviour

To study the effect of lactation stage on grazing behaviour only the data from the strip grazing experiments was used, for the same reason mentioned in the previous section. The cows used in the experiments ranged from 50 to 150 days in milk (DIM), with an average value of 100 DIM. The data set was divided based on the lactation stage of the cows into two categories; early lactation and mid-lactation considering cows with ≤ 100 DIM to be in the early lactation category and cows with > 100 DIM to be in the mid-lactation category. The effect of lactation stage on grazing behaviour parameters is shown in Table 12. The cows in the early lactation category were 77 DIM, whereas those in the mid-lactation category were 114 DIM. The data set was balanced with regard to SSH, which is known to significantly influence daily eating time (Table 11). Dairy cows in early lactation did not show a significantly different grazing behaviour than those in mid-lactation. Other authors (Gibb et al., 1999), however, reported a significant influence of stage of lactation on daily eating time when measured grazing behaviour of lactating and dry cows. They observed that dry cows spent less time grazing daily than lactating cows and related it to the higher nutritional demand of the lactating cows. However, in our experiments the difference in lactation stage was not that big, which might explain the lack of influence observed here, as the difference between categories was on average 36 DIM only.

Table 12. The effect of lactation stage on grazing behaviour of lactating dairy cows

Variable	Lactation stage category Early (≤100 DIM)	 Mid (>100 DIM)	SD[1]	*P* (2-tailed)
	Plant data			
SSH, cm	15.5	16.3	3.0	0.44
Herbage allowance[2]	31.7	34.1	9.5	0.48
	Animal data			
Days in milk	77 ^{a}	114 ^{b}	21	0.001
	Grazing behaviour data			
TET [3], min	528	523	57	0.83
GJM rate, min^{-1}	71	71	5	0.97
Bite rate, min^{-1}	56	57	5	0.53
Chew rate, min^{-1}	15	14	3	0.32
Bites in GJM,%	79	81	4	0.28
Bite mass, g DM	0.62	0.61	0.1	0.77
TRT [4], min	486	452	58	0.09
Rumination rate, min^{-1}	64	62	4	0.22
Boli processing rate [5]	1.2	1.2	0.3	0.92

[1] SD is standard deviation

[2] Herbage allowance is expressed in kg DM/cow/day

[3] TET is total daily eating time (24 h)

[4] TRT is total daily ruminating time (24 h)

[5] Boli processing rate is expressed in boli processed/ min of rumination time

5. Conclusions

The influence of management and season on perennial ryegrass composition, digestibility and nutritive value was larger than that of the variety. The variety explained less than 10 % of the variation observed in chemical composition and nutritive value. Selecting perennial ryegrass varieties for a fast clearing fibre may not necessarily be beneficial in improving DMI and milk yield, as it may lead to a higher passage of the potentially degradable fibre and reduce the utilisation of the ingested fibre. However, selecting for fast degrading fibre seemed to be beneficial in improving DMI, fibre utilisation and milk yield, but it appeared to be very limited for perennial ryegrass as a very small range in fibre degradation rate was observed. In both stall-feeding and grazing situations, high sugar content in perennial ryegrass did not improve DMI and milk yield by dairy cows. However, feeding high sugar grass reduced NH_3-N concentration in the rumen, shifted rumen fermentation towards propionate, did not slow down fibre degradation and clearance and decreased urea concentration in the milk. The observed effect on both rumen NH_3-N and milk urea concentrations was also related to the lower CP content of the high sugar grass, as a strong negative correlation was observed between CP and WSC content in the grass.

The grazing system and daily temperature largely influenced grazing behaviour of dairy cows. Under a daily strip grazing system, dairy cows had the longest grazing bout immediately after the allocation of the new plot, whereas under continuous stocking, dairy cows always had the longest grazing bout at dusk time. Dairy cows' bite rate increased linearly as the day progressed from dawn to dusk, regardless of the grazing system employed. This indicates that dairy cows try to maximise their intake at dusk time by changing the composition of their grazing jaw movements (GJM) into more bites and fewer chews, regardless of the system they are exposed to. Dairy cows further appear to reduce their daily activities when temperature is high by reducing their daytime grazing, reducing the duration of the afternoon grazing bout, reducing their GJM rate, bite rate and chewing rate. The influence of SSH on grazing behaviour was limited to eating time only, as dairy cows on shorter swards spent more time eating, but other grazing behaviour parameters were not significantly influenced.

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Summary

1. Introduction

In temperate environments, perennial ryegrass (*Lolium perenne* L.) is the most widely used species for feeding dairy cows. That is because of its high productivity, palatability, digestibility and nutritive value. At an early stage of maturity perennial ryegrass is highly digestible and has a high nutritive value in terms of energy and protein. However, perennial ryegrass suffers from two main disadvantages: firstly, its unbalanced nutrient content in terms of crude protein (CP) and energy availability at rumen level and secondly, its low dry matter intake (DMI) by high producing dairy cows. Many recent studies reported that cows grazing high quality pasture in the spring consumed much less DM than cows fed a nutritionally balanced total mixed ration (TMR). Furthermore, many earlier studies identified the low grass pasture DMI as a major factor limiting energy intake and hence milk production of high producing cows under grazing systems.

In ruminants, DMI is, in addition to metabolic constraints, a function of the balance between eating motivation, which is strongly related to palatability, on one hand and rumen capacity on the other. Therefore, increasing DMI could be achieved by either improving palatability or increasing rumen capacity or both. Rumen capacity is related to the rate of clearance (Kcl) of material from the rumen, which is the summation of both rates of degradation (kd) and passage (kp). Palatability is mainly a function of flavour and taste, which arise from certain compounds in the grass, especially water-soluble carbohydrates (WSC). This thesis investigated the possibility of improving perennial ryegrass DMI by dairy cows through manipulating both palatability and rumen capacity.

Three main hypotheses were tested in this thesis. The first hypothesis stated that perennial ryegrass DMI would be improved by feeding ryegrass varieties that have a faster rate of removal of fibre from the rumen (Chapters 1, 2 and 3). The second hypothesis stated that perennial ryegrass DMI would be improved by feeding ryegrass varieties that contain a high WSC content (Chapter 4 and 5). The third hypothesis focused on the role rumen fill and fermentation end products may play in signalling the termination of a grazing bout, especially the dusk grazing bout of dairy cows under continuous stocking (Chapter 6).

2. Perennial ryegrass with fast clearing and/or degrading fibre

A fast rate of removal of grass fibre from the rumen increases its capacity to hold more material and delays rumen fill signals, encouraging the animals to consume more and thus have a higher DMI. To be able to select for fast clearing or degrading fibre, varieties should differ in their fibre clearance or degradation rates. Therefore, the first step was to evaluate or screen different varieties of perennial ryegrass for these characteristics (fibre clearance and degradation rates) and define the available ranges for selection. In Chapter 1, an experiment is described, in which six diploid varieties of perennial ryegrass were fermented using the *in vitro* gas production technique to examine if they differ in their fermentation characteristics and rates. The varieties were sampled three times between early July and late August and fermented using the automated gas production technique with rumen liquid from grass-fed cows as an inoculum. Gas curves were fitted using a di-phasic logistic model, which assumes two fractions, which will result in a mathematically determined division of the gas production curves into two phases. The first phase is assumed to arise from the rapidly fermentable fraction, and the second phase from the slowly fermentable fraction. From the results it was found that variation in fermentation characteristics of perennial ryegrass appeared to be mainly due to variation in the rapidly fermentable fraction. Moreover, the slowly fermentable fraction of the grass was less variable, and less influenced by the variety, as the gas production parameters of the second phase were not different among varieties.

In Chapter 2, an experiment is described, in which the six ryegrass varieties were stall-fed to 12 multiparous rumen-cannulated dairy cows during three two-week periods, using a double 3 × 3 Latin square design. The NDF fractional clearance rate (Kcl_{NDF}) and the acid detergent lignin fractional clearance rate (Kcl_{ADL}) were estimated using two consecutive rumen evacuations, separated by a 12-hour period of feed deprivation assuming first order kinetics. The NDF fractional degradation rate (kd_{NDF}) was calculated based on the assumption that NDF fractional passage rate (kp_{NDF}) was equal to Kcl_{ADL}. The Kcl_{NDF} of the different grass varieties was in the range of 5 to 6 %/h and that of kd_{NDF} was in the range of 2 to 3 %/h. Both the Kcl_{NDF} and kd_{NDF} were not significantly different among grass varieties and the difference between the variety with the fastest clearing or degrading rate and that with the slowest rate was less than 1 %/h.

In Chapter 3 an experiment is described, in which the *in situ* NDF degradation characteristics and rates of the six ryegrass varieties were measured by incubating freeze-dried samples from the six varieties into the rumen of grazing dairy cows for 2, 4, 8, 12, 24, 48 and 336 hours. The *in situ* NDF degradation kinetics parameters (U, D and kd) did not differ significantly among varieties. On average, the U-fraction of NDF was around 14 % and the D-fraction around 86 % with a degradation rate of around 2.5 %/h for all varieties.

The three techniques (*in vitro* gas production, *in vivo* rumen evacuation and in *situ nylon* bag) showed that the different varieties of perennial ryegrass used in this study did not differ significantly in their NDF degradation rates and characteristics.

Moreover, the three techniques showed that the difference in fractional degradation rate between the variety with the fastest degrading fibre and the one with the slowest degrading fibre was less than 1 %/h. Therefore, it could be concluded that selecting for fast degrading fibre seemed to be limited for perennial ryegrass as only a small range in NDF degradation rate was observed between varieties.

3. Perennial ryegrass with a high sugar content

The argument that a high sugar content in the grass improves its sweetness and thus palatability, encouraging the animals to have a higher DMI, is counterbalanced by the argument that high sugar intake increases the acid load on the rumen, decreasing fibre degradation and hence reducing intake. To examine this apparent controversy, in this thesis we investigated the effect of feeding high sugar grass on DMI, rumen function and parameters and milk production and composition in lactating dairy cows under stall-feeding (Chapter 4) and under grazing (Chapter 5). In Chapter 4, an experiment is described, in which twelve high-producing Holstein-Friesian dairy cows in mid lactation were stall-fed with six varieties of perennial ryegrass, two of which were characterised by their high levels of water-soluble carbohydrates (WSC). In this experiment, DMI, rumen function, milk production and composition were measured. Contrary to expectations, DMI was not positively influenced by the increased WSC content, as varieties with a high WSC tended to have similar DMI to the other varieties (16.2 vs. 16.6 kg/d). Ruminal pH, neutral detergent fibre fractional clearance (Kcl_{NDF}) and fractional degradation (kd_{NDF}) rates were not reduced as a result of feeding high WSC grasses. Total VFA concentration was not changed, but the VFA composition was altered in favour of propionate at the expense of acetate when feeding high WSC grass. Ruminal ammonia concentration and milk urea concentration were reduced as a result of feeding high WSC grass, which may also be because of the lower N content of the high sugar grass. Milk yield and major constituents (fat, protein and lactose) were not influenced significantly. Feeding high sugar grass did not seem to be beneficial in improving DMI and milk production or in altering the composition of milk.

In Chapter 5, an experiment is described, in which four multiparous rumen cannulated dairy cows were offered four varieties of perennial ryegrass under a daily strip-grazing system using a 4 × 4 Latin square design. Two of the varieties were characterised by their high levels of WSC. Grazing behaviour measurements were done using a solid-state behaviour recorder. Daily intake was estimated using the n-alkane technique. Rumen function was measured using rumen evacuations. In this experiment, caution should be taken when interpreting the results, as many factors were confounded such as herbage allowance, sward surface height (SSH) and WSC content of the grass. The variety with the lowest WSC content had the lowest herbage allowance and the shortest SSH, both of which are known to influence grazing behaviour and DMI.

Cows grazing this variety had a lower DMI and milk yield. However, milk composition, grazing behaviour, rumination behaviour, rumen pool sizes and NDF clearance rates were all not significantly different among varieties. Therefore, the observed trend in DMI among the different varieties in this study cannot be attributed to only one factor (i.e. WSC content), but other factors such as herbage allowance, SSH, and NDF content may have played a role too.

4. Rumen fill and grazing behaviour under continuous stocking

Most of the research concerning the role of rumen fill and fermentation end products in regulating DMI in dairy cows has been performed with animals kept and fed indoors. Few studies about rumen fill and fermentation end products in grazing dairy cows have been conducted, probably due to methodological limitations. Under grazing dairy cows have shown to exercise three main grazing events (bouts) during the day: at morning, afternoon and dusk time. During each of these grazing bouts, the cow usually grazes continuously (non-stop) for more than one hour. Many experiments have shown that the dusk grazing bout is much longer than the other grazing bouts during the day. Moreover, eating behaviour of dairy cows has been shown not to be constant during the day, indicating that dairy cows manipulate their eating behaviour in terms of biting rate and chewing rate as the day progresses and may have a different eating behaviour at dusk time. Many theories to what initiates and terminates a grazing bout and to what controls the short-term daily herbage intake exist. Rumen fill and distension, fermentation end-products concentrations (total VFA, acetate, propionate, butyrate and NH_3) in the rumen and/or blood and rumen osmolality are among the most plausible ones.

In Chapter 6, an experiment is described, in which the behavioural strategies grazing dairy cows use to satisfy their nutritional needs as the day progresses, and the possible role of rumen fill in signalling the termination of a grazing bout were studied. For this purpose, the day was divided into three main periods (0600 to 1200 h, 1200 to 1800 h and 1800 to 2400 h) where the three main grazing bouts (dawn, afternoon and dusk) of dairy cows usually occur. Four late lactating rumen-cannulated dairy cows were used in a repeated measures design, with the grazing bout as the within subjects factor. The cows had access to a 1-ha grass sward under a continuous stocking system. To estimate rumen pool sizes, dry matter intake (DMI), total eating time (TET), bite rate, bite mass (BM) and intake rate at the three bouts, cows were rumen-evacuated at 0600, 1200, 1800 and 2400 h and jaw recorders were fitted to the cows between these time points. To estimate clearance rate (Kcl), which is needed to calculate DMI during the different bouts, cows were deprived of food from 2400 till 0800 h next morning, when rumen evacuations were performed again. The results showed that time spent eating by dairy cows at the dusk grazing bout was much longer than that at the other two grazing bouts and made about 40 % of the daily total eating time. Total grazing jaw movements (TGJM) rate was constant during the day at around 75/min. Bite rate, BM and hence intake rate increased, but chewing rate decreased as the day progressed. The increase in BM was mainly due to the increase in dry matter content of the grass at dusk time rather than increased bite dimensions. Therefore, it could be concluded that the main behavioural strategies dairy cows employ to satisfy their nutritional needs under continuous stocking include manipulating their eating time, biting rate and chewing rate, with little control over TGJM rate and BM. This indicated that dairy cows were trying to maximise their intake at dusk time before darkness falls by increasing their eating time and changing the composition of their TGJM towards more bites and less chews.

Rumen pool sizes were larger ($P < 0.001$) at 2400 h compared to other times of the day (9.5 vs. 13.5 kg DM). Because rumen evacuations in this study were performed at fixed time points during the day and not immediately when grazing ceased, it was important to estimate the fluctuation in rumen fill between the measured points if we want to draw valid conclusions concerning its role in regulating the cessation of grazing. To estimate rumen fill fluctuation during the day, a small dynamic mechanistic model was constructed. This model was based on the measured TET, the exact timing of each individual grazing event, bite rate and BM, Kcl and NDF content in grass and in rumen material. The model consisted of one state variable, which was the rumen NDF pool (Q_{NDFr}). In this model Q_{NDFr} receives input from feed intake (NDF flow into the rumen) and its output is disappearance from the rumen through clearance (the sum of degradation and passage). Both observed and simulated results showed that despite the fact that cows grazed on average 132 min and 175 min during the morning and afternoon grazing bouts, respectively, Q_{NDFr} did not reach the value it reached at 2400 h. This indicates that dairy cows interrupted these two grazing bouts long before reaching their maximal rumen fill capacity. However, rumen pool sizes were always maximal at the time when the dusk grazing bout ceased indicating that rumen fill is more likely to play a major role in signalling the termination of the dusk grazing bout. Furthermore, this study showed that the concentrations of all fermentation end products and rumen pH at the start of a grazing bout were not significantly different from those at the end of a grazing bout. This indicated that rumen fermentation end products and pH might not play a major role in signalling the initiation or termination of a grazing bout.

5. Main Conclusions

1- Among the perennial ryegrass varieties used in this study, variation in fermentation, clearance and degradation characteristics and rates was small, indicating that choosing varieties with a fast degradation rate is very limited.

2- Season and management had a larger influence on perennial ryegrass quality, nutritive value and degradation characteristics and rates than the variety.

3- Feeding perennial ryegrass varieties that are characterised by a high sugar content did not lead to improvements in DMI and milk yield, and did not significantly influence milk composition in terms of fat, protein and lactose. Moreover, it also did not lead to a lower pH and fibre degradation or clearance rate in the rumen. However, feeding high sugar grass varieties reduced rumen ammonia and milk urea concentrations significantly.
4- Grazing behaviour in terms of eating time, rumination time, bite rate, chewing rate, bite mass and ruminative mastication rate of dairy cows grazing different varieties of perennial ryegrass was not different. Herbage allowance and sward surface height had a much larger influence on grazing behaviour than the variety per se.
5- The chemical composition of perennial ryegrass in terms of DM and WSC fluctuated largely during the day. Evening grass was characterised by a higher DM and WSC content compared to morning and afternoon grass.
6- Under continuous stocking, dairy cows spent more time eating during the dusk (evening) grazing bout compared to the morning and afternoon grazing bouts. Eating time during the dusk bout made up more than 40 % of the daily total eating time. Furthermore, dairy cows appeared to alter their eating behaviour in terms of bite rate and chewing rate at dusk time. Total grazing jaw movements' (TGJM) rate, consisting of total number of bites and total number of chews a cow can make per minute, was constant during the day at around 75/min. This indicated that cows could not manoeuvre much by increasing their TGJM rate. However, cows could manoeuvre largely by changing the composition of TGJM between bites and chews and seemed to maximise bite rate and minimise chewing rate at dusk time, in order to maximise intake rate at that time of the day.
7- Under continuous stocking, bite mass (BM) of dairy cows was constant during the day, as the cows seemed to ingest a similar amount of fresh herbage per bite at different times during the day. However, due to the increased DM content of the grass at dusk, BM in mg DM was much higher at that time of the day. This indicated that the change in DM content of the grass as the day progressed was the main factor causing the difference in BM between the grazing bouts under continuous stocking.
8- Rumen pool sizes were significantly larger at 2400 h, when the dusk grazing bout ceased, compared to the other times during the day.
9- Dairy cows interrupted the first two grazing bouts (dawn and afternoon) long before reaching their maximal rumen fill capacity, indicating that rumen fill is less likely to play a significant role in signalling the termination of these two grazing bouts. However, rumen pool size was always maximal at the time when the dusk grazing bout ceased indicating that rumen fill is more likely to play a major role in signalling the termination of the dusk grazing bout.
10- The concentrations of all fermentation end products in the rumen at the start of a grazing bout were not significantly different from those at the end of a grazing bout, indicating that rumen fermentation end products may not play a major role in

initiating or terminating a grazing bout. However, rumen NH_3 concentration was numerically different between the start and the end of a grazing bout. Moreover, the concentration of all fermentation end products fluctuated during the day in response to eating and rumination. This diurnal fluctuation was minor for total VFA, acetate, propionate, butyrate and pH, whereas, the observed fluctuation in NH_3 concentration was major rendering it as a main candidate for a possible role in signalling the termination of a grazing bout.

6- Implications

1- The main concern of the dairy farmer when purchasing perennial ryegrass varieties should be the disease and drought resistance characteristics of these varieties, as these are the main characteristics that contribute largely to grassland productivity and hence dairy farm production. Moreover, these are the only grass characteristics that cannot be manipulated effectively by management. As long as the grass is disease free and drought resistant, the farmer's management plays the main role in determining the yield, quality and nutritive value at grassland level and DMI, milk yield and nutrient utilisation efficiency at animal and farm level.

2- When producing new varieties or mixtures, grass companies should create selection criteria and indices that concentrate on disease and drought resistance, winter hardiness, high yield and high digestibility. Other plant characteristics such as sugar content and degradation rate or animal parameters such as DMI and milk yield are either related to digestibility or are hard to select for, due to the small difference between varieties. Besides, sugar content and degradation rate of grass and DMI and milk yield of grass fed dairy cows can be influenced more effectively by management than by the choice of variety.

3- Dairy cows appear not to utilise their maximum capacity and potential to ingest grass during the morning and afternoon, as they stop eating long before their maximum rumen fill and they do not use their maximum bite rate at those times of the day. Understanding why dairy cows behave in such a way is a key towards designing interventions or grazing systems that allow dairy cows to utilise their maximum potential to ingest pasture grass and hence increase their DMI. Moreover, research that focuses on how to make dairy cows on pasture utilise their maximum potential during the morning and afternoon bouts, by making them eat with their maximum bite rate till their maximum rumen fill, is of utmost importance.

Samenvatting

1. Inleiding

In gematigde streken is Engels raaigras (*Lolium perenne* L.) de meest gebruikte grassoort om koeien mee te voeren. Dit is te danken aan de zeer hoge productiviteit, verteerbaarheid en voederwaarde in termen van energie en eiwit, eigenschappen die reeds in een jong groeistadium worden bereikt. Echter, Engels raaigras heeft ook tekortkomingen. Ten eerste komen (ruw) eiwit en energie niet op een evenwichtige wijze beschikbaar in de pens en ten tweede is de vrijwillige voeropname van droge stof (VVO) uit gras te laag voor hoog productief melkvee. Gebleken is dat koeien die geweid worden op een hoge kwaliteit voorjaarsgras daarvan veel minder drogestof opnemen dan koeien doen van een gebalanceerd totaal gemengd winterrantsoen (TMR). Bovendien werd vastgesteld dat bij grazende koeien de lage drogestof opname de belangrijkste factor is die de energie opname en als gevolg daarvan de melkproductie beperkt.

In herkauwers is de VVO, naast chemische verzadigingssignalen die van invloed zijn, een functie van de balans tussen hun motivatie tot vreten, die sterk beïnvloed wordt door smakelijkheid, en hun penscapaciteit. Een verhoging van de VVO zou daarom bereikt moeten kunnen worden door hetzij de smakelijkheid te verhogen, hetzij de penscapaciteit te vergroten, of beide. Penscapaciteit is afhankelijk van de snelheid waarmee voer de pens verlaat, als gevolg van afbraak of door passage naar de rest van het maagdarmkanaal. Smakelijkheid is vooral een functie van geur en smaak, die ontstaan uit bepaalde componenten van het gras, waarbij vooral aan water oplosbare koolhydraten (WOK) een belangrijke rol wordt toebedacht. Dit proefschrift geeft verslag van een onderzoek naar de mogelijkheid om de VVO van Engels raaigras te verhogen door zowel de smakelijkheid als de penscapaciteit positief te beïnvloeden.

In het onderzoek werden drie hypotheses getest. De eerste hypothese was dat de VVO van Engels raaigras verbeterd kon worden door rassen te ontwikkelen die sneller door de pens verwerkt worden (hoofdstukken 1, 2 en 3). De tweede hypothese was dat de VVO van Engels raaigras verbeterd kon worden door rassen te voeren met een hoger gehalte aan WOK (hoofdstukken 4 en 5). De derde hypothese was gebaseerd op de rol die pensvulling en de eindproducten van pensfermentatie spelen bij het afgeven van signalen om een graasperiode te beëindigen, met name de graasperiode aan het eind van de dag van koeien op een standweide.

2. Engels raaigras met snel verwerkbare celwanden

Een snelle verwijdering van grasvezels uit de pens verhoogt de capaciteit van de pens om materiaal te bevatten en zal ook signalen van maximale pensvulling vertragen, waardoor het dier wordt aangemoedigd om meer gras op te nemen en dus een hogere VVO wordt bereikt. Om op dit gebied succes te kunnen boeken moeten grassen ontwikkeld worden die verschillen in de snelheid waarmee hun vezels of celwanden (NDF) in de pens worden afgebroken of de pens verlaten. Als een eerste stap werden verschillende rassen van Engels raaigras gescreend op deze eigenschappen en werd de mate van variatie vastgesteld.

Van zes rassen Engels raaigras werden de fermentatie eigenschappen en –snelheden onderzocht met de *in vitro* gasproductietechniek (hoofdstuk 1).De rassen werden in de periode tussen begin juli en eind augustus 3 maal bemonsterd en gefermenteerd in een automatisch gasproductiesysteem met pensvloeistof van grazende koeien als inoculum. De gasproductie curven werden gefit met een twee fasen logistisch model. Dit model verdeelt de gasproductie in twee fasen, die geacht worden respectievelijk de snel en langzaam fermenteerbare delen van het gras te weerspiegelen. Uit de resultaten bleek dat de variatie in fermentatie van gras vooral het gevolg was van variatie in de snel fermenteerbare fase. De fermentatie eigenschappen van de langzaam fermenteerbare fase verschilden niet tussen de verschillende rassen.

De zes grasrassen werden via stalvoedering verstrekt aan 12 koeien, waarvan 6 met een penscanule, volgens een dubbel 3x3 Latijns vierkant (hoofdstuk 2). De fractionele verdwijnsnelheden van NDF (Kcl_{NDF}) en ADL (Kcl_{ADL}), waarvan werd aangenomen dat die verliepen volgens een eerste orde kinetiek, werden geschat uit twee opeenvolgende evacuaties van de pensinhoud met een tussentijd van 12 uren, gedurende welke de dieren werden gevast. Aangenomen werd dat de passagesnelheid van de NDF (kp_{NDF}) gelijk was aan Kcl_{ADL}. De Kcl_{NDF} lag in de range tussen 5 en 6 %/uur en die van kd_{NDF} tussen 2 en 3 %/uur. Het maximale verschil tussen de rassen met de snelst en langzaamst afgebroken celwanden bedroeg minder dan 1 % en verschilde niet significant tussen rassen.

De afbraakeigenschappen en -snelheden van de zes rassen Engels raaigras werden, met behulp van nylon zakjes incubaties over periodes van 2, 4, 8, 12, 24, 48 en 336 uur, bepaald in de pens van grazende koeien (hoofdstuk 3). Voor de hieruit verkregen *in situ* NDF afbraak eigenschappen (U, D en kd) werden tussen de rassen geen significante verschillen vastgesteld. De omvang van de U fractie van de NDF was rond de 14 %, die van de D fractie bedroeg 86 %, met een afbraaksnelheid van rond de 2,5 %/uur voor alle rassen. Met geen van de drie gebruikte meettechnieken (*in vitro* gasproductie, *in vivo* pensevacuaties en *in situ* nylon zakjes incubaties) werden tussen de zes grasrassen verschillen van betekenis in de afbraak eigenschappen van NDF vastgesteld. Sterker nog, met alle drie de technieken werden tussen de snelst en langzaamst afbreekbare rassen verschillen gevonden van minder dan 1 %. De conclusie was dat de mogelijkheden om te selecteren op de snelheid van celwandafbraak voor rassen van Engels raaigras tamelijk beperkt zijn.

3. Engels raaigras met een hoog gehalte aan suikers

Tegenover het standpunt dat een hoog gehalte aan oplosbare suikers (WOK) in gras haar zoetigheid en daarmee haar smakelijkheid verhoogt, waardoor er meer van zal worden opgenomen, kan worden ingebracht dat veel WOK bijdragen aan een snelle zuurvorming in de pens, wat de afbraak van met name vezels kan afremmen, en de voeropname weer zal kunnen verlagen. Om deze schijnbare tegenstelling op te helderen werd het effect van het aan koeien verstrekken van vers gras met een hoog gehalte aan WOK op VVO, pensfunctie en productie en samenstelling van melk onderzocht bij zowel stalvoedering (hoofdstuk 4) als onder beweiding (hoofdstuk 5). Twaalf Holstein-Friesian koeien werden in het midden van hun lactatie op stal gevoerd met zes rassen Engels raaigras, waarvan twee een hoog gehalte aan WOK hadden (hoofdstuk 4). In deze proef werden VVO, pensfunctie en de productie en samenstelling van melk gemeten. In tegenstelling tot wat werd verwacht, werd de VVO niet positief beïnvloed door een verhoogd gehalte aan WOK. De dieren die werden gevoerd met rassen met hoog WOK gehalte hadden een VVO die vergelijkbaar was met die van de met laag WOK rassen gevoerde dieren (16,2 vs 16,6 kg drogestof/dag). Ook werden na het voeren van hoog WOK rassen geen lagere waarden vastgesteld voor pens pH, de fractionele verdwijnsnelheid (Kcl_{NDF}) of de fractionele afbraaksnelheid (kd_{NDF}) van de celwanden.

De totale concentratie aan vluchtige vetzuren (t-VFA) was niet veranderd, maar de VFA samenstelling was verschoven ten gunste van propionzuur en ten nadele van azijnzuur. De gehaltes aan ammonia (NH_3) in de pens en ureum in melk waren verlaagd na het voeren van hoog WOK grasrassen, wat mede veroorzaakt kan zijn door een lager N gehalte van het hoge WOK gras. Melkproductie en gehaltes van de voornaamste melkbestanddelen (vet, eiwit, lactose) waren onveranderd. De conclusie wordt getrokken dat het voeren van grasrassen met veel WOK geen voordelen biedt voor wat betreft VVO, melkproductie en melksamenstelling.

Aan vier koeien met een penscanule werden in een 4 x 4 Latijns vierkant proefopzet, onder een dagelijks rantsoenbeweidingssysteem, vier rassen Engels raaigras verstrekt, waarvan twee met een hoog WOK gehalte (hoofdstuk 5). Het graasgedrag van de dieren werd gemeten met graasrecorders en hun grasopname geschat m.b.v. de n-alkanen techniek. De pensfunctie werd gemeten met behulp van evacuaties van de pensinhoud. De resultaten van dit soort proeven moeten met de nodige voorzichtigheid worden uitgelegd, omdat veel factoren met elkaar verstrengeld zijn, zoals grasaanbod, grashoogte en het WOK gehalte van het gras. Het ras met het laagste WOK gehalte had ook het laagste grasaanbod en de kortste grashoogte, allebei factoren waarvan bekend is dat ze het graasgedrag en de VVO beïnvloeden. De koeien die op dit grasras graasden hadden een lagere VVO en melkproductie. Echter, melk samenstelling, graasgedrag, herkauwgedrag, pensinhoud en verdwijnsnelheid van celwanden verschilden geen van alle significant tussen grasrassen. Het zal duidelijk zijn dat de waargenomen trend in VVO tussen de verschillende rassen in deze studie niet aan één enkele factor (bv. het WOK gehalte) kunnen worden toegeschreven, maar dat ook andere factoren zoals grasaanbod, grashoogte and celwandgehalte een rol kunnen hebben gespeeld.

4. Pensvulling en graasgedrag op een standweide

Het meeste onderzoek naar de rol van pensvulling en eindproducten van pensfermentatie bij het reguleren van de drogestof opname is uitgevoerd met dieren die binnen werden gehouden en gevoerd. Als gevolg van methodologische moeilijkheden zijn slechts een beperkt aantal proeven uitgevoerd over pensvulling en de rol van eindproducten van pensfermentatie in grazende koeien. Grazende koeien hebben laten zien dat er drie belangrijke graasperiodes zijn, namelijk vroeg in de ochtend (dawn), rond het middaguur (noon) en tegen de tijd dat het donker wordt (dusk). Gedurende alledrie deze perioden grazen koeien onafgebroken gedurende meer dan een uur. In meerdere proeven is vastgesteld dat de graasperiode tegen de tijd dat het donker wordt veel langer duurt dan de andere graasperiodes. Bovendien is vastgesteld dat het vreetgedrag van koeien niet constant is over de dag, wat een aanwijzing is dat koeien hun vreetgedrag in termen van hap- en kauwsnelheid in de loop van de dag aanpassen. Er zijn vele theorieën over wat het begin en het einde van een graasperiode en daarmee de korte termijn dagelijkse grasopname bepaalt. De vullings- en oprekkingsgraad van de pens, de concentraties in de pens en of in het bloed aan eindproducten van pensfermentatie (totaal VFA, azijnzuur, propionzuur, boterzuur, NH_3), en osmolariteit van de pensvloeistof behoren tot de meest voor de hand liggende factoren.

De gedragspatronen die grazende koeien in de loop van de dag uitoefenen om hun nutritionele behoeftes te bevredigen werden bestudeerd, waarbij met name de rol van pensvulling in het beëindigen van een graasperiode aandacht kreeg (hoofdstuk 6). Voor dit doel werd de dag verdeeld in drie periodes waarin de drie voornaamste graasperiodes vallen, namelijk van 0600 tot 1200 uur, van 1200 tot 1800 uur en van 1800 tot 2400 uur. Een viertal oudmelkte koeien met penscanules werden in een proefopzet van herhaalde metingen gebruikt, met de graasperiode als de binnen koe factor. De koeien graasden op een standweide van 1 ha met een ruim grasaanbod. Voor het schatten van pensinhoud, vrijwillige voeropname (VVO), totale vreettijd (TET), vreetsnelheid, hapgrootte (BM) en opnamesnelheid gedurende de drie periodes, werd de pensinhoud van de koeien geëvacueerd, gemeten en bemonsterd om 0600, 1200, 1800 en 2400 uur en droegen de koeien graasrecorders in de tussenliggende periodes. Om de fractionele verdwijnsnelheid (Kcl) vast te stellen, een gegeven dat nodig is om de VVO in de verschillende periodes te kunnen bepalen, werden de koeien gevast tussen 2400 en 0800 uur de volgende ochtend. Op dat tijdstip werd opnieuw een evacuatie van de pensinhoud uitgevoerd.

Uit de resultaten kwam naar voren dat de tijd die de koeien besteedden aan grazen voor het invallen van de duisternis, veel langer duurde dan in beide andere graasperiodes en ongeveer 40% bedroeg van de totale dagelijkse graastijd. De frequentie van het totale aantal kaakbewegingen (TGJM) was de hele dag ongeveer gelijk en lag op 75 per minuut. De vreetsnelheid en hapgrootte (BM) en dus de opnamesnelheid nam toe, maar de kauwsnelheid nam af met het voortschrijden van de dag. De toename in hapgrootte was meer het gevolg van een toename van het gehalte aan drogestof van het gras bij het invallen van de duisternis dan van een toename van hap dimensies (vreet- en kauwsnelheid). Er werd daarom geconcludeerd dat de voornaamste gedragingen die koeien op een standweide toepassen om hun nutritionele behoeftes te bevredigen bestaan uit het aanpassen van hun vreettijd, hun hapsnelheid en hun kauwsnelheid met weinig verandering in snelheid van kaakbewegingen of hapgrootte. Met andere woorden, de koeien probeerden hun VVO vlak voor het vallen van de nacht te maximaliseren door hun vreettijd te verlengen en hun snelheid van kaakbewegingen aan te passen in de richting van meer happen en minder kauwen.

Vergeleken met de andere tijdstippen was om 2400 uur de pensvulling groter ($P < 0.001$), 13.5 vs 9.5 kg DS. Uitgedrukt in g/kg metabool gewicht (MBW) was de pensvulling zelfs > 100 g/kg MBW en evenaarde dat van koeien gevoerd met winterrantsoenen gebaseerd op silage en krachtvoer. Zulke hoge pensvullingen werden tot nu toe bij grazende koeien niet gevonden; tot nu toe gepubliceerde gegevens houden 75-80 g/kg MBW aan als maximum voor grazende koeien.

Het evacueren van de pensinhoud werd uitgevoerd op vaste tijdstippen en niet onmiddellijk na het stoppen van het grazen. Voor het trekken van geldige conclusies over de rol van pensvulling bij het reguleren van het graasgedrag, werd het van belang geacht een schatting te maken van de fluctuaties in pensvulling tussen de tijdstippen van evacuatie. Voor dit doel werd een klein dynamisch, mechanistisch model gemaakt, gebaseerd op totale vreettijd, de exacte timing van elke individuele graasactie, vreetsnelheid en hapgrootte, Kcl en het celwandgehalte (NDF) van gras en pensinhoud.

Het model bevat één toestandsvariabele (state variable), namelijk de pool aan celwanden in de pens (Q_{NDFr}). In het model krijgt Q_{NDFr} input uit de voeropname (NDF flow naar de pens) en haar output is verdwijning uit de pens (de som van afbraak en passage). Zowel de gemeten als de gesimuleerde resultaten toonden aan dat ondanks het feit dat de koeien gemiddeld 132 en 175 minuten graasden tijdens de ochtend en middag graasperiodes, de Q_{NDFr} niet de omvang bereikte die het om 2400 had. Dit is een aanwijzing dat koeien met grazen stopten lang voordat ze hun maximale pensvullingscapaciteit hadden bereikt. Maar de pensvulling bereikte altijd haar maximale omvang op het moment dat de graasperiode voor het vallen van de nacht werd beëindigd. Dit is een aanwijzing dat de rol van de pensvulling bij het aangeven van een signaal om met grazen te stoppen, zich beperkt tot de graasperiode vlak voor het vallen van de nacht. Bovendien bracht dit onderzoek aan het licht dat de concentraties aan eindproducten van pensfermentatie en de pH in de pens bij het starten van een graasperiode niet significant verschilden van die aan het einde ervan. Dit is een aanwijzing dat eindproducten van pensfermentatie en pH in de pens niet een belangrijke rol spelen bij het geven van een signaal een graasperiode te starten of te stoppen.

5. Voornaamste conclusies

1- Tussen de rassen van Engels raaigras zoals onderzocht in deze studie werd slechts weinig variatie gevonden in fermentatie-, verdwijn- en afbraakeigenschappen, wat aangeeft dat de ruimte voor het selecteren van rassen met een hoge afbraaksnelheid tamelijk beperkt is.

2- Seizoen en management hadden een grotere invloed op kwaliteit, voederwaarde en afbraak eigenschappen dan grasras.

3- Het aan koeien voeren van rassen van Engels raaigras die worden gekenmerkt door een hoog gehalte aan oplosbare suikers leidde niet tot een verbetering van de vrijwillige voeropname of melkproductie en had geen merkbare invloed op de melksamenstelling in termen van vet, eiwit en lactose. Het leidde echter ook niet tot een lagere pH in de pens, een tragere celwandafbraak of verdwijning uit de pens. Rassen met een hoog suikergehalte veroorzaakten wel een lager ammoniakgehalte in de pens en een lager ureumgehalte in de melk.

4- Het graasgedrag van koeien in termen van vreettijd, herkauwtijd, hapsnelheid, kauwsnelheid, hapgrootte en verkleiningssnelheid door herkauwen verschilde niet tussen de diverse rassen Engels raaigras. Grasaanbod en grashoogte hadden een veel grotere invloed op het graasgedrag dan grasras.

5- De chemische samenstelling van Engels raaigras in termen van droge stof (DS) en water oplosbare koolhydraten (WOK) fluctueert sterk gedurende de dag als reactie op weersomstandigheden, plantenfysiologische processen (fotosynthese, transpiratie) en de activiteit van dieren. In vergelijking met dat in de ochtend en de middag wordt gras in de avond gekarakteriseerd door hogere gehaltes aan DS en WOK.

6- Op een standweide spendeerden koeien meer tijd aan vreten tijdens de avond graasperiode in vergelijking met de periodes in de ochtend en in de middag. Van de totale vreettijd op een dag vond 40 % plaats in de avondperiode. Koeien pasten bovendien hun vreetgedrag aan in termen van hap- en kauwsnelheid tijdens de avondperiode. Het totale aantal kaakbewegingen dat een koe kan maken, zijnde de som van het totale aantal beten en het totale aantal kauwbewegingen, was ongeveer constant gedurende de dag en bedroeg

75 per minuut. Een koe kan de verhouding tussen bijtbewegingen en kauwbewegingen echter aanpassen en tijdens de avondgraasperiode werd het aantal bijtbewegingen verhoogd ten koste van het aantal kauwbewegingen, waardoor de opnamesnelheid tijdens dat deel van de dag gemaximaliseerd kon worden.

7- Op een standweide was de hapgrootte aan vers materiaal ongeveer constant op elk tijdstip van de dag. Door een hoger gehalte aan drogestof tegen het vallen van de nacht werd de hapgrootte (in mg drogestof) veel hoger op dat tijdstip van de dag. Dit suggereert dat de toename in het drogestof gehalte van gras in de loop van de dag de belangrijkste factor is die verschillen veroorzaakt in hapgrootte, uitgedrukt in drogestof, tussen de verschillende graasperioden.

8- De hoeveelheid materiaal in de pens was, als de avond graasperiode was gestopt, om 2400 uur groter dan op de andere tijdstippen van de dag.

9- Koeien onderbraken de beide eerste graasperiodes lang voordat ze hun maximale pensvullings capaciteit hadden bereikt. Dit is een aanwijzing dat pensvulling waarschijnlijk niet een belangrijke signaalfunctie vervult om het beëindigen van een graasperiode te bewerkstelligen. Echter de pensvulling was wel altijd maximaal op het moment dat de graasperiode van vlak voor het vallen van de nacht werd beëindigd en hier lijkt het dus wel een belangrijke signaalfunctie te hebben.

10- De concentratie van alle eindproducten van pensfermentatie aan het begin van een graasperiode waren niet significant verschillend van die aan het einde van een graasperiode. Dit wordt beschouwd als een aanwijzing dat eindproducten van pensfermentatie niet een belangrijke rol spelen als signaal voor het stoppen van een graasperiode. Echter de NH_3 concentratie was numeriek verschillend tussen het begin en eind van een graasperiode. Bovendien fluctueerden de gehaltes van alle eindproducten van pensfermentatie als gevolg van voeropname en herkauwen. Deze fluctuatie in de loop van de dag was gering voor het totaal aan VFA, azijnzuur, propionzuur, boterzuur en pH, terwijl de fluctuatie in de NH_3 concentratie aanzienlijk was. Dit maakt de NH_3 concentratie een belangrijke kandidaat voor een mogelijke rol in het signaleren van het einde van een graasperiode.

6. Implicaties

1- De belangrijkste zorg van een melkveehouder wanneer hij rassen van Engels raaigras aankoopt zouden resistentie tegen ziekten en tegen droogte moeten zijn, omdat dit de belangrijkste eigenschappen zijn die bijdragen aan de productiviteit van grasland en dus aan de productie van een melkveehouderijbedrijf. Bovendien zijn dit de enige eigenschappen van gras die niet effectief gemanipuleerd kunnen worden door management. Zolang het gras vrij is van ziekten en bestand tegen droogte zal het management van de boer bepalend zijn voor de opbrengst, kwaliteit en voederwaarde op het niveau van het grasland, en de opname aan droge stof, melkproductie en benutting van nutriënten op het niveau van dier en bedrijf.

2- Bij het ontwikkelen van nieuwe rassen of mengsels zouden graszaadbedrijven selectiecriteria en indicatoren moeten ontwerpen, die zich concentreren op weerstand tegen ziekten en droogte, wintervastheid, een hoge opbrengst en een hoge verteerbaarheid. Andere eigenschappen van planten, zoals suikergehalte en afbraaksnelheid of diergebonden factoren zoals vrijwillige voeropname en melkproductie zijn of gerelateerd aan verteerbaarheid of moeilijk om op te selecteren als gevolg van de geringe variatie tussen rassen. Bovendien kunnen suikergehalte, afbraaksnelheid van gras, VVO en melkproductie op gras effectiever beïnvloed worden door het juiste management dan door de keuze van het juiste ras.

3- Melkgevende koeien lijken hun maximale capaciteit en potentie om gras op te nemen tijdens de ochtend en de middag niet volledig te benutten. Ze stoppen met grazen lang voordat de maximale pensvulling is bereikt en ze gebruiken niet hun maximale vreetsnelheid. Het begrijpen waarom koeien zich op deze manier gedragen is de sleutel tot het ontwerpen van interventies op graslandsystemen die koeien in staat stelt hun maximale potentieel om gras tijdens weidegang op te nemen, te benutten en daardoor hun VVO te verhogen. Bovendien is onderzoek dat zich richt op de vraag hoe koeien er toe zijn te bewegen tijdens weidegang ook in de ochtend en middag hun maximale potentieel te bereiken door met een maximale vreetsnelheid door te gaan tot de maximale pensvulling is bereikt, uiterst belangrijk.

Acknowledgments

I don't know really where to start? I guess I will start from the primary school where it all started. I wish to thank all my primary school teachers, who in addition to teaching me reading, writing, math and science also tried teaching me discipline and honesty. I am not sure whether they succeeded or not, but at least they tried. I also wish to thank all my secondary and high school teachers for their relentless efforts and their patience with my classmates and me. To my English teacher, who I am sure never imagined me writing a book in English, and publishing papers in English peer reviewed journals, to him I say, guess what! I did. To the principal of my high school I wish to thank you for the 20 hard stick-hits you gave me on my hands when I came late to the classroom in the morning, and tell you that it didn't help, I still hate the early morning.

I also wish to extend my thanks and gratitude to all my teachers in Jordan University of Science and Technology, where I obtained my BSc degree. My special thanks go to my Arabic Professor who made Arabic language look so complex even to a native, and who gave me the worst mark I ever got in my life, I want to thank you for letting me pass! My special thanks to Prof. Ismat El Bakri (Egypt), who made physiology, immunology, endocrinology and artificial insemination, look extremely easy and interesting. To him I say you are one of best teachers I have ever had. Many thanks also go to Prof. Daeb Aziz and Refaat El Aani (Iraq), who made me appreciate breeding and ruminant nutrition, and introduced me to the world of research. I also wish to extend special thanks to the great friends I made during my time in Jordan, with who I still keep strong contact till the moment. Sulaiman, Mutasim, Walid, Hussam, Rami and Amer, it is a pity that we cannot see each other on regular basis due to the distance and boarders, but I must tell you that you are always on my mind and heart. I want to thank you for all the support you gave me, all the laughs we had, and all the card games, football and badminton matches we played. Sulaiman and Mutasim you will never manage to beat me in badminton, you need years of training to reach my level, but I must tell you, keep on trying, one day you may succeed!!!!!

Very special words go to the staff of Ramallah Department of Agriculture, where I had my first real job, and where I learned how to apply my theoretical and practical knowledge.

I am indebted to The Netherlands Government and The Netherlands Fellowship Program (NFP) for offering me a full fellowship to continue my education and obtain a Master degree in animal science and nutrition from Wageningen University. Without this fellowship I would have never been able to continue my education and purse my goals. It was during this time in The Netherlands, when I met my supervisor, who later on offered me the PhD position that led to the completion of this thesis and to my PhD degree. Moreover, it was during this time, when I met and fell in love with my lovely wife.

Moreover, I would like to acknowledge all my teachers and tutors in Wageningen University where I matured as a researcher and learned to be scientifically critical and direct. Special thanks go to Henk Udo and Gert Zemmelink for introducing me to the holistic system approach and for the great useful courses I followed with them especially the course "Analysis of livestock systems". I should not forget to extend my gratitude to Prof. Rash who taught me statistics and experimental design and analysis, which appeared to be one of the most important assets for a researcher. During my time in Wageningen I was fortunate to meet people from all over the globe and learn about their culture, tradition and religion. Alexis Ortiz, I wish to tell you that you are more than a friend to me, and that your support and friendship are highly treasured. Thank you for your patience while teaching me Latin American dancing (salsa and maringe), I know that I suck in dancing but because of you, now I suck less. I promise never to tell you again that Chile was discovered by Spain!!!!! Sarah, Jenifer, Shanomae and Megan, your contribution to my English language skills and your enjoyable discussions, company and dinners are highly acknowledged. Sarah, making me steal Megan's bike to give it back to her as a gift for her birthday, was not a good idea after all, was it? Olga Vigiak, I wish to thank you for your friendship and for all the nice moments we spent together, and more importantly I wish to thank you for all the non-alcoholic teramesu you prepared specially for me. Otto and Juan, your friendship is a great asset to me, I will never forget our crazy adventures, especially running in front of the bulls in Pamplona festival (San Fermines), what were we thinking?

Furthermore, I wish to extend my sincere gratitude and thousands of thanks to my supervisor Prof. Dr. ir. Seerp Tamminga. Seerp, I wish to thank you for your trust in me and my work, for the opportunity and independence you gave me, and for being there whenever I needed guidance and advice. Words fail to express how I feel, you were more than of a supervisor to me, you were a father figure and an example to follow. I enjoyed our sailing trip In Holland and our water-pipe session in Egypt. Next time I promise that the tobacco will be stronger and not flavored. I also wish to thank my co-supervisor Jan Dijkstra for introducing me to the world of mechanistic modeling, and for his critical comments, suggestions and remarks. I enjoyed our Mexico travel, and after all, it was not me who was stopped at every check desk in the airports! I wish to extend special words of appreciation to Barbara Williams for introducing me to the *in vitro* gas production technique and for sharing with me her experience about numerous topics including raising bilingual babies. Barbara, I hope you know how to bargain now, and next time the seller tells you 100, you know what to say!!!!!!! 40 not 95. A special word of thanks goes to Anjo Elgersma for her valuable and useful comments, and for the nice educational trip she arranged for me to

Ireland, England and Wales. This section will not be complete without mentioning Huug Boer. Huug, thank you for sailing me through lab equipment and procedures, every thing I know about laboratory equipment and procedures from the milling machine to the fibertec, freeze-dryer, autoclave and spectrophotometer, I learned from you. I loved playing volleyball with you in the warriors team and I am sorry to tell you that after you left, the team was relegated to the lower division (jammer Tamme !!!!).

I shall never forget my two great friends, colleagues and project mates, Bart Tas and Harm Smit. Bart, I consider myself fortunate and lucky to have had the opportunity to see you work and learn from you how to handle free ranging 600-kg cows. The first time I saw you handling cows, I became convinced that you were born next to one and I knew by then that I could not have had a better project mate. Thanks for sharing your massive practical experience with me and for sharing the ups and downs of our project. Also thank you for our two-hour camel-riding trip next to the Pyramids in Egypt, which turned out to be one of the most enjoyable and memorable things, I've ever done. Harm, thank you for your openness and friendship and for all the hard work you have done to assure that the grass grows sufficiently and the project runs smoothly. I also want to thank you for the swimming lesson you gave me on the beaches of Cancun.

The lab personnel at the Animal Nutrition Group (ANU), Truus, Meijke, Jane-Martine and Dick Bongers, are highly acknowledged for their professionalism, support and help in sample analyses. Further, the help of the numerous MSc students who participated in the project is highly appreciated. I also wish to thank all the PhD students and staff members of ANU for the laughs, nice moments and memories. Thomas, you deserved the first prize for the playback show, isn't that true Ajay?

A special word of appreciation goes to the spiritual father of ANU, Prof. Dr. ir. Martin Verstegen (dank u wel jongen). I was fortunate to have you as my teacher during my Master degree and as my friend during my PhD degree.

Many thanks also go to Laurens Beerepoot, Nicolaas van Hanja and David Long from Barenbrug company for their interest, discussions and support during the various phases of the project.

Finally, the love and support of my wife (Ula) and family is always treasured, you are always my source of inspiration. Ula, thanks for your patience and tolerance especially when I came back home so many times smelling like a rumen. Without your support I would have never reached where I am today.

PhD Education Plan	**Graduate School WIAS**

Name	Hassan Z. H. Taweel
Group	Animal Nutrition Group
Period	01-09-2000 until 01-09-2004
Supervisor(s)	Seerp Tamminga
Daily advisor	Seerp Tamminga & Jan Dijkstra

The Basic Package (minimum 3-7 cp)	year	cp
❍ WIAS Common Course (mandatory)	2001	2.0
❍ WIAS Course Philosophy of Science and Ethics (mandatory)	2001	1.0
❍ Course on Laboratory Animal Science	2001	3.0
SUBTOTAL		**6.0**

International Exposure (conferences, seminars and presentations, minimum 5 cp)	year	cp
❍ **International Conferences** (minimum 2 cp)		
Annual meeting of the EAAP 2001, Budapest, Hungary	2001	0.8
Annual meeting of the EAAP 2002, Cairo, Egypt	2002	0.8
European grassland federation conference (EGF) 2002, La Rochell, France	2002	0.8
Symposium on the Nutrition of Herbivores 2003, Merida, Mexico	2003	1.2
Annual meeting of ADSA, ASAS and PSA, St. Louis Missouri, USA	2004	1.0
❍ **WIAS Seminars Plus** (minimum 0.5 cp)		
Organic farming, a challenge for animal production systems research	2000	0.3
Potential use of Stable Isotopes related to Studies on Stress and Metabolic Adaptation.	2001	0.3
Fats and Seafood for Health	2003	0.2
❍ **Other Seminars** (minimum 0.5 cp)		
WIAS science day 2001	2001	0.2
WIAS science day 2002	2002	0.2
WIAS science day 2003	2003	0.2
PhD retreat 2003	2003	0.4
WIAS science day 2004	2004	0.2
❍ **Presentations** (minimum 4 original presentations of which at least 1 oral)		
EAAP 2001, Budapest, Hungary, oral presentation	2001	0.5
EGF 2002, La Rochell, France, oral presentation	2002	0.5
EAAP 2002, Cairo, Egypt, oral presentation	2002	0.5
Symposium Herbivorous nutrition 2003, Merida, Mexico, poster presentation	2003	0.5
WIAS science day 2004, oral presentation	2004	0.5
WIAS science day 2004, poster presentation (Awarded best poster prize)	2004	0.5
The 29th dutch speaking animal nutritionist annual meeting, oral presentation	2004	0.5
Annual meeting of ADSA, ASAS, PSA, St. Louis Missouri, USA, 2 oral presentations	2004	1.0
SUBTOTAL		**11.1**

In-Depth Studies (disciplinary and interdisciplinary courses, minimum 4 cp)	year	cp
❍ WIAS Advanced Statisitics Course: Design of Animal Experiments	2000	0.6
❍ WIAS Debating course Biology underpinning Animal Sciences: Broaden your Horizon	2001	0.8
❍ WIAS course 'Stable Isotopes in Studies of Nutrient Dynamics' related to Stress and Metabolic Adaptation	2001	0.6
❍ VLAG Advanced Course on Ecophysiology of the Gastro-Intestinal Tract	2001	1.0
❍ Nutrient Dynamics (modelling classes of the undergraduate course ANU-30304)	2003	2.0
❍ Wild and Domestic Herbivore Diet Characterization, satellite meeting	2003	0.4
❍ Genetic Algorithms applied to Animal Breeding	2003	0.2
SUBTOTAL		**5.6**

Professional Skills (support courses, minimum 2 cp)	year	cp
❍ Wageningen University Language Centre course: English Scientific Writing.	2001	1.2
❍ WIAS Course Techniques for Writing and Presenting a Scientific Paper.	2001	0.8
SUBTOTAL		**2.0**

TOTAL	**24.7**

One credit point (cp) equals a study load of approximately 40 hours

Publications of the author

Full papers and articles

1. Intake regulation and grazing behavior of dairy cows under continuous stocking. 2004. **H. Z. Taweel,** B. M. Tas, J. Dijkstra and S. Tamminga. J. Dairy Science .**87** (in press).

Conference and Symposia proceedings and abstracts

1. Fermentation kinetics of different varieties of perennial ryegrass measured using the cumulative gas production technique. 2001. **H. Z. Taweel** and S. Tamminga. In: the 52nd annual meeting of the European Association for Animal production (EAAP), Budapest, Hungary, 29 August 2001.
2. The effect of genotype and season on rumen fill and clearance in dairy cows fed different varieties of perennial ryegrass. 2002. **H. Z. Taweel**, B. M. Tas, H. J. Smit, J. Dijkstra and S. Tamminga. In: Grassland Science in Europe, Vol. 7, the 19th general meeting of the European Grassland Federation (EGF), La Rochelle, France, 27- 30 May 2002.
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4. A comparison between rumen evacuation and gas production techniques in screening forages. 2002. **H. Z. Taweel**, B. M. Tas, B. A. Williams, J. Dijkstra and S. Tamminga. In: Journal of Animal Science Vol. 80, Suppl. 1/ Journal of Dairy Science Vol. 85, Suppl. 1.
5. Fermentation kinetics of different grazing horizons of perennial ryegrass measured using the automated gas production technique. 2003. **H. Z. Taweel**, B. M. Tas, B. A. Williams, J. Dijkstra and S. Tamminga. In: the 6th International Symposium on the Nutrition of Herbivores, Merida, Mexico, 19 October 2003.
6. Grazing behavior of late lactating dairy cows under continuous stocking. 2004. **H. Z. Taweel,** B. M. Tas, J. Dijkstra and S. Tamminga. In: J. Dairy Science Vol. 87, Suppl. 1.
7. Rumen fill and intake regulation of grazing dairy cows under continuous stocking. 2004. **H. Z. Taweel,** B. M. Tas, J. Dijkstra and S. Tamminga. J. Dairy Science Vol. 87, Suppl. 1.
8. Influence of grass variety on herbage intake and milk production. 2001. H. J. Smit, H. Schlepers, A. Brok, L. Westra, B. M. Tas, **H. Z. Taweel** and A. Elgersma. In: the proceedings of the International Occasional Symposium of the European Grassland Federation, Witzenhausen, Germany, 10- 12 July 2001
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Curriculum Vitae

Hassan Z. H. Taweel was born on the 30th of May 1974 in Jerusalem, Palestine. He attended primary, secondary and high schools in Ramallah and Jerusalem. After passing the scientific stream high school state exam in 1991, he travelled to Jordan where he was enrolled in the Faculty of Agriculture of Jordan University of Science and Technology. In June 1995, he obtained a BSc degree in Animal Science with honours. Shortly after that, he returned back to Palestine and was appointed by the Ministry of Agriculture as an extension agent. In August 1998, he was awarded a full fellowship from The Netherlands Fellowship Programme (NFP) to continue his education at Wageningen University, in The Netherlands. In January 2000, he successfully concluded his studies and obtained an MSc degree in Animal Science (Animal Nutrition) with distinction from Wageningen University. After obtaining his MSc degree from the Netherlands, he returned back to Palestine and was appointed as the director of the animal production department in the National Agricultural Research Centre in Jericho, Palestine. In September 2000, he was appointed by the Animal Nutrition Group of Wageningen University in The Netherlands as a PhD researcher and started his PhD research. After four years of experimentation that included five animal experiments, two *in vitro* gas production experiments and one *in situ* experiment, he fulfilled the requirements of a PhD degree in Animal Nutrition (Ruminants Nutrition) and defended his thesis on the 14th of September 2004.

The research described in this thesis was conducted as a joint effort between the Animal Nutrition Group and the Crop and Weed Ecology Group of Wageningen University in The Netherlands.

The experiments were carried out at the experimental fields of Unifarm (Plant Sciences Department), using cows and animal housing facilities of de Ossekampen (Animal Sciences Group).

This research was sponsored jointly by Barenbrug Holland BV and the Ministry of Economic Affairs (Economische Zaken) in The Netherlands, project BTS 99170, Senter, EZ.

Cover design: Urszula Kudla

Printing: Universal Press