Perennial ryegrass for dairy cows:
intake, milk production and nitrogen utilisation

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Proefschrift

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Propositions

1. Growing conditions and farmers' management have more effect on the nutritive value of grass and grass intake and milk production by dairy cows than grass cultivars do. *(This thesis)*

2. The milk urea N content is a good indicator, but a poor predictor of the individual N excretion in the urine and faeces. *(This thesis)*

3. Attributing the differences in feed intake and milk production solely to the water-soluble carbohydrate content of two grasses, and neglecting differences in cell wall and protein content, has led to a distorted idea of the effect of cultivars with an elevated water soluble carbohydrate content. *(Miller et al., 2001. Grass Forage Sci. 56:383-394)*

4. Trying to explain feed intake behaviour of cattle by excluding some factors such as maximum rumen fill, and integrating all other factors in the "satiety concept", does not increase our understanding of feed intake behaviour. *(Tolkamp et al., 2002. Animal Science 74:369-382)*

5. The future of animal husbandry depends less on the behaviour of citizens as consumers than on their behaviour as tax payers

6. "If you do what you say, you don't lie..." *(Fragment from the television serial Bartje, at the moment Bartje has to take care of his own)*

7. With Turkey, the European Union gets in the Trojan Horse.

Pertaining to the thesis of Bart Tas:

"Perennial ryegrass for dairy cows: intake, milk production and nitrogen utilisation"

Wageningen, 27th April, 2005

Stellingen

1. De groeiomstandigheden en het management van de boer hebben meer invloed op de voederwaarde van het gras en de opname en melkproductie door koeien dan het grasras.
   (Dit proefschrift)

2. Het melk ureum N gehalte is een goede indicator, maar een slechte voorspeller van de N-uitscheiding in de mest en urine.
   (Dit proefschrift)

3. Door verschillen in voeropname en melkproductie enkel toe te schrijven aan het suikergehalte van twee grassen, zonder aandacht te schenken aan verschillen in celwand- en eiwitgehalte, is een verkeerd beeld ontstaan van het effect van grasrassen met een hoog suikergehalte.
   (Miller et al., 2001. Grass Forage Sci. 56:383-394)

4. Het trachten te verklaren van voeropnamegedrag van koeien door een aantal factoren, waaronder maximale pensvulling, uit te sluiten en de overige factoren onder te brengen in het "concept van verzadiging", vergroot niet ons inzicht in het voeropnamegedrag.
   (Tolkamp et al., 2002. Animal Science 74:369-382)

5. De toekomst van de veehouderij hangt minder af van het gedrag van de burger als consument, dan van de burger als belastingbetaler.

6. "Als je doet wat je zegt, dan lieg je niet..."
   (Fragment uit de serie Bartje, wanneer Bartje op eigen benen gaat staan)

7. De Europese Unie haalt met Turkije het Paard van Troje binnen.

Behorende bij het proefschrift van Bart Tas:

"Perennial ryegrass for dairy cows: intake, milk production and nitrogen utilisation"

Wageningen, 27 april 2005

Abstract

Bart M. Tas, 2005. Perennial ryegrass for dairy cows: intake, milk production and N utilisation. Ph.D. Thesis, Wageningen University, The Netherlands,

In the Netherlands, grass is one of the main roughages in the diet of high productive dairy cows. Grass is associated with two main problems: the limited dry matter intake (DMI) and the low N utilisation by dairy cows. Grassland is renovated every 3- 15 years and is usually resown with a mixture of mainly perennial ryegrass cultivars. The aim of the research described in this thesis was to evaluate the effects of perennial ryegrass cultivars on 1) degradation of herbage in the rumen of dairy cows, 2) the DMI, digestibility and milk production by dairy cows, and 3) the N utilisation by dairy cows. These effects were then related to sward characteristics and chemical composition of these cultivars, in order to identify new selection criteria that may be used in grass breeding programmes. Therefore, the effects of eight cultivars in a stall feeding experiment and four cultivars in a grazing experiment on intake, grazing behaviour, digestibility, milk production, milk composition and N utilisation were determined. The perennial ryegrass cultivars consistently differed in their water soluble carbohydrate (WSC) content. Among cultivars, the largest differences were found in WSC content, whereas the differences in crude protein and neutral detergent fibre content were small. An increased WSC content was expected to increase the palatability, and this may increase the intake. However, there was no effect of cultivar on intake in three of the four years of experiments. In one year, the lower intake of two cultivars was associated with a lower WSC content, but also with a severe crown rust infestation. However, the grazing behaviour did not differ among cultivars. The milk production and milk composition were almost not affected by cultivar, with the exception of the year with differences in intake. The cultivars differed only slightly in the degradation characteristics in the rumen. The high crude protein content and the relatively high fractional degradation rate, in comparison with organic matter and neutral detergent fibre, resulted in a high supply of N to rumen microbes. This was expected to be balanced by a higher supply of energy from an increased WSC content. However, the milk urea N content and the N utilisation by dairy cows did almost not differ among cultivars.

Key words: perennial ryegrass, dairy cows, intake, digestibility milk production, nitrogen utilisation.

Preface

The research described in this thesis is the product of collaboration between the Animal Nutrition Group and the Crop and Weed Ecology Group of Wageningen University and the grass breeding company Barenbrug Holland B.V. A research project was developed to evaluate the effects of perennial ryegrass cultivars on intake, digestion, nutrient utilisation and milk production by dairy cows. The experiments were conducted on the experimental farms Unifarm and De Ossekampen of Wageningen University. At the start of the research, three Ph.D. students were appointed, who in close cooperation have conducted the research. Each Ph.D. has focussed on specific parts of the research.

In this thesis, the results are described of the effects of perennial ryegrass cultivars on intake, rumen degradation, milk production and nitrogen utilisation of dairy cows. Experiments were conducted by feeding fresh grass in the barn to dairy cows and by grazing on pasture. In four chapters and in the general discussion different parts of the research are described and discussed.

Bart Tas
March, 2005

Contents

Chapter 1

General Introduction

General Introduction

Grasslands cover approximately 25 % of the world land area, including natural, semi-natural and cultivated grassland (FAO, 2004). In the Netherlands, grassland covers around 30 % of the land area and it is mainly used to feed dairy cows, either by grazing, zero-grazing (summer feeding fresh grass) or cutting grass for silage. A large proportion of this grassland is intensively managed to obtain a high herbage yield per unit land area. The aim is to provide feed with a high nutritive value to ruminants and to produce a high amount of milk and/ or meat per unit of land.

Grassland renovation

In the Netherlands, the mean total grassland in use by dairy farms was around 950.000 ha and on average 120.000 ha was ploughed and sown with grass each year (CBS, 2004). The proportion of grassland that is renovated depends on the soil type. In 1999, the proportion of renovated grassland was 18 %, 10 % and 3 % on sandy, clay or peat soils, respectively, and this equals renovation each 5, 10 and 30 years, respectively (Schils et al., 2002). One of the reasons for resowing, especially on sandy soils, is that grassland is used in ley-arable rotations with other forage crops, mainly maize. The reason for renovation is in general that the performance of the existing sward is lower than the potential performance. The most important criterion for grassland renovation is the sward composition, and renovation is recommended when the proportion of perennial ryegrass (*Lolium perenne* L.) in the sward is below 50 % and /or when the sward contains more than 10 % couch grass (*Elymus repens* L.) (Schils et al., 2002). In general, new grassland is sown with a mixture of grass species, and sometimes white clover, and these mixtures contain a high proportion of perennial ryegrass. Grass breeders have improved grass aiming at a consistent production of herbage during the growing season with a high feeding value. An increase in annual DM yield of perennial ryegrass of 2.5 to 6 % per decade has been made (Wilkins and Humphreys, 2003). Many cultivars of perennial ryegrass have been selected and these cultivars have been tested for agronomic performance. Only candidate cultivars that are distinct, uniform, and stable, and that have sufficient agronomic performance and added value for cultivation, can enter the National list of recommended varieties and are approved to become commercially available. The characteristics that are tested are heading date, persistence, winter hardiness, resistance to crown rust (*Puccinia coronata*), relative DM yield of the first cut and relative annual DM yield (Bonthuis and Donner, 2001). However, the chemical composition and nutritive value of cultivars for dairy cows are not yet included in these cultivar evaluation tests or in breeding programmes. This thesis aims to identify selection criteria for grass breeding programmes, based on the utilisation of grass by lactating dairy cows. The importance of grass for dairy farming in the Netherlands, and current problems associated with grass-fed and grazing dairy cows and associated research questions are outlined below.

Dairy farming in The Netherlands

In The Netherlands, the average farm size in 2001 was 38 ha, of which 34 ha was used as grassland and the other 4 ha were covered with other forage crops, mainly maize. On average, the milk production was more than 12,000 kg/ha and the stocking density was between 1.5 and 1.9 dairy cows/ha (CBS, 2004). To produce grass and grass products, the grasslands are generally well-drained and receive a high amount (250 - 400 kg/ha) of N, from inorganic fertilizer and manure. In 2001, on average the grassland was mown slightly more than twice a year. Around 81 % of the cut herbage was used to make silage, 9 % to make hay and 9 % was fed as fresh grass in the barn. To make silage, the swards were cut at on average about 2800 kg DM/ha, resulting in around 6 ton DM/ha per year. The annual herbage yield was high, with a mean of 10.4 ± 0.7 ton DM/ha per year under cutting and grazing (CBS, 2004). This would indicate that around one-third to half of the annual herbage production is used for grazing and half to two-thirds to make grass silage. The grazing season in The Netherlands is around 165 d/yr and, depending on the stocking density and annual herbage yield. A grazing dairy cow consumes around 9 to 18 kg DM/d. The mean DMI of grass silage during the winter period, is around 15 kg DM/d per cow during 2001 (CBS, 2004).

Table 1 Proportions of concentrate feed and roughage in the diet, and proportions of fresh grass, grass and maize silage in the roughage, in summer and winter in the South-East and North-West in The Netherlands.

Diet composition	summer		winter	
	South-East	North-West	South-East	North-West
Concentrate feed	0.23	0.24	0.41	0.41
Roughage	0.77	0.76	0.59	0.59
fresh grass	0.43	0.75	-	-
grass silage	0.16	0.16	0.49	0.74
maize silage	0.41	0.09	0.51	0.26

Source: CBS, 2004.

The diet of dairy cows consists on average of two-third roughage and one-third concentrate feed. The main roughages are grass, grass silage and maize silage. Due to climatic conditions, soil type and farming styles, there is a regional difference in the ability to grow maize silage and therefore in the South-East a higher proportion of the roughage consists of maize silage than in the North-West of the Netherlands (Table 1). In summer the proportion of roughage is

17-18 % higher than in winter, with no difference between regions. This difference can partly be attributed to the higher energy content of fresh grass (6.6 to 7.2 MJ/kg DM) in comparison with grass silage and maize silage (6.3 MJ/kg DM) (CVB, 2003).

In the Netherlands, the production costs on farms with grazing are lower than on farms with summer feeding (Jager and Van Everdingen, 2004). A higher proportion of grass in the diet of dairy cattle may reduce the use of relatively expensive concentrates and hence reduce the input of minerals to the farm with these concentrates. Moreover, the composition of milk fat is affected by the diet. Grazing dairy cows have higher contents of unsaturated fatty acids - with in particular conjugated linoleic acid- than dairy cows fed grass silage (Kelly et al., 1998; Elgersma et al., 2003). Dairy products are important contributors to the fat consumption in humans, and high contents of saturated fatty acids in these products are associated with increased risks for coronary heart diseases and related health problems (McGuire and McGuire, 1999). Grazing, therefore, could reduce these risks. Furthermore, the health and welfare of dairy cows is of increasing concern by the public. As grazing is the natural behaviour of cows, it is associated with a higher welfare than permanent indoor housing of cows. Moreover, the Dutch landscape is characterised by grassland and grazing dairy cows. Therefore, grazing has become important for the acceptance of intensive dairy farming by society (Van den Pol-Van Dasselaar et al., 2002; LTO, 2004).

However, the general tendency is to reduce the proportion of fresh grass in the diet and to increase the proportion of maize silage and concentrates. Furthermore, the daily time spent grazing is restricted (at around 50 to 60 % of the farms) or cows are permanently housed indoors (at around 10 % of the farms) (Van den Pol-Van Dasselaar et al., 2002, CBS, 2004). Among the many reasons for this decrease in fresh grass feeding and grazing, two main reasons are a more efficient grassland use and a more balanced and constant diet composition for dairy cows. In general, herbage losses are lower under cutting than under grazing, and the application of organic manure by injection into the soil results in a higher N utilisation by the sward than by defecation and urination of grazing dairy cows (Vellinga et al., 2001). A high proportion of fresh grass in the diet, either by stall feeding or grazing, is associated with two main problems. Firstly, the milk production (MP) of grazing dairy cows is lower than potentially possible in comparison to stall-fed dairy cows with nutrient-balanced diets. This is associated with a limited dry matter intake (DMI) of grass, and with an imbalance between absorbed nutrients when the diet contains a high proportion of grass. Secondly, the utilisation of N by grass-fed is low, and grazing dairy cows are associated with N losses, due to the relatively high crude protein content in comparison with the energy content in grass.

Dry matter intake and milk production

The efficiency of conversion of nutrients into milk by dairy cows increases with the level of MP, due to proportionally lower maintenance costs (Tamminga, 1996). Therefore, dairy cows have been continuously selected for this trait and a linear increase in MP of on average 1.25 % per animal per year has been achieved (LEI/CBS, 2004). However, the increase in DMI was less than half the amount needed to cover the increased requirements for MP (Veerkamp, 1998). Dairy cows with a high proportion of forages in their diet (Veerkamp et al., 1994) or grazing intensively managed grassland had a lower MP than potentially possible (Kolver and Muller, 1998; Peyraud et al., 2004). With grazing only, dairy cows can reach a MP with a maximum of 28 to 30 kg/d (Van Vuuren, 1993; Kolver and Muller, 1998). Comparing grazing with a nutrient-balanced diet, a reduced MP of grazing dairy cows was observed, that was attributed for 61 % to a reduced digestible DMI. The remaining part was attributed to the imbalance between absorbed nutrients, of which the metabolizable energy was found to be first limiting (Kolver and Muller, 1998).

The regulation of feed intake is very complex and depends on dietary and animal factors and their interactions (Forbes, 1996). The palatability of dietary components (aversions or preferences) may affect diet selection, the DMI, and nutrient balance (Forbes and Provenza, 2000). Cultivars of perennial ryegrass differ in their resistance to crown rust (Bonthuis and Donner, 2001) and cows seem to avoid severely infected herbage (Kimbeng, 1999). Furthermore, preferences among cultivars of tall fescue (*Festuca arundinacea*) by grazing dairy cows were associated with higher water-soluble carbohydrate (WSC) contents (Mayland et al., 2000). Perennial ryegrass cultivars differ in their WSC content and WSC content is a consistent and heritable trait (Humphreys, 1989). Cultivars with increased WSC contents may, therefore, be preferred to cultivars with lower WSC contents. It is not clear if a higher preference results in a higher DMI when there is no opportunity for selection. Moreover, the sward structure influences the grazing behaviour (e.g. Wade, 1991; Gibb et al., 1999; Orr et al., 2004) and differences among cultivars may influence intake characteristics of grazing animals (Orr et al., 2003).

Besides the effect of palatability, the main factor controlling intake is the post-ingestive feed back (Forbes, 1996; Faverdin, 1999; Forbes and Provenza, 2000) and this is an integration of signals from receptors that respond to distension of the rumen, ruminal pH and osmolality and metabolized nutrients, most likely by additivity of signals (Forbes, 1996). The digestibility of forages and the rumen fill are strongly related with the cell wall content and the lignification of the cell wall (Van Soest, 1994; Mertens, 1994). A perennial ryegrass cultivar with a lower cell wall content and a higher WSC content than a control cultivar resulted in a higher digestible DMI and MP (Miller et al., 2001). Differences among perennial ryegrass cultivars in sward characteristics may affect the digestible DMI and hence MP by grass-fed and grazing dairy cows.

N utilisation by dairy cows

Nitrogen (N) utilisation on intensive dairy farms is low, with an average efficiency of N retention in milk between 12 and 26 % (Castillo et al., 2000). The main processes in the conversions of N and their efficiencies are N fertilisation (organic N from manure and/ or inorganic N from fertilizer) to the soil and uptake by the herbage (N efficiency of 70 %), herbage N into ingested feed N (fresh and ensilaged grass; N efficiency of 85 %), and ingested N into milk N (N efficiency of 20 %) by the dairy cow (Oenema et al., 2000). The most limiting factor in the N utilisation on the farm is the conversion of feed into milk by the dairy cow. Although the theoretical maximum of efficiency of N utilisation may reach 40 to 45 % of N intake (Van Vuuren and Meijs, 1987), the N utilisation of grass-fed or grazing dairy cows is often lower than 25 % (Van Vuuren, 1993; Kolver, 2003). Grasslands receive high amounts of inorganic fertilizer-N and manure-N, and the grass is cut or grazed by cows in a young and leafy stage. Generally, such grass contains a relatively high crude protein (CP = N × 6.25) content that is rapidly degraded in the rumen in comparison with the energy-yielding substrates. This leads to an excess of N in the rumen for microbial protein synthesis, and more than 80 % of this excess-N will be excreted with the urine (Van Vuuren, 1993). Furthermore, grass contains a relatively high intestinal digestible protein (DVE) content in comparison with the content of net energy available for lactation (NEL). When MP is limited by NEL, the absorbed protein in surplus of requirements for maintenance and milk protein synthesis will not be utilised, but excreted in the urine. The high N losses in the rumen and in the intermediary metabolism of grass-fed or grazing dairy cows result in a high N excretion, that has a negative impact on the environment. Nitrogen utilisation by grass-fed dairy cows can be improved by either a decrease of the N fertilisation of grass or harvesting the grass in a later stage of growth and hence decrease the CP content (Van Vuuren, 1993; Peyraud and Astigaragga, 1998; Valk et al., 2000) or by partially replacing grass in the diet by a low-N roughage or a high energy concentrate feed (Bargo et al., 2003). Miller et al. (2001) showed that a perennial ryegrass cultivar with an increased WSC content in comparison with a control resulted in a higher N utilisation by lactating dairy cows, although CP content was low (100 g/kg DM) in that experiment. The efficiency of microbial protein synthesis measured in vitro increased with WSC content of grass (Lee et al., 2002[a]). This may indicate that the energy supplied by WSC reduced the surplus of N in the rumen and hence increased the N utilisation.

Aim of the research and outline of the thesis

The aim of the research described in this thesis was to evaluate the effects of perennial ryegrass cultivars on 1) degradation of the herbage in the rumen of dairy cows, 2) the DMI, digestibility and MP by dairy cows, and 3) the N utilisation by dairy cows. These effects were

then related to sward characteristics and chemical composition of these cultivars, in order to identify new selection criteria that may be used in grass breeding programmes.
Therefore, eight cultivars of perennial ryegrass were mown daily and fed to twelve dairy cows housed in a tie-stall during two summers (2000 and 2001). During the first summer (2000), samples of the herbage were collected and these samples were incubated in the rumen of dairy cows. The results of the degradation characteristics of the eight cultivars during the growing season are described in Chapter 2. The intake, digestibility and MP of twelve dairy cows fed eight perennial ryegrass cultivars are described in Chapter 3, and the N utilisation of the dairy cows of six cultivars in Chapter 4. After two years of stall feeding, four cultivars out of the eight were selected based on contrasting chemical composition, and were sown in the autumn of 2001. In the summers of 2002 and 2003, the DMI, grazing behaviour, MP and N utilisation by twelve grazing dairy cows were determined and the results are described in Chapter 5. In the general discussion, the data of the stall-feeding experiment and the grazing experiment are compiled and the effects of cultivar and growing period on the DMI and MP of dairy cows are discussed. Moreover, the effect of differences between years and within years in N fertilisation level on herbage growth and chemical composition of the herbage are described. Finally, the N metabolism in the grass-fed and grazing dairy cows, and the opportunities to use milk urea N to predict urinary N excretion are discussed.

Chapter 2

Rumen degradation characteristics of perennial ryegrass cultivars during the growing season

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Abstract

The objective of this experiment was to evaluate the effects of sward characteristics and chemical composition of eight diploid perennial ryegrass cultivars during the growing season on degradation characteristics in the rumen of dairy cows. As part of an indoor-feeding experiment with dairy cows conducted in summer 2000, dry matter (DM) yield and morphology of grass plants were measured and grass samples were collected of eight cultivars in seven two-week periods during the summer. The sward characteristics, chemical composition and rumen degradation characteristics were determined and tested for differences among cultivars and periods.

Cultivars tended to differ in DM yield. Very small variation was found among cultivars in neutral detergent fibre (NDF) content and NDF degradation in the rumen. Of the eight cultivars, two had higher water soluble carbohydrate (WSC), intermediate to low crude protein (CP), and lower NDF content, and intermediate to high fractional degradation rates of organic matter (OM), CP and residue (RES = OM – CP - NDF). In both cultivars effectively degraded OM (ED_{OM}) and RES (ED_{RES}) were higher and ratios between effectively degraded N and OM or carbohydrates (CB = NDF + RES) available for microbial growth in the rumen were lower.

DM yield, plant morphology and chemical composition of the grass differed more among periods than among cultivars. Grass in the reproductive stage had a higher NDF content and a lower fractional degradation rate of NDF. A higher CP content in grass resulted in a higher degradation rate and this was associated with a higher effectively degraded CP (ED_{CP}) and ED_{OM} and higher N:OM and N:CB ratios in the rumen. The ratios N:OM and N:CB exceeded the theoretical optimum for microbial growth and this may lead to rumen excess of N, which will be excreted in the urine.

Keywords: perennial ryegrass; cultivar; rumen degradation; nitrogen; water soluble carbohydrate.

Introduction

In Western Europe, grass and grass products are primary forages for dairy cows, because of their high dry matter yield and high nutritive value. The nutritive value is determined by the chemical composition of grass, which may vary considerably and is influenced by many genetic and environmental factors. Among these factors are grass species and variety, climate and growing conditions (e.g. temperature, rainfall and light intensity), soil type, and grassland management (e.g. fertilisation level, harvest date, regrowth stage, and growing days). These factors also determine the degradation characteristics in the rumen of dairy cows (Van Straalen and Tamminga, 1990), which play an important role in the nutritive value of grass. In intensive sytems, grasslands are predominantly pure grass swards and perennial ryegrass (*Lolium perenne* L.) is the most abundant grass species. Fertilisation levels are high (250 - 400 kg N/ha per yr) and grass is cut or grazed frequently at a young and leafy stage during the growing season (Whitehead, 2000).

The young and leafy grass is characterised by a high content of soluble proteins with a high rate and extent of degradation by microbial proteases in the rumen (Tamminga et al., 1990). As their primary source of energy, rumen microbes use carbohydrates that are in fresh grass mainly present as soluble sugars, fructosans and cell wall polysaccharides. Once released from the cells, the water soluble carbohydrate (WSC) fraction (i.e. free sugars and fructosans) is rapidly, almost instantly and completely degraded (Boudon et al., 2002). Cell wall polysaccharides are however degraded at a lower rate and extent, which depends on the organisation and structure of the cell wall (Chesson and Forsberg, 1988). Differences in the protein to carbohydrate ratio in grass, as well as differences in solubility and rate of degradation may cause an imbalance between rumen degradable protein and energy. The excess of rumen degraded protein is absorbed as ammonia through the rumen wall, converted to urea in the liver and excreted with urine (Tamminga, 1996), resulting in a loss of up to 35 % of protein intake (Van Vuuren et al., 1992).

Grass breeding may be a way to balance the supply of protein and energy for microbial synthesis. Within different grass species it has been shown that cultivars of perennial ryegrass differ in their in vitro digestibility (Beerepoot et al., 1994; Wilkins, 1997) and that breeding for digestibility can improve the utilisation by the dairy cow (Vogel and Sleper, 1994). Moreover, cultivars of perennial ryegrass have been selected for an elevated WSC content (Humphreys, 1989), which may lead to less protein losses due to changes in microbial degradation (Miller et al., 2001). Due to differences among different plant parts (e.g. leaf blade, stem, and pseudo-stem) in chemical composition, digestibility (Wilman and Altimimi, 1982), intake and rumen degradation (Poppi et al., 1981), others have focused more on the morphological structure of grass cultivars and its effect on intake and digestibility (Hazard et al., 1998).

More information is needed on differences among cultivars of perennial ryegrass in their rumen degradation to identify potential traits to include in grass breeding and selection programmes. Therefore, this study aimed to evaluate the sward canopy characteristics, the chemical composition and the degradation characteristics of OM, CP, NDF and RES in the rumen of dairy cows of eight perennial ryegrass cultivars, which were sequentially harvested after re-growth periods of three to four weeks during the summer.

Materials and methods

Samples and preparations

Rumen degradation characteristics were determined with nylon bag incubations of eight cultivars (1 to 8) of diploid perennial ryegrass (*Lolium perenne* L.) during spring 2001 in The Netherlands. Grass samples originated from an indoor feeding experiment conducted in summer 2000, in which fresh grass was mown and fed daily to twelve dairy cows. The eight cultivars were selected from the Dutch List of Recommended Varieties (Bonthuis and Donner, 2001), based on intermediate (May 25 to June 1; cultivars 1, 2, 6, 7 and 8) and late (June 7 to 10; cultivars 3, 4, and 5) heading dates. Cultivars further varied in DM yield, persistency, winter hardiness, and crown rust resistance, and cultivar 1 was also chosen because it was bred for an elevated WSC content.

This experiment was divided in two sub-experiments: I and II. In experiment I, cultivars 7 and 8 were mown in each of four periods on June 21 and 22 (period 1), July 19 and 20 (period 3), August 16 and 17 (period 5), and September 13 and 14 (period 7). Cultivars 1 to 6 in experiment II were mown in three periods on July 5 and 6 (period 2), August 2 and 3 (period 4) and August 30 and 31 (period 6). See Table 1 for information about cutting and fertilisation management and growing conditions. The grass was mown between 13.00 and 15.00 h and the DM yield/ha was determined by weighing first the total fresh weight of grass. Then of each cultivar two samples were taken with a grass core (i.d. 3 cm), one sample was oven-dried at 70 °C overnight and the other sample was stored in the freezer at –20 °C. The frozen samples of the two consecutive days were pooled to one sample for each cultivar in each period, and in total 26 grass samples were obtained. Another sample of the grass was taken by hand, grass plants were divided in leaf blade, pseudo-stem, stem, ear and dead plant parts and these parts were dried at 70 °C overnight and different plant parts were expressed as proportion of total DM weight.

The frozen grass samples were freeze-dried and cut with a paper cutter at a length of approx. 1 cm. Nylon bags with an inner size of 14.5 × 8 cm and a pore size of 40 μm (PA 40/30, Nybolt, Switzerland) were filled with approx. 5.3 g DM of these freeze-dried grass samples. During filling of the bags, grass was sampled and later ground (1 mm sieve; Retsch, ZM1), for chemical analysis.

Table 1 Cutting and fertilisation management and weather conditions during the growing periods.

Periods	1	2	3	4	5	6	7
Date	21-22 June	5 - 6 July	19 - 20 July	2 -3 August	16-17 August	30-31 August	13-14 September
Growing days	22/ 23	21/ 22	23/ 24	22/ 23	23/ 24	26/ 27	26/ 27
N fertilizer (kg/ha)	45	45	47	47	40	40	40
In growing period							
Irradiation ($MJ/m^2/d$)	19.6	17.6	13.8	14.5	15.4	15.1	10.7
Temperature (°C/d)	16.8	15.9	14.3	15.6	17.8	17.2	15.7
Precipitation (mm)	84.9	36.9	73.5	94.6	48.0	21.5	20.5

Rumen incubations

Rumen incubations were carried out with twelve rumen cannulated dairy cows and the handlings with the cows were approved by the Experimental Animal Committee of Wageningen University, The Netherlands. The grass samples were incubated in the rumen for 2, 4, 8, 16, 24, 48 and 336 h. Six cows, 168 ± 19 days in milk and producing 28.7 ± 0.3 kg/d, were used for short time incubations (2 to 48 h). Cows were continuously stocked on a pasture predominated by perennial ryegrass, milked twice a day and fed 2 kg DM of concentrate feed after each milking. The other six cows were used for the long time incubations (336 h). These cows were housed indoors and fed a grass-maize silage diet supplemented with concentrate feed.

The all-out method was applied. For incubation times 2, 4 and 8 h one bag, 16 and 24 h two bags and 48 h three bags of each sample were incubated in each cow. After removal from the rumen, bags were immediately placed in ice water for 5 minutes to stop fermentation, and later rinsed with tap water. Then the bags were washed in a washing machine (50 min, 70 L cold water without centrifuging (wool program), including not-incubated bags (0 h) to determine the washable (W) fraction. After washing, bags were frozen at –20 °C, freeze-dried, acclimatised to air and weighed. Residues were pooled within incubation time of each cultivar in each period and pooled residues were ground through a 1 mm sieve (Retsch, ZM1).

Chemical analyses

Grass samples and pooled residues were analysed for dry matter (DM), inorganic matter (ASH), CP (6.25 × N), and NDF. Moreover, grass samples were analysed for acid detergent lignin (ADL) and WSC. DM was determined by drying at a constant weight at 103 °C (ISO 6496), and ASH by combustion at 550 °C (ISO 5984). N was determined with $CuSO_4$ as catalyst (ISO 5983). NDF was analysed according to a modified method of Van Soest et al. (1991) as described by Goelema et al. (1998), and ADL according to Van Soest (1973). WSC was analysed as described by Van Vuuren et al., (1993), but soluble sugars were extracted with water instead of ethanol.

Calculations of degradation characteristics

Degradation characteristics were calculated for organic matter (OM = DM - ASH), CP, NDF and residue (RES = OM – CP – NDF). Organic matter, CP, NDF, and RES were classified in three fractions: a W fraction by the disappearance of material after washing in the washing machine which is assumed to be rapidly degradable; a truly undegradable fraction (U), measured as the asymptote of the degradation curve at infinite incubation time; and a potentially degradable fraction (D = 1 - W - U). The fractional rate of degradation of the D fraction (k_d, in /h) was calculated using a first order model without a lag time as described by Robinson et al. (1986): $Rt = U + D \times e^{(-kd \times t)}$ where Rt is the residue after incubation and t is time of incubation (in h). Data was also fitted with a model including a lag time, but results are not presented because the lag time was small and no improvement in the curve fit was observed. The contents of effectively degraded (ED) OM, CP, NDF and RES in the rumen were calculated as $ED = W + (D \times k_d) / (k_d + k_p)$ as described by Van Vuuren et al. (1990), assuming a fixed fractional passage rate (k_p) of 0.045/h for OM, CP and RES (Tamminga et al., 1994) and 0.02/h for NDF (Van Vuuren et al., 1992). The hourly effective degradation was calculated $ED = W + [(D \times k_d) / (k_d + k_p)] \times [1 - e^{-t \cdot (k_d + k_p)}]$ according to Sinclair et al. (1993). The difference in cumulative amounts degraded at successive hours was regarded as the amount degraded each hour. The ratios N to OM and N to carbohydrates (CB = NDF + RES) (both in g/kg) were calculated for the contents in grass (N:OM and N:CB), the contents washed out ($W_{N:OM}$ and $W_{N:CB}$), and the extent of effective degradation ($ED_{N:OM}$ and $ED_{N:CB}$).

Statistical analyses

The fractional rate of degradation and the U fraction were estimated with the NLIN procedure in SAS 6.12 (SAS, 1989). Due to different growing conditions, sampling plot within paddock and different paddocks between cultivars 1 to 6 and cultivars 7 and 8, period was regarded as an independent variable. Therefore, data from experiments I and II were analysed separately. Both experiments were analysed according to a two-way ANOVA with the GLM procedure, with cultivar and period as independent variables in the model. If the means of cultivars or periods differed significantly ($P < 0.05$) then the all-pairwise comparisons test of Student-Newman-Keuls was used to separate the means. Regression analyses were done with the REG procedure in SAS.

Results and discussion

Cultivars: chemical composition and degradation characteristics

The cultivars means for sward characteristics, chemical composition, and degradation characteristics are presented in Table 2. Cultivars 7 and 8 in experiment I had a very similar DM yield, chemical composition and degradation characteristics. Only U_{CP} differed significantly. In experiment II, DM yield differed more than 300 kg DM/ha among cultivars and there was a tendency (P = 0.06) for a lower DM yield of cultivar 3. The OM content varied only slightly (10 g/kg), but the $k_{d,OM}$ of cultivar 1 was higher (P < 0.05) than of cultivar 6 (0.054 vs. 0.045/h). The mean CP content of cultivars 1 to 6 was relatively low compared to that of cultivars 7 and 8 and also compared to what has been reported by others dealing with similar soil type, N fertilisation level and DM yield (Van Vuuren et al., 1991) and days of re-growth (Valk et al., 1996). The difference between experiment I and II may be caused by a different N supply from the soil between the two paddocks, due to another history in cultivated crops and fertilisation management. The difference among cultivars in CP content was 8.8% of the mean, with cultivar 3 being higher than cultivars 4 and 5. The $k_{d,CP}$ was almost 0.02/h higher than the $k_{d,OM}$ and similarl to what was found for OM, cultivars 1 and 5 had a higher $k_{d,CP}$ than the other cultivars, although for OM the differences were smaller and not significant. The mean NDF content ranged from 400 to 422 g/kg DM and the difference among cultivars was only 5 % of the mean. The mean $k_{d,NDF}$ of 0.025/h was low in comparison with Van Vuuren et al. (1992) and Valk et al. (1996) with similar NDF contents and the difference in $k_{d,NDF}$ among cultivars was only 0.003/h.

The RES was calculated by subtraction and due to either a higher OM (cultivar 1), or a lower CP (cultivar 4) and lower contents of NDF (cultivar 1 and 4), these two cultivars had a higher RES content (P < 0.05). Similar differences (some 40 g/kg DM) were found for the WSC contents among cultivars, which agrees with differences among cultivars of perennial ryegrass as found by Smith et al. (1998) and Miller et al. (2001). The higher RES and WSC contents of cultivars 1 and 4 were associated with a 0.02 to 0.03/h higher $k_{d,RES}$ than the other cultivars, although this difference was not significant.

Periods: chemical composition and degradation characteristics

Means of sward characteristics, chemical composition and degradation characteristics in the different periods of cutting are presented in Table 3. Herbage growth rate depends largely on light intensity (irradiation), temperature and rainfall and their interaction. Normally, the maximum growth rate and yield are reached in July and then decrease (Deinum 1966). The decrease in growth rate with the progression of the season was found in both experiments, but the decrease in herbage yield was compensated by the 3 to 5 days more regrowth in the last

Table 2 Means of cultivars in sward and plant characteristics, chemical composition and degradation characteristics.

	Experiment II						Experiment I	
Cultivars[1]	1	2	3	4	5	6	7	8
Herbage yield (kg DM/ha)	2111	2199	1891	2132	1995	2129	1998	2007
Growth rate (kg DM/ha/d)	90	95	81	91	85	91	84	84
Leaf blades[2]	0.88	0.79	0.89	0.88	0.82	0.76	0.76	0.77
OM (g/kg DM)	890.3^{a}	885.1^{ab}	884.3^{ab}	886.1^{ab}	882.6^{ab}	879.5^{b}	892.4	891.9
W_{OM}	0.100	0.101	0.099	0.112	0.111	0.113	0.113	0.103
U_{OM}	0.172	0.156	0.152	0.154	0.163	0.157	0.154	0.165
$k_{d,OM}$ (/h)	0.054^{a}	0.047^{ab}	0.046^{ab}	0.050^{ab}	0.051^{ab}	0.045^{b}	0.045	0.047
CP (g/kg DM)	165.3^{abc}	165.1^{abc}	172.5^{a}	158.0^{c}	163.3^{bc}	167.1^{ab}	188.7	188.9
W_{CP}	0.069	0.061	0.067	0.067	0.060	0.081	0.089	0.097
U_{CP}	0.181^{a}	0.152^{b}	0.174^{ab}	0.170^{ab}	0.167^{ab}	0.158^{ab}	0.141^{b}	0.151^{a}
$k_{d,CP}$ (/h)	0.073	0.066	0.067	0.067	0.071	0.067	0.067	0.071
NDF (g/kg DM)	400.3^{b}	412.4^{ab}	411.1^{ab}	401.4^{ab}	408.8^{ab}	422.1^{a}	424.5	423.6
ADL (g/kg DM)	16.8	16.4	15.9	17.0	15.2	17.0	18.0	18.3
U_{NDF}	0.144	0.138	0.125	0.137	0.145	0.144	0.142	0.154
$k_{d,NDF}$ (/h)	0.025	0.024	0.025	0.025	0.027	0.025	0.026	0.024
RES (g/kg DM)	324.7^{a}	307.6^{abc}	300.7^{bc}	326.7^{a}	310.5^{ab}	290.3^{c}	279.2	279.4
WSC (g/kg DM)	181.3^{a}	160.3^{b}	152.7^{b}	180.2^{a}	156.6^{b}	140.8^{c}	119.9	120.5
W_{RES}	0.279	0.315	0.303	0.307	0.310	0.305	0.319	0.297
U_{RES}	0.142	0.143	0.150	0.137	0.138	0.139	0.138	0.137
$k_{d,RES}$ (/h)	0.151	0.131	0.117	0.143	0.130	0.120	0.105	0.108

a,b,c,d Means within row and experiment with different superscripts differ significantly ($P < 0.05$).

1 OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADL = acid detergent lignin, RES = residue (OM – CP – NDF), W is washable fraction at t = 0; U is undegradable fraction estimated at t = ∞; k_d is fractional rate of degradation of the potential degradable fraction.

2 DM weight of leaf blades as proportion of total DM weight of the plants.

two periods. Remarkably, the highest yield ($P < 0.05$) and growth rate were measured in period 3, whereas in this period the lowest temperature and one but lowest radiation were measured (Table 1). In experiment I, OM content varied among periods and small but significant differences were observed. The $k_{d,OM}$ was more than 0.01/h higher (*n.s.*) in period 3 than in periods 5 and 7. This may be due to the higher CP and lower NDF content ($P < 0.05$) in period 3 than in the last two periods. Although the CP content differed between periods 1 and 3 ($P < 0.05$), degradation characteristics were similar (except the U_{CP}). The W_{CP} was lower but the U_{CP} was higher in periods 5 and 7 than in periods 1 and 2 and therefore the D fraction was only slightly higher in periods 5 and 7.

The $k_{d,CP}$ was, however, more than 0.02/h lower in the last period than in the first two periods (*n.s.*). The opposite was found for the $k_{d,NDF}$ where in period 1 and 7 the NDF content was almost similar, but in period 1 the $k_{d,NDF}$ was lower and U_{NDF} higher ($P < 0.05$) than in period 7. This was most likely due to the higher proportion of stems and a higher lignification of the NDF in period 1 than in period 7. The ADL content was 10 g/kg DM higher in period 1 than in the other periods ($P < 0.05$) and ADL as fraction of NDF was 57.4 and 35.7 g/kg in periods 1 and 7, respectively. Changes in the rumen degradation characteristics of NDF (k_{dNDF}, U_{NDF}) therefore may result from changes in the leaf blade to stem ratio and from changes in the NDF structure. In general the stems of perennial ryegrass have higher cell wall contents and are less digestible than leaves (Wilman and Altimimi, 1982). In vitro fungal degradation of leaf cell walls was found to be slightly higher than stem cell walls of young ryegrass (Sijtsma and Tan, 1996). Akin (1989) argued that the lower fractional rate of degradation may be caused by the presence of highly lignified tissues, but also by the presence of chemical barriers within cell walls. The lower NDF content in period 3 also resulted in a higher $k_{d,NDF}$ ($P < 0.05$). The RES content and the W_{RES} were lower and the U_{RES} was higher in period 7 than in the other periods, but the $k_{d,RES}$ did not differ significantly among periods. WSC content varied with the season and did not decrease as RES content did in the last period.

In experiment II, CP content was highest in period 2 and lowest in period 6 (Table 3). The fertilisation level was similar in all periods as shown in Table 1, and growing conditions were poorly related to the CP content. With this decrease in CP content, the $k_{d,CP}$ decreased whereas the U_{CP} increased. Most likely due to the lower proportion of leaf blades in period 2, the NDF ($P < 0.05$) and ADL (*n.s.*) contents were higher in this period than in the other periods. As a consequence of the higher CP and NDF content ($P < 0.05$), RES content ($P < 0.05$) was lower in period 2 ($P < 0.05$). Similar as RES content, WSC content increased with the season ($P < 0.05$).

Effect of chemical composition on degradation characteristics

Although OM content varied among the six cultivars in experiment II and among the periods in experiment I ($P < 0.05$), differences in OM content were small. Furthermore, very small differences were found among cultivars and periods in NDF content and as a result no relationships were found between the content and the degradation characteristics. The ADL content was an almost constant proportion (0.288 ± 0.019) of the U_{NDF}, regardless cultivar or period. Moreover, $k_{d,NDF}$ was only slightly ($R^2 = 0.30$) related with $k_{d,OM}$ in our experiment, whereas calculated from their data, Van Vuuren (1992) and Valk et al. (1996) found a much higher relation ($R^2 = 0.93$). In their experiments the average $k_{d,NDF}$ was also only slightly lower (0.0065 - 0.007/h) than $k_{d,OM}$, whereas we have found a more than 0.02/h lower $k_{d,NDF}$ than $k_{d,OM}$.

In experiment II, CP content showed a positive relation with $k_{d,CP}$ ($R^2 = 0.69$) and a negative relation with U_{CP} ($R^2 = 0.71$), whereas there was no relation with W_{CP}. These relationships were mainly found due to the relatively large difference between period 2 and periods 4 and 6 in comparison with the small differences among cultivars. Between cultivar 7 and 8, U_{CP} was also negatively related with the CP content ($R^2 = 0.60$), but there were no relations with W_{CP} and $k_{d,CP}$. Van Vuuren et al. (1991) and Valk et al (1996) found an increase of W_{CP} with an increasing CP content, but these differences were mainly found at higher N levels (400 and 700 kg N/ha per yr and 450 kg N/ha per yr, respectively) than the 300 kg N/ha per yr in our experiment. At lower N levels, Van Vuuren et al. (1991) also did not observe a higher W_{CP}. In agreement with Van Vuuren et al. (1991) and Valk et al. (1996), U_{CP} decreased with an increasing CP content. This was caused by the season and not by cultivar differences, because the only significant difference in U_{CP} was found between cultivar 1 and 2 with a similar CP content. Except the high U_{CP} in period 1, U_{CP} increased with the progression of the season in agreement with Steg et al. (1994). Together with a decrease in CP content with the season, this resulted in an almost constant amount of U_{CP}. The amount of degradable CP was positively related ($R^2 = 0.43$) with $k_{d,CP}$, in agreement with Valk et al. (1996).

In contrast with $k_{d,NDF}$, the $k_{d,CP}$ was positively related ($R^2 = 0.59$) with the $k_{d,OM}$, whereas it was not related with the $k_{d,NDF}$. This indicates that in contrast with Valk et al. (1996), the $k_{d,OM}$ depended in this experiment more on the $k_{d,CP}$ and thus CP content rather than on $k_{d,NDF}$ and NDF content. In the experiment of Valk et al. (1996), part of the determined NDF must have originated from CP based on relatively high neutral detergent insoluble N (NDIN) contents in CP of 350 to 500 g/kg. These high values were most likely caused by oven-drying, where freeze-drying resulted in lower NDIN contents. The freeze-dried samples in our experiment resulted in a lower average $NDIN_{CP}$ (NDIN × 6.25) content of 28.8 ± 5.6 g/kg DM and $NDIN_{CP}$ as proportion of CP in grass of 0.17 – 0.18. The $NDIN_{CP}$ increased with CP content ($R^2 = 0.65$) and was related with the U_{CP} ($R^2 = 0.32$), whereas $NDIN_{CP}$ as proportion of CP did not show a relation with both CP content and U_{CP}. After incubation, samples were also

Table 3 Means of periods in sward and plant characteristics, chemical composition and degradation characteristics.

	Experiment I				Experiment II		
Periods[1]	1	3	5	7	2	4	6
Yield (kg DM/ha)	2102[ab]	2253[a]	1904[bc]	1751[c]	1993	2088	2147
Growth rate (kg DM/ha/d)	93[a]	96[a]	81[a]	66[b]	93[a]	93[a]	81[b]
Leaf blades	0.46[c]	0.78[b]	0.90[a]	0.91[a]	0.71[b]	0.89[a]	0.90[a]
OM (g/kg DM)	911.7[a]	890.5[b]	878.2[c]	888.2[b]	885.8	882.2	886.0
W_{OM}	0.117	0.138	0.094	0.084	0.087[b]	0.112[a]	0.118[a]
U_{OM}	0.187[a]	0.140[c]	0.146[c]	0.166[b]	0.167[a]	0.149[b]	0.160[a]
$k_{d,OM}$ (/h)	0.047	0.054	0.043	0.041	0.052	0.048	0.047
CP (g/kg DM)	181.1[b]	219.0[a]	169.3[c]	185.9[b]	200.7[a]	150.4[b]	144.5[c]
W_{CP}	0.127[a]	0.122[a]	0.052[b]	0.070[b]	0.07.5	0.062	0.066
U_{CP}[2]	0.142[c]	0.110[d]	0.159[b]	0.173[a]	0.139[c]	0.174[b]	0.189[a]
$k_{d,CP}$ (/h)	0.079	0.080	0.062	0.057	0.078[a]	0.064[b]	0.063[b]
NDF (g/kg DM)	442.7[a]	386.9[b]	424.3[a]	442.4[a]	416.9[a]	405.7[b]	405.6[b]
ADL (g/kg DM)	25.4[a]	15.6[b]	15.8[b]	15.8[b]	17.6	15.5	16.1
U_{NDF}	0.189	0.133	0.128	0.142	0.148[a]	0.126[b]	0.143[a]
$k_{d,NDF}$ (/h)	0.021[c]	0.029[a]	0.025[b]	0.025[b]	0.026	0.025	0.024
RES (g/kg DM)	287.9[a]	284.7[a]	284.6[a]	259.9[b]	268.2[b]	326.1[a]	335.9[a]
WSC (g/kg DM)	131.5	115.5	120.7	113.2	120.5[c]	171.5[b]	193.9[a]
W_{RES}	0.331[a]	0.359[a]	0.298[ab]	0.244[b]	0.305	0.303	0.302
U_{RES}	0.126[a]	0.111[a]	0.136[a]	0.177[b]	0.144	0.136	0.144
$k_{d,RES}$ (/h)	0.120	0.107	0.095	0.105	0.112[b]	0.141[a]	0.143[a]

[a,b,c,d] Means within row and experiment with different superscripts differ significantly ($P < 0.05$).

[1] OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADL = acid detergent lignin, RES = residue (OM − CP − NDF), W is washable fraction at t = 0; U is undegradable fraction estimated at t = ∞; k_d is fractional rate of degradation of the potential degradable fraction.

[2] DM weight of leaf blades as proportion of total DM weight of the plants.

freeze-dried and this might be an explanation for the lower fractional rate of degradation of the NDF in comparison with Van Vuuren et al. (1991), Van Vuuren et al. (1992) and Valk et al. (1996). Calculated from data of Valk et al. (1996), $NDIN_{CP}$ was on average 89.9 ± 5.1 g/kg DM and NDF corrected for this fraction equalled 399.1 ± 7.7 g/kg DM, which corresponds well with the freeze-dried NDF we obtained in our experiments. Valk et al. (1996) assumed that NDIN might be degraded at a higher rate than cell walls and thus have increased the average $k_{d,NDF}$. This is in line with the absence of a relation between $k_{d,CP}$ and $k_{d,NDF}$ in our results, whereas Valk et al. (1996) found a high relation ($R^2 = 0.88$).

WSC showed a high linear relationship with RES (WSC = -165.8 + 1.04 × RES; $R^2 = 0.92$). The WSC content as proportion of RES ranged from 0.43 to 0.56, which is higher than the W_{RES} which ranged from 0.24 to 0.38. This indicates that at most 62 % of the WSC was washed out of the bags, which is similar as found by Boudon and Peyraud (2001) who reported that less than 60 % of total soluble carbohydrates disappeared after ingestive mastication. Furthermore, RES and WSC content were positively related with the amount of W_{RES} ($R^2 = 0.44$ and $R^2 = 0.28$). Only 28 % of the variation in the amount of W_{RES} could be explained by the WSC content and this may have been a result from the presence of intact cells in the freeze-dried samples before rumen incubation.

Sample preparation and degradation characteristics

The W_{OM} and W_{CP} fractions were small in our experiment. Sample preparation is an important factor, which influences the W fraction, notably that of CP. Huntington and Givens (1997) showed that freezing grass silage before rumen incubation decreased the soluble fraction in comparison with freshly incubated grass silage. Van Vuuren et al. (1993) and Lopez et al. (1995) reported an increase of the soluble fraction of fresh grass after drying and grinding at 3 mm and 2.5 mm, respectively, in comparison with frozen grass cut at approx. 1 cm with a paper cutter. In our experiment, after freeze drying grass was cut with a paper cutter at a length of approx. 1 cm as was done by Van Vuuren et al. (1991), Steg et al. (1994), and Valk et al. (1996) with fresh grass. The lower washable fractions may be caused, however, by using freeze-dried grass in comparison with frozen and thawed grass. In the freeze dried grass many intact plant cells were present and the soluble part of these intact cells was most likely smaller than in fresh grass. This may also support the lower W_{CP} than W_{OM} due to the lack of loss of CP from intact plant cells.

Table 4 Comparison of means of cultivars in the extent of effective degradation (ED) in the rumen of OM, CP, NDF and RES and the ratio between N and energy (OM and CB) in the grass, in the washable fraction (W) and in the extent of ED.

	Experiment II						Experiment I	
Cultivars[1]	1	2	3	4	5	6	7	8
ED_{OM} (g/kg DM)	442.5^{a}	425.9^{ab}	422.8^{ab}	440.9^{a}	439.2^{a}	420.3^{b}	428.2	423.6
ED_{CP} (g/kg DM)	88.5	88.3	90.5	83.5	88.0	90.0	104.4	105.2
ED_{NDF} (g/kg DM)	189.5	192.1	200.1	193.0	199.8	198.8	203.3^{a}	196.1^{b}
ED_{RES} (g/kg DM)	236.0^{ab}	221.0^{bcd}	209.8^{cd}	238.1^{a}	223.5^{abc}	206.1^{d}	195.5	194.6
N:OM (g/kg)	29.7	29.9	31.2	28.5	29.6	30.4	33.8	33.9
$W_{N:OM}$ (g/kg)	22.1^{ab}	18.7^{ab}	22.6^{ab}	17.9^{ab}	17.2^{b}	23.7^{a}	26.0^{b}	31.5^{a}
$ED_{N:OM}$ (g/kg)	32.1^{ab}	33.2^{a}	34.2^{a}	30.3^{b}	32.0^{ab}	34.3^{a}	38.7	39.5
N:CB (g/kg)	36.7	37.0	39.0	34.9	36.6	37.7	43.1	43.1
$W_{N:CB}$ (g/kg)	21.2^{ab}	17.4^{b}	21.6^{ab}	17.3^{b}	17.8^{b}	24.8^{a}	29.8	35.6
$ED_{N:CB}$ (g/kg)	33.9^{ab}	34.8^{ab}	35.7^{a}	31.3^{b}	33.8^{ab}	36.0^{a}	41.8	43.0

[a,b,c,d] Means within row and experiment with different superscripts differ significantly ($P < 0.05$).

[1] OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADL = acid detergent lignin, RES = residue (OM – CP – NDF), ED = extent of degradation, N:OM, $W_{N:OM}$ $ED_{N:OM}$ = ratio between N and OM in the grass, in the washable (W) fraction and in the extent of degradation, respectively. N:CB, $W_{N:CB}$ $ED_{N:CB}$ = ratio between N and carbohydrates (CB = NDF + RES) in the grass, in the washable (W) fraction and in the extent of degradation, respectively.

Effective degradation in the rumen

The mean contents of effectively degraded OM (ED_{OM}), CP (ED_{CP}), NDF (ED_{NDF}) and RES (ED_{RES}) in the rumen are presented in Table 4 for cultivars and in Table 5 for periods. In experiment I, cultivars differed only slightly, though significantly in ED_{NDF}. In experiment II, the contents of ED_{OM} and ED_{RES} of cultivars 1, 4, and 5 were significantly higher than of cultivar 6. Differences among cultivars in ED_{CP} and ED_{NDF} were small and not significant. In experiment I, ED_{OM} and ED_{CP} contents were highest ($P < 0.05$) in period 3 and lowest ($P < 0.05$) in periods 5 and 7 (Table 5). The content of ED_{NDF} was lower ($P < 0.05$) in period 1 than in periods 5 and 7 with similar NDF contents, due to the lower $k_{d,NDF}$ in period 1. The ED_{RES} was lower in the last period due to a lower RES content and W_{RES} and a higher U_{RES}. In experiment II, the ED_{OM} did not differ among periods and only a small but significant difference in ED_{NDF} was found. The ED_{CP} was around 1.5 times higher in period 2 than in periods 4 and 6, whereas the opposite was found for ED_{RES}.

The mean ED_{OM} contents of 430 ± 4.0 g/kg was similar as found by Steg et al. (1994), but in their experiment grass was harvested in a more mature stage with higher DM yields and higher NDF contents. Van Vuuren et al. (1991) and Valk et al. (1996) had higher ED_{OM} contents due to higher degradation rates. The W_{CP} in the experiments of Van Vuuren et al. (1991) and Valk et al. (1996) were similar as in our experiments, but the $k_{d,CP}$ was higher and as a result the ED_{CP} were on average 0.69 and 0.65 of CP content in the grass, respectively. In our experiments, the average ED_{CP} of CP in grass was less and equalled 0.54. This may indicate that supply of feed CP in the small intestine was higher in our experiment than compared with Van Vuuren et al. (1991) and Valk et al. (1996). However, it might be better explained by the relationship between the determined fractional rates of degradation and the assumed fixed fractional rates of passage.

Table 5 Comparison of means of periods in the extent of effective degradation (ED) in the rumen of OM, CP, NDF and RES and the ratio between N and OM and between N and CB in grass, the washable fraction (W) and the extent of ED.

	Experiment I				Experiment II		
Periods[1]	1	3	5	7	2	4	6
ED_{OM} (g/kg DM)	429.8^{b}	474.7^{a}	407.0^{c}	392.1^{c}	429.5	435.5	430.8
ED_{CP} (g/kg DM)	107.3^{b}	134.1^{a}	86.2^{c}	91.5^{c}	115.2^{a}	76.9^{b}	72.3^{c}
ED_{NDF} (g/kg DM)	184.5^{c}	199.5^{b}	204.1^{ab}	210.7^{a}	199.9^{a}	196.5^{ab}	190.2^{b}
ED_{RES} (g/kg DM)	208.9^{a}	208.4^{a}	194.2^{a}	168.6^{b}	187.0^{b}	237.4^{a}	242.9^{a}
N:OM (g/kg)	31.8^{bc}	39.3^{a}	30.9^{c}	33.5^{b}	36.3^{a}	27.3^{b}	26.1^{c}
$W_{N:OM}$ (g/kg)	35.2^{a}	34.8^{a}	17.3^{c}	27.8^{b}	31.6^{a}	15.0^{b}	14.5^{b}
$ED_{N:OM}$ (g/kg)	40.0^{b}	45.2^{a}	33.9^{c}	37.4^{bc}	42.9^{a}	28.3^{b}	26.9^{b}
N:CB (g/kg)	39.7^{bc}	52.2^{a}	38.2^{c}	42.3^{b}	46.9^{a}	32.9^{b}	31.2^{c}
$W_{N:CB}$ (g/kg)	38.9^{a}	42.2^{a}	16.7^{b}	33.0^{a}	29.9^{a}	15.1^{b}	15.1^{b}
$ED_{N:CB}$ (g/kg)	43.6^{b}	52.6^{a}	34.7^{c}	38.6^{bc}	47.7^{a}	28.4^{b}	26.7^{b}

a,b,c Means within row and experiment with different superscripts differ significantly ($P < 0.05$).

1 OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADL = acid detergent lignin, RES = residue (OM – CP – NDF), ED = extent of degradation, N:OM, $W_{N:OM}$ $ED_{N:OM}$ = ratio between N and OM in the grass, in the washable (W) fraction and in the extent of degradation, respectively. N:CB, $W_{N:CB}$ $ED_{N:CB}$ = ratio between N and carbohydrates (CB = NDF + RES) in the grass, in the washable (W) fraction and in the extent of degradation, respectively.

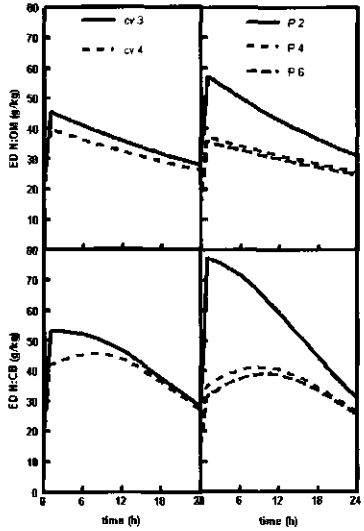

Figure 1 The mean hourly ratio between effectively degraded N and OM and between effectively degraded N and carbohydrates (CB = NDF + RES) of the cultivars 3 and 4 and of the periods 2, 4, and 6.

Ruminal degradability of N and energy

Microbes require nitrogen and energy for their growth, where it was found that optimal microbial growth in the rumen occurred at around 25 g N/kg OM truly digested in the rumen (Czerkawski, 1986) and at 32 g N/kg CB degraded in the rumen (Sinclair et al., 1991). At higher than these optimal levels, the extra N will not be captured in microbial protein but will increase the ammonia pool in the rumen. Ammonia will be absorbed into the blood and after conversion in the liver to urea it will be excreted with the urine. This N excretion can be decreased by partial replacement of grass by low protein/ high energy feeds (e.g. Van Vuuren et al., 1993; Bargo et al., 2003) but another possibility might be by balancing the readily available N by increasing the WSC content in grass (Miller et al., 2001). Miller et al (2001) used therefore two cultivars of perennial ryegrass differing 39 g/kg DM in WSC content and they found a higher proportion of N intake excreted in the milk (0.07) of dairy cows fed the high WSC cultivar compared to the control. This was explained by a higher efficiency of rumen microbial protein synthesis by an improved balance or synchrony of N and energy-yielding substrates. However, Kolver et al. (1998) did not find an effect of synchronisation on N utilisation by dairy cows fed a synchronous and an asynchronous diet. With our data, this asynchrony is illustrated in Figure 1, where the ratio between the hourly ED_N and ED_{OM} and between ED_N and ED_{CB} is shown of cultivars 3 and 4 and of the three periods in experiment II. During the first hours of rumen degradation the largest differences between cultivars and periods were found. The $ED_{N:OM}$ and $ED_{N:CB}$ were lower for cultivar 4 than cultivars 3 and 6 (Table 4) and this was mainly an effect of the lower ($P < 0.05$) CP and higher ($P < 0.05$) RES content of cultivar 4 than of the small differences in degradation rates. In both experiments, the differences in chemical composition and in degradation characteristics were larger among periods than cultivars. In experiment I, the difference in CP content and $k_{d,CP}$ between periods 3 and 5 clearly resulted in a difference in the $ED_{N:OM}$ and $ED_{N:CB}$ (Table 5). A similar result was found in experiment II with an almost double $ED_{N:CB}$ in period 2 in comparison with period 6 (Table 5). The difference in the hourly $ED_{N:CB}$ were even more pronounced (Figure 1). If an increased WSC content among cultivars will lead to a more efficient microbial protein synthesis and a higher N utilisation by dairy cows should be confirmed in a feeding experiment with these cultivars.

Conclusions

The more pronounced differences among periods than among cultivars indicated that weather conditions and grassland management had more effects on chemical composition and rumen degradation than genotype of the cultivar. Differences among cultivars showed that with a reduced CP content and an increased WSC content, the calculated supply of N in excess of energy for microbial protein synthesis can be reduced. Whether there are also consistent differences among cultivars in their nutrient supply to or N utilisation by dairy cows, however, should be tested in feeding trials.

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Chapter 3

Effects of perennial ryegrass cultivars on intake, digestibility, and milk production by dairy cows

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Abstract

The effects of eight diploid perennial ryegrass (*Lolium perenne* L.) cultivars on dry matter intake (DMI), digestibility (*d*) and milk production (MP) of dairy cows were evaluated in the summer of 2000 and 2001. Each summer, herbage was harvested daily and stall-fed to twelve dairy cows during six periods of two weeks each. Six cultivars were fed in three periods (1, 3, and 5) according to a double 3 x 3 Latin square design. In the other periods (2, 4, and 6), two cultivars were fed in a repeated measurement design. Herbage mass and proportion of leaf blades in the sward canopy varied among cultivars, but differences were not consistent between years. The largest differences in herbage composition were found in water soluble carbohydrate (WSC) content, followed by crude protein (CP) content and only small differences were found in the neutral detergent fibre (NDF) content. A higher WSC content was found in two cultivars in both years, whereas ranking of cultivars in CP and NDF content was not consistent with years. There was no effect of cultivar on DMI and MP. In both years the DM digestibility (*d*DM) was high (> 77 %), with very small differences among cultivars in 2000 (< 0.5 %) and larger differences in 2001 (up to 4 %). This was associated with a delayed heading date in 2001 resulting in larger differences in proportion of leaf blades and in NDF content among cultivars.

Keywords: perennial ryegrass, cultivar, intake, milk production

Introduction

In temperate areas of the world, intensively managed grasslands are dominated by perennial ryegrass. This grass has a high DM yield per hectare and provides feed for dairy cows of a high nutritive value at low costs. Because of the limited DMI of grass in high productive dairy cows, the energy requirements for MP of these cows can not be met by grass intake alone. DMI and *d*DM both determine the digestible DMI, which represents the amount of nutrients that can be absorbed and utilised by the dairy cow for MP. DMI and *d*DM are interrelated, where DMI seems to be the most important factor in determining digestible DMI (Waldo, 1986). It is known that digestibility of forages is strongly related with cell wall content (NDF) and its lignification (Van Soest, 1994). The NDF and acid detergent lignin (ADL) are thought to limit digestible DMI due to the physical fill of the gastro intestinal tract (Waldo, 1986), of which the rumen is supposed to be the most limiting compartment. Also, the absorbed nutrients from end products of microbial fermentation in the rumen and digestion in the small intestine may act as metabolic constraints in DMI (Illius and Jessop, 1996). From several trials with grazing cows, Chilibroste (1999) concluded that not one single factor, but a combination of physical and metabolic factors will constraint DMI.

The yield and nutritive value of perennial ryegrass are both determined by genetic factors (grass species and variety) and environmental factors (e.g. climate and weather conditions, soil type, fertilisation level, grazing and cutting management). There are many different varieties of perennial ryegrass and they vary in their sward canopy characteristics. Some authors reported small but consistent differences between grass varieties in in vitro digestibility (Reed and Sutherland, 1994; Beerepoot et al., 1997) and such findings indicate that there is potential for selection on this trait. Others focus more on the morphogenetic traits as leafiness and leaf length to improve productivity of grass (Hazard et al., 1996), but also on the nutritive value and effect on intake with grazing animals (Carrère et al., 2001). Moreover, WSC content of perennial ryegrass cultivars was found to be a consistent and heritable trait (Humphreys et al., 1989) and elevated WSC content increased the in vitro *d*DM (Radojevic et al., 1994) and efficiency of microbial protein synthesis (MPS) in vitro (Lee et al., 2002[a]) and resulted in a higher digestible DMI and MP by dairy cows (Miller et al., 2001). The focus of grass breeding has been mainly on sward and plant characteristics, but it is recognised that maximising DMI and optimising rumen function should be included in future breeding programmes (Beever and Reynolds, 1994).

The aim of this paper was therefore to evaluate if cultivars of diploid perennial ryegrass under similar management and growing conditions differ in their sward canopy characteristics and nutritive value and if the intake, digestibility and milk production of dairy cows were affected by cultivar. If significant and consistent differences were found among cultivars, there might be a potential for grass breeders not only to include sward canopy characteristics, but also its nutritive value and effects on dairy cow's production in their breeding and selection schemes of new cultivars.

Materials and methods

Herbage

In autumn 1999, eight diploid perennial ryegrass (*Lolium perenne* L.) cultivars (cultivars 1 to 8) were sown on three adjacent paddocks with a clay soil. In summer 2000 and 2001 two feeding experiments were conducted, in which grass cultivars were daily cut and fed to twelve high productive Holstein-Friesian dairy cows. The individual DMI, apparent digestibility of the diet (*d*), and MP were measured. An adaptation period of two weeks preceded the experiments, which lasted twelve weeks divided in six periods of two weeks. In 2000, the experiment was conducted from June 28 until September 15 and in 2001 from July 7 until September 20. Cultivars 1, 2, 3, 4, 5, and 6 were fed in periods 1, 3, and 5, whereas cultivar 7 and 8 were fed in periods 2, 4, and 6.

The eight cultivars were selected from the Dutch List of Recommended Varieties (Bonthuis and Donner, 2001). Cultivars 1, 2, 6, 7 and 8 were chosen from the intermediate heading date class and heading dates range from May 25 to June 1, whereas cultivars 3, 4, and 5 were chosen from the late heading date class and heading dates range from June 7 to 10. These cultivars further varied in herbage yield, persistency, winter hardiness, and crown rust resistance. Cultivar 1 was also chosen because it was selected for an elevated WSC content. Moreover, cultivars 7 and 8 were selected, because cultivar 7 showed a 2 % higher in vitro digestibility than cultivar 8 (Beerepoot et al., 1997), and this will be compared with the in vivo digestibility measured in the experiments.

Cultivars 1 to 6 were sown on paddock A previously cultivated with grass seed. This paddock was divided in 21 blocks and each block was divided in six strips of 2 m wide. Within each block, the six cultivars were assigned at random to a strip. Cultivars 7 and 8 were sown on paddock B, previously cultivated with barley. This paddock was divided in 19 blocks and each block was divided in two strips of 6 m wide. At random cultivar 7 and 8 were assigned to one of the two strips within a block. On the third paddock (C) cultivars 1 to 6 and cultivars 7 and 8 were sown in similar designs as on paddocks A and B, respectively.

Animals

Each year, twelve Holstein-Friesian dairy cows were used with approval of the Experimental Animals Committee of Wageningen University. In 2000 all cows were multiparous, ranging from their second to tenth lactation and an expected 305 days fat and protein corrected milk production (FPCM) of 8379 ± 250 kg/ 305 d. At the start of the experiment cows were 118 ±

11 DIM and BW was 587 ± 12 kg. In 2001 six heifers and six multiparous cows in their second and third lactation were used. The heifers had an expected FPCM of 7776 ± 185 kg/305 d, were 140 ± 16 DIM and BW was 522 ± 14 kg. The average expected FPCM of the multiparous cows was 9053 ± 607 kg/ 305 d, cows were 115 ± 17 DIM and BW was 571 ± 17 kg at the start of the experiment.

Herbage cutting and sampling

The number of re-growth days of the grass (20 to 30 days) was adjusted to obtain a herbage mass of approximately 2,000 kg DM/ha. The grass was mown daily between 13.00 and 15.00 h at 5 cm above ground level and the herbage mass (kg DM/ha) was determined. One sample of the herbage was taken by hand for analyses of morphology of the sward canopy by determining percentages of leaf, stem, pseudo-stem and dead material based on DM weight. Another herbage sample was taken with a grass core (i.d. = 3 cm), and oven-dried at 70 °C to determine DM content. Half of the amount of grass to be fed was stored in the barn at room temperature, whereas the other half was stored in a cooling unit at 4 °C overnight until the feeding the next morning.

Table 1 Weather conditions during the regrowth periods, the total days of regrowth, and N fertilisation per cut in the six periods and the mean of periods 1, 3 and 5 (cultivars 1 to 6) and periods 2, 4, and 6 (cultivars 7 and 8) in 2000 and 2001.

periods	1	2	3	4	5	6	1, 3, and 5	2, 4, and 6
In 2000								
Irradiation (MJ/m^2/d)	18.6	15.1	15.4	16.0	16.0	12.5	16.7	14.5
Temperature (°C/d)	16.3	14.4	15.8	17.9	17.2	16.0	16.4	16.1
Total rainfall (mm)	63	85	72	51	21	16	52	51
Regrowth days (d)	22	24	23	24	27	27	24	25
N fertilisation (kg N/ha)	48	48	41	40	38	40	42	43
In 2001								
Irradiation (MJ/m^2/d)	20.1	17.2	17.8	16.9	15.6	12.0	17.8	15.4
Mean temperature (°C/d)	18.1	18.0	18.5	18.5	18.0	15.3	18.2	17.3
Total rainfall (mm)	12	58	18	12	3	24	11	31
Regrowth days (d)	29	27	19	24	21	26	23	26
N fertilisation (kg N/ha)	100	53	55	76	81	59	79	63

Animal Housing, Feeding and Sample Collection

Cows were housed in a tie stall, where temperature was maintained between 15 °C and 20 °C. Drinking water was always available. Cows received ad libitum fresh grass. Each day, from 15.00 h onwards the grass stored in the barn at room temperature was fed. At 06.00 h the next morning, orts were removed and the grass stored in the cooling unit was fed. In addition a concentrate feed (4.1 kg DM/d in 2000 and 2.5 kg DM/d in 2001) was fed. The OM, CP, NDF and ADL content of concentrate feed in 2000 were 941.1, 154.5, 230.8, and 20.3 g/kg DM, respectively, and in 2001 these contents were 941.6, 135.1, 259.1, and 20.9 g/kg DM, respectively. Orts were collected daily and DM content was determined. Individual DMI was calculated by subtracting the DM weight of orts from the DM weight of grass fed to the cows. During milking, concentrate feed was fed in two equal portions. Cows were milked twice a day at 6.00 h and 16.00 h, and individual MP was recorded (in kg/d). Daily individual milk samples were taken, in which fat and protein contents were determined. The FPCM was calculated as (0.337 + 0.116 × fat % + 0.06 × protein %) × MP. Cows were weighed once every period. Individual apparent digestibility of DM, OM, CP, and NDF of the diet was determined by quantitative collection of the faeces of each cow on days 11, 12, 13 and 14 of each period. In 2000, each day faeces was collected in two periods of 3 h and in 2001 each day faeces was collected during one period of 6 h. In both years, faeces was collected for in total 24 h and covering one full day. The total amount of faeces excreted by each cow was weighed and a sample of 3 % in 2000 and 5 % in 2001 of fresh weight was taken, added to a pooled sample per cow per period and stored at –20 °C.

Sample Handling and Chemical Analysis

The oven-dried grass samples of day 10, 11, 12, and 13 in each period of each cultivar were ground through a 1 mm sieve. These samples were pooled based on equal DM weight and analysed for DM, inorganic matter (ASH), CP (6.25 × N), NDF, ADL, and WSC. The frozen faeces samples were thawed overnight and in the fresh faeces the contents of DM and CP were determined. Subsequently, sub samples were taken, which were freeze dried, ground through a 1 mm sieve and analysed for DM, ASH, NDF, and ADL content.
DM was determined by drying at a constant weight at 103 °C (ISO 6496), and ASH by combustion at 550 °C (ISO 5984). N was determined following ISO 5983 with $CuSO_4$ as catalyst. NDF was analysed according to a modified method of Van Soest et al., 1991 described by Goelema et al. (1998) and ADL was analysed according to Van Soest (1973). WSC was analysed by determination of the content of reducing sugars (Van Vuuren et al., 1993), but water instead of ethanol was used for extraction. Milk fat and protein content were determined (ISO 9622) at the Melkcontrolestation, Zutphen, The Netherlands.

Experimental Design and Statistical Analyses

Herbage mass and morphology of the sward canopy were analysed using data from day 6 to 13 in each period. Chemical composition of the grass was determined in a pooled sample of days 10 to 13 in each period. The DMI and MP per cow in each period were calculated as the mean of day 6 to day 13 in each period. Digestibility was calculated based on days 10 to 13, when faeces was collected and these days corresponded with the sampling days of the herbage. Data from herbage mass and sward canopy were analysed with cultivar and period as independent variables and days within period as repeated measurements. These data of cultivars 1, 2, and 3, of cultivars 4, 5, and 6 and of cultivars 7 and 8 were analyzed separately, in agreement with animal data. The twelve cows were divided in two groups of six cows, based on parity, and within each group cows were ranked on pre-experimental FPCM. Cows from different groups with equal ranking numbers were assigned to a pair of cows. At random, one of the two cows of each pair was assigned to cultivar 7 and the other to cultivar 8. The experimental design was a stratified block design with repeated measurements with cultivar and period as independent variables. Cultivars 1 to 6 were analysed according to two Latin squares (LS), where in LS I cultivars 1, 2 and 3 and in LS II cultivars 4, 5, and 6 were fed in three periods to six cows. The independent variables in each Latin square were cultivar, period and cow. All data was analysed using the GLM procedure of SAS 6.12 (SAS, 1989) and the Tukey test was used to test for all-pair-wise comparisons among means.

Results and Discussion

Sward Canopy

Weather conditions varied within and between years as shown in Table 1. June and July in 2001 were relatively warm and the rainfall was low, and this decreased the herbage growth rate. Therefore N fertilisation and/ or regrowth days were increased in these periods. The nitrogen fertilisation level per cut was in 2001 higher (20 to 40 kg N/cut) than in 2000 and differed in 2001 between the two paddocks.

The sward canopy and the chemical composition of the eight cultivars in 2000 and in 2001 are presented in Table 2. In 2000, herbage mass varied slightly among the cultivars and ranged from 1993 to 2238 kg DM/ha. Herbage mass of cultivar 8 was higher ($P < 0.05$) than of cultivar 7. The mean herbage mass was slightly higher in 2001, with a similar range but not ranking among cultivars. In 2001, herbage mass of cultivar 4 was higher ($P < 0.05$) than of cultivar 6. In contrast with 2000, cultivar 7 and 8 had a very similar herbage mass in 2001. The relative differences among cultivars in herbage mass were similar as in the Dutch List of recommended varieties (Bonthuis and Donner, 2001), although ranking of cultivars was not

Table 2 Means (± standard error (SE)) of herbage mass (HM) and percentage of leaf blades in the sward canopy and the chemical composition of cultivars in 2000 and 2001.

	Latin square I				Latin square II				Block		
Cultivars	1	2	3	SE	4	5	6	SE	7	8	SE
In 2000											
HM (kg DM/ha)	2173	2149	1993	*78*	2185	2099	2159	*81*	2103[b]	2238[a]	*34*
Leaf blades (%)	82	78	86	*1.3*	88	81	80	*1.5*	81	82	*0.7*
OM (g/kg DM)	889.6[a]	887.4[a]	881.0[b]	*1.6*	884.5	882.5	878.8	*1.9*	880.3	875.9	*1.5*
CP (g/kg DM)	160.2[ab]	157.3[b]	166.2[a]	*1.6*	150.4[b]	156.4[a]	158.6[a]	*1.3*	197.8[a]	195.0[b]	*0.1*
WSC (g/kg DM)	192.3[a]	170.1[b]	158.1[b]	*4.1*	195.3[a]	171.6[b]	151.7[c]	*3.2*	105.9	101.9	*1.1*
NDF (g/kg DM)	399.0[b]	423.4[a]	414.3[a]	*2.6*	400.4	412.2	428.7	*5.8*	428.0	429.5	*3.4*
ADL (g/kg DM)	16.7	16.5	16.9	*0.7*	15.3	17.2	18.2	*0.7*	16.4	16.8	*0.4*
In 2001											
HM (kg DM/ha)	2277	2295	2403	*66*	2540[a]	2384[ab]	2231[b]	*72*	2122	2142	*57*
Leaf blades (%)	85[a]	81[a]	73[b]	*1.8*	75[b]	75[b]	83[a]	*1.7*	83[b]	87[a]	*0.9*
OM (g/kg DM)	891.8	886.5	887.3	*2.4*	886.7	886.9	883.7	*1.9*	885.3	885.7	*0.6*
CP (g/kg DM)	204.3	197.5	194.2	*5.3*	190.0	193.4	203.0	*7.1*	193.9[a]	187.5[b]	*0.6*
WSC (g/kg DM)	130.7[a]	109.8[b]	92.5[c]	*3.9*	112.8	99.8	98.4	*4.3*	105.0	116.7	*3.0*
NDF (g/kg DM)	420.0	438.5	462.5	*14*	456.8	453.1	437.4	*13*	439.9	432.5	*6.5*
ADL (g/kg DM)	22.1	23.0	24.1	*1.6*	21.3	25.0	21.4	*1.3*	22.3	19.9	*0.8*

[a,b,c] Means of cultivars within a row and within Latin square or Block with different superscripts differ significantly ($P < 0.05$).

similar as to that in the list. The higher herbage mass in the second year may have been caused by a combination of a one-year older sward with a higher tiller density (data not shown) and a 100 kg/yr higher N fertilisation.

In both years cultivars varied in their percentage of leaf blades. In 2000, cultivar 3 tended to have a higher ($P = 0.07$) percentage of leaf blades than cultivar 2, but in 2001 cultivar 3 had the lowest ($P < 0.05$) percentage of leaf blades. A similar contrast between years was found in LS II: cultivar 4 had a higher ($P < 0.05$) percentage of leaf blades in 2000, whereas in 2001 cultivar 6 had a higher ($P < 0.05$) percentage of leaf blades than the other cultivars. The lower percentage of leaf blades in 2001 than in 2000 and the different ranking of cultivars may be associated with grassland management between the experiments. In winter and spring sheep continuously grazed the paddocks, and this may have affected grass growth and retarded the heading dates of the –according to the List of Recommended Varieties- earlier heading date cultivars.

Grass Composition

In Table 2 the chemical composition of the cultivars in 2000 and in 2001 is presented. In 2000, the six cultivars differed slightly ($P < 0.04$) in their CP content, had a relatively high WSC content and larger differences among cultivars (more than 40 g/kg DM) were found in WSC content than in CP content. Two cultivars (1 and 4) had a higher ($P < 0.01$) WSC content and a slightly lower NDF content (LS I: $P < 0.01$ and LS II: $P = 0.06$) than the other cultivars. Chemical composition of cultivars 7 and 8 was very similar, with a small difference ($P < 0.05$) in CP content. The relatively high CP content of cultivars 7 and 8 compared with the other six cultivars was associated with a lower WSC content. The ADL content was low and did not differ significantly among cultivars. In 2001, CP content varied slightly ($P > 0.05$) among the cultivars in both Latin squares. In line with the observations in 2000, cultivar 7 had a slightly higher ($P < 0.05$) CP content than cultivar 8. WSC content ranged from 92.5 to 130.7 g/kg DM with a 20 to almost 40 g/kg DM higher WSC content of cultivar 1 than cultivars 2 and 3. In LS II, the difference in WSC content between cultivar 4 and cultivars 5 and 6 was less than in 2000 and also not significant ($P = 0.13$). Cultivar differences in NDF and ADL content were relatively small and not significant.

In 2000, the mean N fertilisation per cut was similar (Table 1), but the CP content of cultivar 7 and 8 was higher than of the six cultivars. A possible explanation may be a different N supply from the soil between the two paddocks due to different crops and fertilisation management in years prior to the experiments. The higher CP content of the six cultivars in 2001 than in 2000 was most likely associated with the higher N fertilisation level in 2001 (79 kg N/cut) compared with 2000 (42 kg N/cut). In contrast, CP content of cultivars 7 and 8 was almost similar in both years, whereas in 2001 the mean fertilization level was 20 kg N/cut higher than in 2000. The higher mean CP content in 2001 was associated with a lower mean WSC content, in agreement with the known inverse relationship between CP and WSC content (e.g. Wilman and Wright, 1978; Valk et al., 2000). This inverse relation was, however, observed between years and also between paddocks in 2000, but was absent within cultivars in agreement with Radojevic et al. (1994). The mean NDF content was slightly increased in 2001 in comparison with 2000, which was associated with the relatively high percentage of stems in the sward canopy in the first two periods in 2001 as mentioned before.

This was also shown in an increased ADL/NDF ratio which was on average 50.6 and 40.2 g ADL/kg NDF in 2000.

In both years the differences among cultivars in chemical composition were relatively largest for WSC content, intermediate for CP content, and smallest for NDF content. Moreover, cultivars 1 and 4 had in both years the highest WSC content and cultivars 3 and 6 the lowest. A similar ranking of cultivars for WSC content between years led Humphreys et al. (1989) conclude that WSC content was a consistent and heritable trait.

Table 3 Mean (± standard error (SE)) diet and herbage dry matter intake (DMI), and apparent digestibility (*d*) by dairy cows of eight cultivars in 2000 and 2001.

	Latin square I				Latin square II				Block		
Cultivars	1	2	3	SE	4	5	6	SE	7	8	SE
In 2000											
Diet DMI (kg/d)	20.2	21.5	21.4	*0.4*	20.1	20.9	20.7	*0.4*	20.5	20.6	*0.3*
Herbage DMI (kg/d)	16.2	17.4	17.4	*0.4*	16.1	16.8	16.6	*0.4*	16.4	16.6	*0.3*
Diet DMI ($kg/BW^{0.75}/d$)[1]	167.8	177.4	177.3	*3.1*	165.4	171.9	169.3	*2.0*	171.8	163.6	3.4
*d*DM (%)	83.7	83.5	83.7	*0.9*	82.3	82.2	82.0	*0.8*	83.0	82.3	*0.7*
*d*OM (%)	85.2	85.0	85.4	*0.8*	84.1	83.9	83.8	*0.7*	84.8	84.4	*0.6*
*d*NDF (%)	86.6	86.0	87.3	*0.8*	86.1	85.7	86.4	*0.8*	88.1	87.7	*0.5*
*d*CP (%)	77.7	78.7	78.9	*1.3*	75.2	76.0	76.2	*0.9*	80.6	79.4	*0.8*
In 2001											
Diet DMI (kg/d)	17.1	17.3	16.8	*0.3*	16.1	16.5	17.1	*0.40*	17.2	16.7	*0.2*
Herbage DMI (kg/d)	14.7	14.9	14.4	*0.3*	13.7	14.0	14.7	*0.40*	14.8	14.2	*0.2*
Diet DMI ($kg/BW^{0.75}/d$)[1]	152.4	153.5	149.0	*1.9*	148.0	152.4	157.1	*3.01*	155.3	148.8	1.7
*d*DM (%)	80.9^{a}	79.2^{ab}	77.3^{b}	*1.0*	80.4	77.3	79.6	*1.0*	80.3	80.7	*0.6*
*d*OM (%)	82.9^{a}	80.7^{ab}	78.6^{b}	*0.9*	81.6	78.7	81.2	*0.9*	82.2	82.4	*0.6*
*d*NDF (%)	85.7^{a}	83.9^{ab}	80.6^{b}	*0.9*	84.3^{a}	80.4^{b}	84.2^{a}	*0.9*	86.1	85.8	*0.5*
*d*CP (%)	79.5	77.8	76.4	*1.1*	78.1	76.3	78.5	*1.0*	77.5	77.2	*0.7*

[a,b] Means of cultivars within a row and Latin square or block with different superscripts differ significantly ($P < 0.05$).

[1] BW = body weight.

Dry Matter Intake and Digestibility

The intake and digestibility of the cultivars in 2000 and in 2001 are presented in Table 3. In 2000 the mean DMI of herbage was 16.6 ± 0.2 kg/d with additionally 4.1 kg/d of concentrate feed. There were only small (up to 1.2 kg DM/d) and non-significant differences ($P > 0.05$) among the cultivars. Cultivars 1 and 4, with the higher WSC content, had the lowest DMI ($P > 0.05$). The apparent digestibilities of DM, OM and NDF were high (above 80 %) for all diets and assuming that the digestibility of the concentrate part of the diet was not affected by cultivar, the cultivars varied only slightly ($P > 0.05$) in their digestibility. In 2001, the herbage DMI was 14.4 ± 0.2 kg/d and cows were supplemented 2.5 kg DM/d of concentrate feed. Differences among cultivars in herbage DMI were small, within 1 kg DM/d, and not significant. The apparent digestibility of the diet was high (above 77 %) and cultivars differed up to 6 % in average *d*NDF. The lower mean *d*NDF of diets with cultivars 3 and 5 ($P < 0.03$) were related with the lower percentage of leaf blades and higher NDF and ADL contents in the first period as the *d*NDF was lower in this period. This effect was not found for the diet with cultivar 4, with a similar percentage of leaf blades and NDF content as the diets containing cultivars 3 and 5 in the first period. The lignification of the cell walls therefore may be an explanation as the ADL/NDF ratio of cultivars 1, 2, 4, and 6 was approximately 54 g/kg but this ratio was higher for cultivars 3 and 5 with 60 and 64 g/kg, respectively. The 2 % higher in vitro digestibility of cultivar 7 in comparison with cultivar 8, as found by Beerepoot et al. (1997), was not found in vivo in our experiments.

The regulation of intake by the animal has been the topic of many experiments, and the general concept is that intake is under an integrated physical and metabolic control, depending on diet characteristics and physiological state of the animal (Forbes, 1996). Physical control depends on the fill capacity of the gastro intestinal tract, which is in general found to be inversely related with NDF content of forages (i.e. grass and legumes) (Waldo, 1986; Mertens, 1994). In both years, DMI was negatively and weakly related with NDF content, most likely due to the small differences in NDF content among cultivars. Moreover, NDF of the total diet was highly digestible (> 80 %) for all cultivars. Therefore, cultivars with a higher DMI resulted in a higher NDF intake and thus physical fill of the gastro intestinal tract did not seem to limit DMI among cultivars within an experiment. Moreover, no differences were found among the cultivars in the rate and extent of NDF degradation in the rumen (Taweel, 2004), as this might affect the DMI (Forbes, 1996). The high digestibilities and small differences in *d*DM among cultivars did not affect DMI in agreement with Valk et al. (2000). Miller et al. (2001) found a higher digestible DMI ($P < 0.05$) of a cultivar with a high WSC content in comparison with a cultivar with a normal WSC content, which was explained by the difference in WSC content and digestible fiber. Our results suggested an opposite effect: cultivars 1 and 4 with the higher WSC contents had the lowest DMI in 2000 and cultivar 4 also in 2001. Grass differing in WSC content did not influence DMI with indoor-feeding (Valk et al., 2000) and with rotational grazing (Orr et al., 1997). Increased WSC contents in grass therefore do not seem to increase the daily DMI. The small differences in CP content were not related with differences in DMI and this is in agreement with Van Vuuren et al. (1992) and Valk et al. (2000) with grass that had larger differences in CP content.

Table 4 Mean (± standard error (SE)) milk production (MP), fat and protein corrected milk production (FPCM), milk fat and protein yields and contents and the feed conversion (FC) by dairy cows of eight cultivars in 2000 and 2001.

	Latin square I				Latin square II				Block		
Cultivars	1	2	3	SE	4	5	6	SE	7	8	SE
In 2000											
MP (kg/d)	26.9	28.1	26.3	*1.20*	26.8	27.9	28.2	*0.86*	27.9	26.3	*0.76*
FPCM (kg/d)	27.0^{ab}	27.8^{a}	26.0^{b}	*1.16*	26.6	27.3	27.5	*0.79*	27.6	27.1	*0.70*
Fat yield (g/d)	1072^{ab}	1098^{a}	1008^{b}	*50.9*	1053	1063	1072	*30.4*	1067	1120	*31.9*
Protein yield (g/d)	917	940	899	*37.9*	887	931	930	*21.6*	970^{a}	873^{b}	*22.9*
Fat (g/kg)	40.5	39.2	38.3	*1.06*	39.7	38.5	37.9	*0.90*	38.6^{b}	42.7^{a}	*0.88*
Protein (g/kg)	34.3	33.7	34.1	*0.43*	33.4	33.8	32.9	*0.45*	35.0^{a}	33.3^{b}	*0.36*
FC (kg DMI/kg MP)	0.77	0.79	0.83	*0.02*	0.76	0.77	0.73	*0.02*	0.74	0.80	*0.03*
In 2001											
MP (kg/d)	24.7	24.8	23.8	*0.79*	22.5	23.3	23.9	*1.10*	22.7	23.2	*0.62*
FPCM (kg/d)	23.7	23.7	23.5	*0.75*	21.9	23.0	23.4	*0.90*	22.6	23.0	*0.56*
Fat yield (g/d)	921	931	950	*34.8*	878	936	940	*32.7*	916	931	*23.4*
Protein yield (g/d)	780	765	739	*18.3*	683	718	744	*28.9*	718	730	*16.0*
Fat (g/kg)	37.4^{b}	37.5^{b}	39.9^{a}	*0.66*	39.7	40.5	39.8	*0.88*	40.5	40.5	*0.54*
Protein (g/kg)	32.0^{a}	31.1^{b}	31.3^{b}	*0.57*	30.5	31.0	31.5	*0.43*	31.7	31.8	*0.35*
FC (kg DMI/kg MP)	0.71	0.71	0.72	*0.01*	0.73	0.72	0.74	*0.01*	0.77	0.74	*0.02*

a,b,c Means of cultivars within a row and Latin square or block with different superscripts differ significantly ($P < 0.05$).

Milk Production and Milk Composition

The mean milk production and its composition of the cultivars in 2000 and in 2001 are presented in Table 4. In 2000, cultivars showed small (less than 2 kg/d) and non-significant differences in MP. The FPCM and milk fat yield were, however, higher for cultivar 2 than for cultivar 3. In 2001, MP and FPCM among cultivars varied ($P > 0.05$) only slightly (less than 1.5 kg/d). In LS I, small differences in milk fat ($P = 0.03$) and protein content ($P = 0.02$) among the three cultivars were found. Comparing both years, cows produced the lowest amount of milk on cultivars 3 and 4 and the highest amount on cultivars 2 and 6. Differences between cultivars 7 and 8 in MP and its composition were small and results were inconsistent when both years were compared.

The digestible DMI and the chemical composition of the diet determine both the amount and type of nutrients available for milk production. A positive linear relationship between DMI and MP within years ($R^2 = 0.44$ in 2000 and $R^2 = 0.40$ in 2001) was found. The conversion of the diet into milk varied ($P > 0.05$) among cultivars, up to 6 % in 2000, but in both years no significant cultivar effect was present.

The composition of the diet may have an effect on the yield and content of milk fat and protein. The main end product of NDF fermentation in the rumen is acetate, which is a precursor of milk fat synthesis. The milk fat yields differed only slightly, most likely due to small differences in NDF content and NDF intakes. In general, dietary effects on milk protein content are much smaller than effects on milk fat content (DePeters and Cant, 1992). The milk protein yield may be stimulated by a higher propionate production by rumen fermentation, a higher amount of glucose absorption in the small intestine, or a higher amino acids absorption in the small intestine. Increasing the WSC content in the diet may increase the proportion of propionate and butyrate and reduce acetate and reduce the non-glucogenic to glucogenic ratio (NGR) in the rumen (Lee et al., 2002[b]). In our experiments, the NGR was lower ($P = 0.04$) of cultivar 1 than cultivars 2 and 3 in 2000, but this effect was absent in 2001 with a similar difference in WSC content. Moreover, in both years cultivar 4 with also a higher WSC content did not show a lower NGR than cultivars 5 and 6 (Taweel, 2004). Amounts of glucose absorbed from the small intestine are likely small, since all WSC are assumed to be degraded in the rumen and the starch content of the total diet is small. Amino acids in the small intestine can originate from RUP from the feed and from rumen microbial protein. Differences among cultivars in RUP were small (Tas et al., unpubl.) and therefore do not seem to explain differences among cultivars in milk protein. A more efficient MPS may increase the amount of microbial protein synthesized in the rumen and thus the amount that flows to the small intestine, where the microbial protein will be digested and absorbed and thus provide more amino acids available for milk protein synthesis. The MPS depends on the N and energy supply for the microbes in the rumen. Within grass, an increased WSC content is believed to supply readily available energy next to the readily degradable CP and this is believed to increase the efficiency of MPS in the rumen. However, Dijkstra et al. (1998) argued that in diets with a sufficient CP content, an improved balance between degradable energy and N is unlikely to increase the efficiency of MPS. According to Miller et al. (2001), the assumed increased efficiency could explain the differences found in MP between cultivars differing (39 g/kg DM) in WSC content, but Lee et al. (2002[b]) did not show a more efficient MPS with even larger differences in WSC content (83 g/kg DM) between cultivars. Although the WSC content of cultivar 1 and 4 was higher than the other cultivars, the digestibilities did not differ, indicating that there was not a higher MPS because of elevated levels of degradable carbohydrates in the rumen. Moreover, in our experiments the DMI and the efficiency of MPS may have had a confounding effect on the amount of microbial protein synthesized and this may have reduced the effect of the differences in chemical composition of the cultivars on MP and milk fat and protein yields and contents.

Conclusions and Implications

In both years, herbage mass, percentage of leaf blades and the chemical composition varied slightly among the cultivars. The largest differences among cultivars in herbage composition were found in WSC content, followed by CP content and only small differences were found in the NDF content. A higher WSC content was found in two cultivars in both years, whereas ranking of cultivars in CP and NDF content was not consistent with years. The DMI and *d*DM by the dairy cows was not affected by the cultivar that was fed. Digestibility of NDF in 2001 was lower for two cultivars with a delayed heading date and lower percentage of leaf blades and higher NDF and ADL contents. There was no effect of cultivar found on the MP and only small differences among cultivars were found on the milk fat and protein content and yield. Based on these results, it may be concluded that the eight cultivars used in these two experiments do not provide grass breeders with encouraging evidence to include selection criteria for an increase in DMI, digestibility, and MP in their grass breeding schemes.

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Chapter 4

Utilisation of N in perennial ryegrass cultivars by stall-fed lactating dairy cows

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Abstract

In the summers of 2000 and 2001, the effect of six diploid perennial ryegrass (*Lolium perenne* L.) cultivars (cultivars 1 to 6) on the N utilisation by twelve high productive dairy cows was determined. Both experiments were conducted according to a double 3 x 3 Latin square design, within each Latin square three cultivars were fed to six cows during three periods of two weeks each. Two cultivars showed in both years a higher water soluble carbohydrate (WSC) content than the other cultivars. Differences in crude protein (CP) and neutral detergent fibre (NDF) content among cultivars were smaller than in WSC content and not consistent with years. In both years dry matter intake (DMI) and milk production (MP) varied only slightly among cultivars and no differences among cultivars were found in the excretion of N in milk (in g/d and as % of N intake). Furthermore, in both experiments two cultivars had a lower N intake and this was associated with a lower milk urea N (MUN) content and urinary N excretion. Strong linear relationships were found between MUN and urinary N excretion with N intake, and with N content in grass, whereas only poor relationships with WSC content were found. These strong relationships were, however, more associated with differences among periods and cows than with cultivar differences.

Keywords: perennial ryegrass, cultivar, nitrogen, water soluble carbohydrate, milk urea N.

Introduction

Nitrogen (N) utilisation on intensive dairy farms is low, with an average efficiency of N retention in milk between 12 and 26 % (Castillo et al., 2000). In temperate areas of the world, high quality forages are the cheaper components in dairy diets. Perennial ryegrass (*Lolium perenne* L.) is among the main forages used in intensive dairy farming. In these intensive systems, grass is highly fertilised and offered to the (grazing) dairy cow in a young and leafy stage. Generally, such grass contains a high N content that is rapidly degraded in the rumen. This leads to an excess of N in the rumen (Van Vuuren, 1993), which will mainly be excreted with the urine and this results in substantial N losses to the environment. Urinary N is predominantly present as urea, which is easily converted to ammonia and to a lesser extent to nitrous oxide (N_2O), both of which contribute to air pollution. When converted to nitrate, it contributes to water pollution through leaching to rivers and ground water resources (Castillo et al., 2000). In many countries, dairy farming is the main source of this N pollution (Tamminga, 1992). Moreover, N is one of the more expensive feed ingredients. Increasing the N utilisation of grass, therefore, results in reduced feed costs and increased profits and reduced N losses to the environment.

Nitrogen utilisation by grass-fed dairy cows can be improved by either a decrease of the N fertilisation of grass (Van Vuuren, 1993; Peyraud and Astigaragga, 1998; Valk et al., 2000) or by partially replacing grass in the diet by a low-N roughage or high energy concentrate feed (Bargo et al., 2003). The main aim of such measures is to reduce N intake (NI) and to balance the rumen degradation of N and carbohydrates, without adversely affecting dry matter intake (DMI), digestibility, and milk production (MP). Another possible way may be to include the optimisation of rumen degradation in forage breeding programmes (Beever and Reynolds, 1994).

In temperate areas, intensively managed grasslands are often monocultures of perennial ryegrass. The last decade, efforts have been made to breed ryegrass cultivars with an increased water soluble carbohydrate (WSC) content. These efforts aimed to decrease the asynchronous supply of nitrogen and energy in the rumen for microbial protein synthesis (Humphreys et al., 1989). Cultivars with an elevated WSC content have been shown to increase the efficiency of microbial protein synthesis in vitro (Lee et al., 2002[a]), but this effect could not be demonstrated in vivo with steers (Lee et al., 2002[b]). Feeding grass with an elevated WSC content increased weight gain of lambs (Lee et al., 2001), and increased MP and N retention in milk of late lactating dairy cows (Miller et al., 2001).

The aim of the two experiments described in this paper was to evaluate the effect of six diploid perennial ryegrass cultivars on the N utilisation of lactating dairy cows.

Materials and methods

Experimental design

Twelve lactating dairy cows were stall fed with fresh grass of six perennial ryegrass cultivars in 2000 from June 24th till September 1st and in 2001 from June 29th till September 7th. Both experiments were conducted as a double 3 x 3 Latin square design. In each Latin square, three cultivars were fed to six dairy cows during three periods. Prior to the start and in between the three periods, cows were fed during two weeks fresh grass from two other cultivars of perennial ryegrass.

Animals

In both years, twelve Holstein-Friesian dairy cows were used with the approval of the Experimental Animal Committee of Wageningen University. In 2000, cows were multiparous ranging from their second to tenth lactation, were in mid-lactation with 118 ± 11 days in milk (DIM) and had a body weight (BW) of 587 ± 12 kg at the start of the experiment. The MP prior to the experiment was 31.3 ± 1.2 kg/d. In 2001, six heifers and six multiparous cows in their second and third lactation were used. Heifers and multiparous cows were in mid-lactation with 140 ± 16 and 115 ± 17 DIM, respectively. Heifers were smaller (522 ± 12 kg BW) and produced less milk (23.5 ± 0.9 kg/d) than the multiparous cows which had a BW of 571 ± 17 kg and produced 28.4 ± 1.2 kg/d. Cows were housed in a tie-stall with rubber mats and temperature in the stall was maintained at 15-20 °C with cooling fans.

Diets, feeding and milking

Six cultivars of perennial ryegrass (cultivars 1 to 6) were sown in autumn 1999 on paddock with a clay soil. Cultivars 1, 2, and 6 had intermediate heading dates (May 25 - 28) and cultivars 3, 4 and 5 had late heading dates (June 7 – 10). Cultivar 1 was bred for an elevated WSC content. All cultivars received a similar management and the annual fertilisation was 300 kg N/ha in 2000 and 400 kg N/ha 2001. The N applied for the three consecutive periods was 48, 41, and 38 kg N/ha per cut in 2000 and 100, 55 and 81 kg N/ha per cut in 2001. In both years, regrowth period of the herbage was approximately 24 days, aiming at 2,000 kg DM/ha at cutting. Grass was cut daily between 13.00 and 15.00 h, weighed and sampled. Small containers were filled with 10 to 12 kg of fresh grass, weighed and stored either in the barn for feeding in the afternoon and evening or in a cooling unit at 4 °C for feeding the next morning. Grass was fed ad libitum and in addition a concentrate feed was fed at 4.1 kg DM/d in 2000 and 2.5 kg DM/d in 2001 in two equal portions at milking. Ingredients and composition of the concentrate feed are shown in Table 1. Before the afternoon milking, feed residues of each cow were collected, weighed and sampled. Cows were milked twice a day at

6.00 h and 16.00 h, MP of each cow was recorded and two individual milk samples were taken from the afternoon and consecutive morning (1:1). Drinking water and salt lick blocks were available at all times. Cows were weighed once every period.

Table 1 Ingredients and chemical composition of concentrate feed.

Ingredients		Chemical composition	
Beet pulp (%)	21.0	DM (g/kg fresh)	883.0
Maize (%)	21.0	Ash (g/kg DM)	58.9
Barley (%)	21.0	CP (g/kg DM)	154.5
Soya hulls (%)	11.5	WSC (g/kg DM)	113.6
Coconut peels (%)	10.0	NDF (g/kg DM)	230.8
Beet molasses (%)	8.0	ADF (g/kg DM)	141.4
Argentinean Soya (%)	7.5	ADL (g/kg DM)	20.3
		Fat (g/kg DM)[1]	27.0
		Starch (g/kg DM)[1]	261.0
		Resistant starch (g/kg DM)[1]	74.0
		VEM (unit/kg DM)[2]	1050
		DVE (g/kg DM)[3]	96
		OEB (g/kg DM)[4]	-16

[1] Calculated by feed manufacturer based on ingredient composition

[2] VEM is feed unit lactation, which contains 6.9 kJ net energy for lactation.

[3] DVE is the sum of digestible feed and microbial true protein available in the small intestine corrected for endogenous protein losses.

[4] OEB is the degradable protein balance in the rumen reflecting the difference between the potential microbial protein synthesis based on degraded feed crude protein and that based on energy available for microbial fermentation.

Sample collection, handling and chemical analyses

Grass samples were taken daily with a grass core (i.d. 3 cm), oven-dried at 70 °C and ground through a 1 mm sieve (Peppink, hammer mill). A pooled sample was made of each cultivar from days 10, 11, 12 and 13 of each period. This pooled grass sample was analysed for DM, Ash, N, WSC, neutral detergent fibre (NDF), and acid detergent lignin (ADL). On days 10, 11, 12 and 13 of each period, faeces was collected quantitatively from each cow during eight times of three h in 2000 and four times of six h in 2001, covering one complete day. After each collection time, faeces was weighed, a sample of a fixed percentage was taken and added to a pooled sample per cow per period and stored in a freezer at –20 °C. The pooled faeces samples were thawed overnight and in the fresh faeces the DM and CP content were determined. Sub-samples of the faeces were taken and these sub-samples were freeze-dried, ground through a 1 mm sieve (Retsch ZM100) and analysed for DM, Ash, NDF, and ADL. One of the two individual milk samples was analysed for fat, protein, and lactose content (ISO 9622) and the other was analysed for milk urea N (MUN) content with the pH difference method (ISO 14637) (Melkcontrolestation, Zutphen, The Netherlands).

DM was determined at 103 °C (ISO 6496) and Ash by combustion at 540 °C (ISO 5984). N was analysed according to ISO 5983 with $CuSO_4$ as catalyst. NDF was analysed according to a modified method of Van Soest et al. (1991) as described by Goelema et al. (1998), and ADL was analysed according to Van Soest (1973). WSC was analysed by determining the reducing sugars as described by Van Vuuren et al. (1993), but WSC was extracted with water instead of 40 % ethanol.

Calculations and statistical analyses

The DMI was calculated as the difference between grass offered and grass residue. Apparent digestibilities of OM, NDF, and CP were calculated by (intake-faeces)/ intake (all in kg/d). The urinary N excretion was calculated as the difference between NI and N excretion in faeces and in milk (N = CP / 6.38), assuming a zero balance of N retained in the body. Fat and protein corrected milk (FPCM) was calculated as $(0.337 + 0.116 \times \text{fat \%} + 0.06 \times \text{protein \%}) \times \text{MP}$.

Data from both years (2000 and 2001) were analysed separately, because there were different growing conditions, the sward age and sward management differed, and 12 different cows were used. The double 3 × 3 Latin square designs in each year were also analysed separately. In each year, data were analysed of cultivars 1, 2, and 3 in the first Latin square (LS I) and data of cultivars 4, 5, and 6 in the second Latin square (LS II). The GLM procedure of SAS 6.12 (SAS, 1989) was used for the statistical analyses. The chemical composition of the herbage was analysed with a two-factor main effects model with cultivar and period as classification variables. The animal data was analysed with a three-factor main effects model with cultivar, period and cow as classification variables. When a significant effect of cultivar was found, the Tukey test was used to test for all pair-wise comparisons among the least square means. The regression analyses were done within years, using the REG procedure of SAS 6.12.

Table 2 Chemical composition (in g/kg DM) of six perennial ryegrass cultivars in 2000 and 2001.

	Latin square I					Latin square II				
Cultivar	1	2	3	*SE*[1]	*P*[1]	4	5	6	*SE*[1]	*P*[1]
2000										
OM	889.6[a]	887.4[a]	881.0[b]	*1.6*	*0.04*	884.5	882.5	878.8	*1.9*	*0.22*
CP	160.2[ab]	157.3[b]	166.2[a]	*1.6*	*0.04*	150.4[b]	156.4[ab]	158.6[a]	*1.3*	*0.03*
WSC	192.3[a]	170.1[b]	158.1[b]	*4.1*	*0.01*	195.3[a]	171.6[b]	151.7[c]	*3.2*	*0.01*
NDF	399.0[b]	423.4[a]	414.3[a]	*2.6*	*0.01*	400.4	412.2	428.7	*5.8*	*0.06*
ADL	16.7	16.5	16.9	*0.7*	*0.95*	15.3	17.2	18.2	*0.7*	*0.08*
2001										
OM	891.8	886.5	887.3	*2.5*	*0.35*	886.7	886.9	883.7	*1.9*	*0.47*
CP	204.3	197.5	194.2	*5.3*	*0.47*	190.0	193.4	203.0	*7.1*	*0.47*
WSC	130.7[a]	109.8[b]	92.5[b]	*3.9*	*0.01*	112.8	99.8	98.4	*4.3*	*0.13*
NDF	420.0	438.5	462.5	*14.0*	*0.22*	456.8	453.1	437.4	*13.1*	*0.58*
ADL	22.1	23.0	24.1	*1.6*	*0.67*	21.3	25.0	21.4	*1.3*	*0.20*

[a,b,c] Means within a row and Latin Square with different superscripts differ significantly ($P < 0.05$).

[1] SE = standard error of the mean, P = Probability.

Results and discussion

Chemical composition of grass

The chemical composition of the six cultivars is shown in Table 2. In 2000, differences among cultivars were found in CP and WSC content. The differences between extremes in WSC content were higher, both in absolute (more than 30 g/kg DM) and relative (more than 20 %) terms than differences between extremes of CP (8 g/kg DM and 6 %) and NDF (25 g/kg DM and 6 %) contents. Cultivar 1 had a higher ($P = 0.01$) WSC content, an intermediate CP content and lower NDF ($P = 0.01$) content than cultivars 2 and 3. Cultivar 4 had a higher ($P = 0.01$) WSC content, a slightly lower ($P = 0.06$) NDF content and a lower ($P = 0.03$) CP content than cultivars 5 and 6. In 2001, WSC content of cultivar 1 and cultivar 3 differed ($P = 0.01$) almost 40 g/kg DM in absolute and 35 % in relative terms. The WSC content of cultivar 4 was only slightly (approx. 13 g/kg) and not significantly ($P = 0.13$) higher than cultivars 5 and 6. In 2001, cultivars showed similar differences in CP and NDF content as in 2000, although these were not significant.

The higher fertilisation level of 36 kg N/ha per cut in 2001 than in 2000 was most likely the main reason for the higher CP and lower WSC contents in 2001 than in 2000. It is well known that an increase in CP content is accompanied by an up to similar decrease in WSC content (e.g. Wilman and Wright, 1978; Valk et al., 2000). However, in the present experiment the decrease in WSC content (66 g/kg DM) was more than 1.5 times the increase in CP content (39 g/kg DM). Moreover, cell wall content and composition (NDF and ADL) are almost not affected by N fertiliser level, but more by stage of maturity of the grass (Peyraud and Astigaragga, 1998). Although days of regrowth and DM yield were similar in both years, the heading date of grass seemed to be delayed in 2001 and this resulted in a higher proportion of stems in the sward canopy (data not presented) and higher NDF and ADL contents in the first period. As a result, the mean NDF content was higher in 2001 than in 2000. Humphreys et al. (1989) showed that WSC content is a consistent and heritable trait and that there are opportunities to breed cultivars for an increased WSC content. In agreement, cultivars 1 and 4 consistently had a higher WSC content in our experiments, although in 2001 WSC content of cultivar 4 was not significantly higher than cultivars 5 and 6. The differences in CP and NDF contents among cultivars were relatively small and the ranking was not consistent between years.

Table 3 Dry matter intake (DMI) of diet and herbage, and apparent digestibilities (*d*) of OM and NDF of the diet of six cultivars of perennial ryegrass in 2000 and 2001.

	Latin square I					Latin square II				
Cultivar	1	2	3	*SE*[1]	*P*[1]	4	5	6	*SE*[1]	*P*[1]
2000										
DMI (kg/d)	20.2	21.5	21.4	*0.4*	*0.11*	20.1	20.9	20.7	*0.3*	*0.18*
Herbage DMI (kg/d)	16.2	17.4	17.4	*0.4*	*0.09*	16.1	16.8	16.6	*0.3*	*0.19*
*d*OM (%)	85.2	85.0	85.4	*1.0*	*0.98*	84.1	83.9	83.8	*1.6*	*0.98*
*d*NDF (%)	86.6	86.0	87.3	*1.0*	*0.81*	86.1	85.7	86.0	*1.4*	*0.96*
2001										
DMI (kg/d)	17.1	17.3	16.8	*0.2*	*0.29*	16.1	16.5	17.1	*0.4*	*0.20*
Herbage DMI (kg/d)	14.7	14.9	14.4	*0.2*	*0.31*	13.7	14.0	14.7	*0.4*	*0.18*
*d*OM (%)	82.9[a]	80.7[ab]	78.6[b]	*0.9*	*0.03*	81.6	78.7	81.2	*0.9*	*0.10*
*d*NDF (%)	85.7[a]	83.9[a]	80.6[b]	*0.9*	*0.01*	84.3[a]	80.4[b]	84.2[a]	*0.9*	*0.02*

[a,b,c] Means within a row and Latin square with different superscripts differ significantly ($P < 0.05$).

[1] SE = standard error of the mean, P = Probability.

Dry matter intake and digestibility

The diet DMI, herbage DMI and apparent digestibility of OM (*d*OM) and NDF (*d*NDF) of the diets are shown in Table 3. In 2000, cows consumed 0.6 to 1.2 kg DM/d less herbage from the cultivars with the higher WSC content (1 and 4) than from the other cultivars ($P = 0.11$ in LS I and $P = 0.18$ in LS II). The *d*OM and *d*NDF were high (> 82 %) for all cultivars and this was associated with the relatively low cell wall contents (NDF and ADL) in grass. Only small differences in NDF and ADL content among cultivars were found and this was related with non-significant differences among cultivars in *d*OM and *d*NDF. In 2001, DMI varied slightly among cultivars, within 1 kg/d, and differences were again not significant. Two cultivars with a higher WSC content (cultivars 1 and 4) showed an intermediate (cultivar 1) or the lowest (cultivar 4) DMI. The *d*OM and *d*NDF were lower ($P = 0.02$) of cultivars 3 and 5 than the other cultivars and this was mainly related to the higher NDF content in the first period. In this period, the late-heading cultivars 3 and 5 did not have a leafy sward with more than 30 % of the canopy consisting of stems and as a result the NDF content was higher (data not presented). The digestibility of these cultivars was lower and this reduced the mean digestibility (Table 3).

In recent years, three experiments with dairy cows fed perennial ryegrass indoors were published (Van Vuuren et al., 1992; Peyraud et al., 1997; Valk et al., 2000). The results showed that reducing N fertilisation from high to moderate levels decreased the CP content of the grass, increased its WSC content and slightly affected NDF content, but intake and digestibility by dairy cows were almost not affected. The difference between the two years in DMI can be attributed to the different groups of cows and the slightly different chemical composition of grass. The mean digestibilities of OM, NDF and CP in both years were high and slightly higher than reported by Van Vuuren et al. (1992) and Valk et al. (2000).In agreement with these authors the digestibility was not associated with DMI.

Milk production and composition

Milk production differences among cultivars were small, within 2 kg/d, and not significant (Table 4). In LS I, cows produced more FPCM and milk fat ($P \leq 0.05$) when fed cultivar 2 than cultivar 3 in 2000. Differences among cultivars in 2001 were small, with the only significant differences among cultivars 1 to 3 in the milk protein and fat contents. The differences - though n.s.- in MP among cultivars were consistent between years, in that cultivars 2 and 6 gave the highest and cultivars 3 and 4 the lowest MP. The DMI and digestibility together determine the amount of nutrients that can be utilised by a dairy cow for MP. Because in both years only small differences in intake and digestibility were found among cultivars, no differences in MP were to be expected.

Table 4 Milk production (MP), fat and protein corrected milk (FPCM), and milk composition of six cultivars of perennial ryegrass in 2000 and 2001.

	Latin square I					*Latin square II*				
Cultivar	1	2	3	*SE*[1]	*P*[1]	4	5	6	*SE*[1]	*P*[1]
2000										
MP (kg/d)	26.9	28.1	26.3	*0.5*	*0.08*	26.8	27.9	28.3	*0.58*	*0.26*
FPCM (kg/d)	27.0[a,b]	27.8[a]	26.0[b]	*0.4*	*0.05*	26.6	27.3	27.5	*0.42*	*0.31*
Fat (g/kg)	40.5	39.2	38.3	*0.7*	*0.17*	39.7	38.5	37.9	*0.56*	*0.12*
Protein (g/kg)	34.3	33.7	34.1	*0.3*	*0.46*	33.3	33.8	32.9	*0.30*	*0.18*
Fat (g/d)	1072[a,b]	1098[a]	1008[b]	*22.8*	*0.04*	1053	1063	1072	*13.1*	*0.61*
Protein (g/d)	917	940	899	*20.1*	*0.38*	887	931	930	*17.1*	*0.17*
MUN[2] (mg/dL)	8.8	9.4	9.6	*0.4*	*0.26*	7.3[b]	8.8[a]	8.7[a]	*0.22*	*0.01*
2001										
MP (kg/d)	24.6	24.7	23.8	*0.5*	*0.31*	22.5	23.3	23.9	*0.6*	*0.23*
FPCM (kg/d)	23.7	23.7	23.5	*0.4*	*0.88*	21.8	23.0	23.4	*0.5*	*0.09*
Fat (g/kg)	37.4[b]	37.5[b]	39.9[a]	*0.6*	*0.03*	39.7	40.5	39.8	*0.8*	*0.74*
Protein (g/kg)	32.0[a]	31.0[b]	31.3[b]	*0.2*	*0.02*	30.5	31.0	31.5	*0.3*	*0.14*
Fat (g/d)	921	931	949	*15.6*	*0.46*	878	936	940	*16.4*	*0.05*
Protein (g/d)	780	765	739	*15.3*	*0.24*	683	718	744	*18.9*	*0.13*
MUN[2] (mg/dL)	17.4	17.4	16.4	*0.6*	*0.41*	16.3	16.7	17.2	*0.2*	*0.06*

[a,b,c] Means within a row and Latin square with different superscripts differ significantly ($P < 0.05$).

[1] SE = standard error of the mean, P = Probability.

[2] MUN = Milk urea N.

Nitrogen utilisation

Nitrogen intake (NI) and excretion of N in milk, faeces and urine (in g/d and as % of NI) in both years are presented in Table 5. In 2000, the NI differed among cultivars with lower ($P \leq 0.05$) NI's of cultivars 1 and 4, which was caused by a combination of an intermediate to low CP content and a lower DMI (though n.s.) (Table 4). The amount of N excreted with the faeces and with the milk showed, however, very small differences among cultivars. The lower NI of cultivars 1 and 4 was related to a numerically lower (n.s; $P = 0.12$ and $P = 0.42$, respectively) urinary N excretion of around 30 g N/d in comparison with the other cultivars. In contrast with 2000, in 2001 cultivar 1 had a slightly higher ($P = 0.13$) NI than cultivars 2 and 3, and this resulted in a slightly higher amount ($P = 0.03$) of N excreted with the urine. The more than 60 g/d lower ($P = 0.03$) NI of cultivar 4 than of cultivar 6 in 2001 tended ($P = 0.051$) to a lower urinary N excretion of cultivar 4 than cultivar 6.

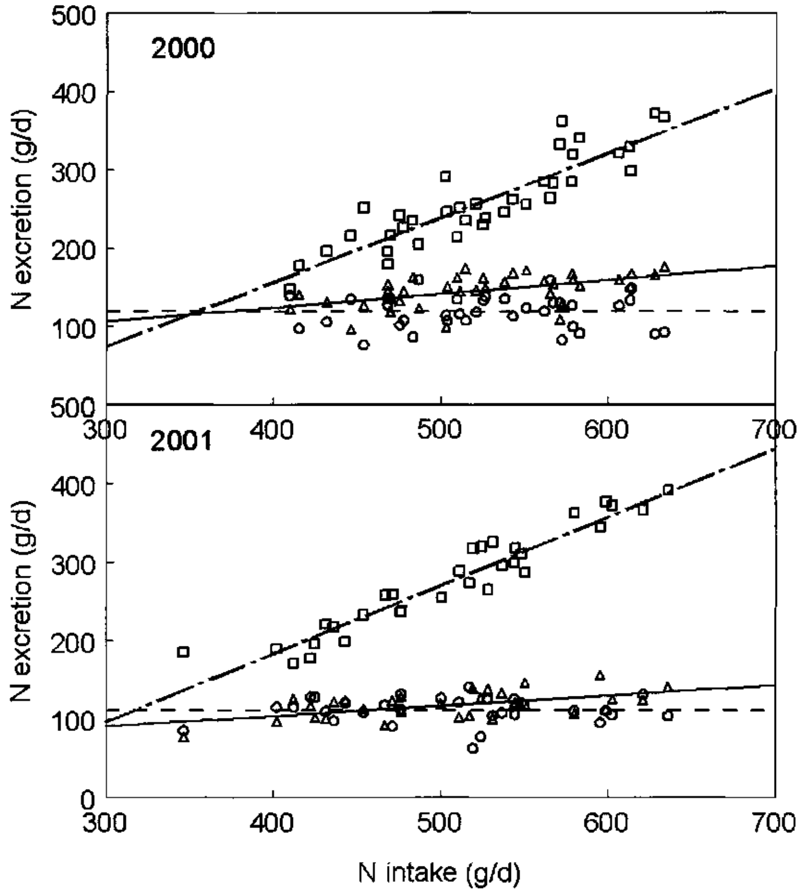

Figure 1 Relationships between N intake (in g/d) and the N excretion (in g/d) in milk (Δ; solid line), faeces (○; dashed line) and urine (□; dash-dotted line) in 2000 and 2001. The fitted lines in 2000: N milk= 0.17 (± 0.05) NI + 55.6 (± 26.19), R^2 = 0.27; N faeces = 119.3 (± 31.5); N urine = 0.83 (± 0.073) NI – 174.9 (± 38.3), R^2 = 0.79, and in 2001: N milk = 0.13 (± 0.033) NI + 51.0 (± 17.99), R^2 = 0.30; N faeces = 113.2 (± 21.5);N urine = 0.88 (± 0.047) NI – 164.3 (± 24.25), R^2 = 0.91.

Although DMI was lower in 2001 than in 2000, NI was similar in both years due to the higher N content in the grass in 2001. In both years, DMI was not influenced by the N content of the grass. Faecal N excretions were almost similar in both years with 118 ± 3.5 g/d in 2000 and 111 ± 2.8 g/d in 2001 and in both years there was no relation between NI and faecal N excretion (Figure 1). Nitrogen excreted in milk was weakly, but positively related with the NI (R^2 = 0.25 in 2000 and R^2 = 0.27 in 2001). A higher NI, however, resulted mainly in an increase in the N excreted with the urine with R^2 = 0.79 in 2000 and R^2 = 0.91 in 2001 (Figure 1). This is in agreement with data reviewed by Castillo et al. (2000), who reported a linear to exponential increase of the amount of N excreted with milk and urine with NI's above 400 g/d, respectively. Van Vuuren (1993) showed a linear relationship of rumen N loss with N contents above 25 g/kg DM and a loss in the rumen of 79 % of extra N in grass, which will be excreted in the urine. The NI is the product of DMI and the N content of the grass. Therefore, the amount excreted in urine was expressed as g N/ kg DMI per day and related to the N content in grass (Figure 2). In both years, a positive relationship was found between urinary N excretion (in g/kg DMI) and N content in grass (Figure 2; R^2 = 0.58 in 2000 and R^2 = 0.82 in 2001), which is in agreement with Van Vuuren (1993). The differences between years in slope (0.71 vs. 0.88) and correlation coefficient were most likely related with the lower mean N content in 2000 than in 2001. In 2000, the mean N content in the three consecutive periods was 32.1, 24.1 and 23.1 g/kg DM, respectively, and in the latter two periods thus below the level of 25 g N/kg DM and this may have resulted in the lower N losses from the rumen. In 2001, N content was in all periods above 25 g/kg DM.
In comparison with the N content, in both years urinary N excretion (in g/kg DMI) was weaker related to the ratio's between N and digestible OM (R^2 below 0.40) and between N and WSC (R^2 below 0.45). Moreover, the negative relationship with WSC content in grass was weak (R^2 below 0.18) (Figure 3). This indicates that the high N contents of grass had more effect on urinary N excretion than the ratio between N and energy or the WSC content. This suggests that reducing N content of grass has more effect on the amount of N excreted with urine than increasing the WSC content.

In our calculations, BW changes of the cows were not taken into account and a zero N balance for BW was assumed. The BW was measured only once each period and was influenced by rumen fill. In both years the cows gained on average 0.3 to 0.5 kg BW/d and the requirements for this weight gain equals approximately 10 to 16 g N/d (CVB, 1991) and thus 2 to 3 % of NI. In agreement, Van Vuuren and Meijs (1987) reported an N retention of up to 5 % of NI and in the review of Castillo et al. (2000) 98 % of NI was excreted with milk, faeces and urine. This was considered as negligible and therefore no corrections were made for the N balance of BW.

The relationships between N excreted in the faeces, milk and urine with NI presented above were based on relatively wide ranges in NI and N excretions, due to seasonal effects and cow effects (Figure 1). In comparison, the differences between the means of cultivars were limited (Tables 2 and 5). Moreover, there was a confounding effect of the N content of the grass in the three periods, due to fertiliser use, cutting management and weather conditions, with the progression of DIM of the cows during the experiment. Within the three months of the experiments, the MP may have decreased more than the DMI and hence the N partitioning was affected by DIM. Nevertheless, from the relationships mentioned above, it may be concluded that lowering NI by reducing the N content in grass is the most efficient way to reduce urinary N excretion and increase the efficiency of ingested N excreted in milk of dairy cows.

Table 5 Nitrogen intake (NI) and excretion in faeces, milk and urine (in g/d and % of NI) of six cultivars of perennial ryegrass in 2000 and 2001.

	Latin square I					Latin square II				
Cultivar	1	2	3	*SE*[1]	*P*[1]	4	5	6	*SE*[1]	*P*[1]
2000										
NI (g/d)	516.1[b]	539.9[ab]	561.2[a]	*10.5*	*0.046*	487.4[b]	521.9[a]	523.2[a]	*7.7*	*0.02*
N faeces (g/d)	113.9	112.4	115.9	*6.4*	*0.93*	119.7	124.7	124.9	*8.1*	*0.88*
N milk (g/d)	143.6	147.3	140.9	*3.1*	*0.36*	139.1	146.0	145.7	*2.7*	*0.14*
N urine (g/d)	258.5	280.2	304.4	*13.5*	*0.12*	228.7	251.2	252.6	*13.4*	*0.42*
N faeces (% NI)	22.3	21.3	21.1	*1.4*	*0.80*	24.8	24.0	23.8	*1.8*	*0.92*
N milk (% NI)	28.1[a]	27.2[ab]	25.1[b]	*0.7*	*0.04*	28.6	28.1	28.0	*0.8*	*0.86*
N urine (% NI)	49.6	51.5	53.8	*1.8*	*0.31*	46.5	47.9	48.2	*2.3*	*0.88*
2001										
NI (g/d)	533.7	522.9	501.1	*10.2*	*0.13*	468.5[b]	488.2[ab]	531.2[a]	*13.7*	*0.03*
N faeces (g/d)	107.9	114.5	116.4	*6.3*	*0.62*	101.2	112.3	112.4	*4.9*	*0.23*
N milk (g/d)	122.2	120.0	115.9	*2.6*	*0.23*	107.1	112.6	116.7	*3.1*	*0.13*
N urine (g/d)	303.7[a]	288.4[ab]	268.8[b]	*7.4*	*0.03*	260.3	263.3	302.2	*11.0*	*0.051*
N faeces (% NI)	20.5	22.2	23.6	*1.1*	*0.21*	21.9	23.7	21.5	*1.0*	*0.32*
N milk (% NI)	23.1	23.1	23.5	*0.3*	*0.61*	22.8	23.3	22.1	*0.6*	*0.38*
N urine (% NI)	56.4	54.7	52.9	*1.1*	*0.15*	55.3	53.0	56.4	*1.4*	*0.25*

[a,b,c] Means within a row and Latin square with different superscripts differ significantly ($P < 0.05$).

[1] SE = standard error of the mean, P = Probability.

Milk Urea N content

There is a passive diffusion of urea N from the blood into milk and several authors, as listed by Hof et al. (1997), have reported a very strong relationship between blood urea N levels and MUN levels. Urea is synthesized in the liver from NH_3, which originates from microbial fermentation in the rumen and the oxidation of amino acids. The regulation of ureagenesis is still not clear, but it seems that removal of the toxic NH_3 has priority above amino acids absorbed in excess of requirements. The concentration in rumen fluid and amount produced of NH_3 in the rumen depends on the balance in the supply of N and energy for microbial fermentation. Therefore MUN is an indicator of the excess of N in the rumen for microbial fermentation or/ and excess of amino acids in the intermediate metabolism in the animal (Lobley et al., 2000).

In 2000, MUN content was low with means below 10 mg/dL, with cultivar 4 giving a lower ($P = 0.02$) MUN content than cultivars 5 and 6 (Table 4). The low mean MUN in 2000 was associated with the N content below 25 g N/kg DM in periods 2 and 3 and thus a balance between N and energy supply in the rumen. In 2001, the N content in the diet was high with 30.3 ± 0.57 g N/kg DM and well above the optimal level for microbial protein synthesis in the rumen. The MUN content was also high with 16.8 ± 0.33 mg/dL, and again differences among cultivars were small (Table 4). Cultivars 3 and 4 had the lowest N content and this was associated with a tendency ($P = 0.06$) to a lower MUN content.

In 2000, a positive relationship was observed between MUN and N content in the diet (Figure 2; $R^2 = 0.45$) whereas a negative relationship was found between MUN and WSC content ($R^2 = 0.22$). In larger datasets including more observations than in our experiments, strong linear ($R^2 = 0.84$) (Broderick and Clayton, 1997) or quadratic relationships ($R^2 = 0.80$) (Broderick, 2003) with N content of the diet were found. However, in 2001 no relationship between MUN and N content in the diet was found. An upper limit to hepatic ureagenesis and/ or a more active renal removal of urea from the blood to prevent too high levels of urea in the blood may lead to a quadratic response of MUN on the increase of N content in the diet. This is supported by the quadratic relation between N of the diet and MUN as found by Broderick (2003) and the tendency for a quadratic slope at high N contents in the diet for legume forages as reported by Nousiainen et al. (2004).

Miller et al. (2001) showed that after feeding a cultivar with an elevated WSC content, a higher % of NI was excreted as N in milk. In agreement, we found in our experiment in 2000 that cultivar 3 with the highest CP content ($P = 0.04$) and the lowest WSC content ($P = 0.01$) showed the lowest milk N excretion as % of NI and that the two cultivars (1 and 4) with the higher WSC content had a higher milk N excretion as % of NI. Lee et al. (2002[b]) concluded that cultivars selected for an elevated WSC content compared with a control cultivar increased DMI and rumen digestible OM, which resulted in an increase in non-ammonia-N and microbial N flow to and absorption from the small intestine. However, Lee et al. (2002[b]) did not find an increase in the efficiency of microbial protein synthesis in the rumen. In these studies, cultivars with an increased WSC had often a lower NDF (45 to 83 g/kg DM) content and digestibility, whereas N content was lower (2.2 g/kg DM) or slightly higher (0.7 g/kg DM). However, the N content of both cultivars was low and ranged from 14.7 to 17.0 g N/kg DM. Furthermore, an additional increase in WSC content in grass is mainly an increase in fructosan content (Peyraud and Astigaragga, 1998). Although the release of these fructosans is slower than that of free sugars, 30-45 % of the fructosans and up to 60 % of total WSC were released from cell contents after ingestive mastication (Boudon and Peyraud, 2001) and WSC was completely released after a residence time of 1.5 h in the rumen (Boudon et al., 2002). Only up to one third of the nitrogen was released after ingestive mastication (Boudon and Peyraud, 2001) and the release in the rumen was slower and not yet complete after 7.5 h (Boudon et al., 2002). This indicates that energy supply from WSC might only balance N supply during eating and up to 1.5 h after eating and that there is no effect of an increased WSC content at longer residence times in the rumen. This may supports our findings that in 2000 there seemed to be an effect of cultivars with an increased WSC content on MUN and N utilisation, but an effect of these cultivars was absent in 2001 with a similar difference in WSC content among cultivars. Therefore it may be concluded that the WSC content had less effect on MUN and N utilisation than the N content of the diet and NI.

Milk urea N and urinary N excretion

Several authors have reported strong linear relationships between the MUN concentration and urinary N excretion (Jonker et al., 1998; Kauffmann and St-Pierre, 2001, Broderick, 2003, Nousiainen et al., 2004). In agreement, a linear relationship was found in 2000 (Figure 4) although in their equations the regression coefficients were lower and Jonker et al. (1998) and Kauffmann and St-Pierre (2001) did not find an intercept significantly differing from zero. The differences in slopes and the lower or absence of an intercept may be related with different roughages, diet composition and N content of the diet, lower digestibilities of their diets, the DIM and parity of the cows and to different methods used for MUN analyses. However, no relationship (P = 0.11) between MUN and urinary N excretion was found in 2001 (Figure 4). In spite of the high relationships between MUN and N excreted in urine (g/d) reported in literature, the different slopes and the absence or presence of an intercept in their equations and data from our experiments -a similar N excretion with urine in both years (262.6 ± 9.4 g/d in 2000 and 281.1 ± 10.7 g/d in 2001) with almost a double MUN in 2001 (16.9 ± 0.3 mg/dL) than in 2000 (8.7 ± 0.3 mg/dL)- suggest that predicting urinary N excretion solely with MUN is not valid.

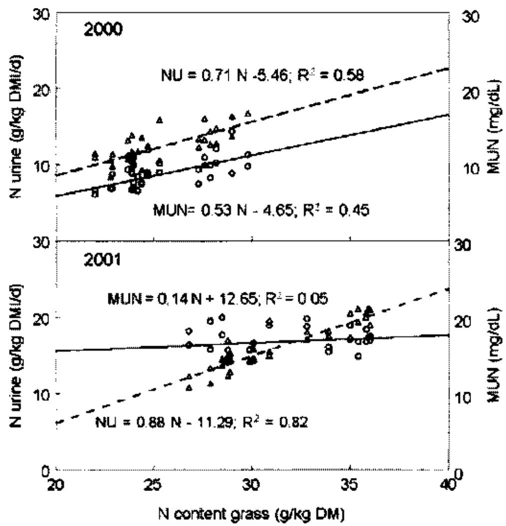

Figure 2 Effect of N content in grass in g/kg DM on the urinary N excretion in g/kg DMI (Δ; dashed line) and MUN in mg/dL (○; solid line) in 2000 and 2001.

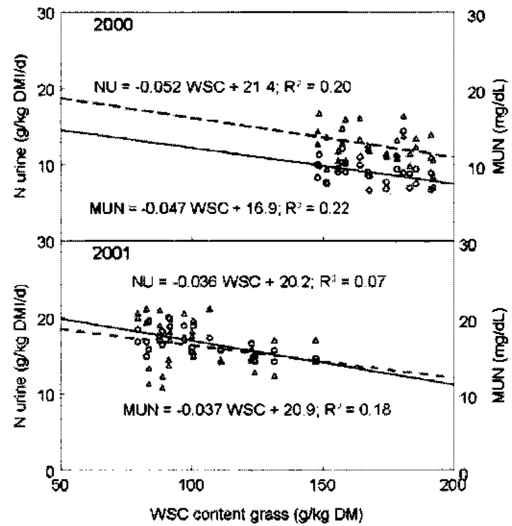

Figure 3 Effect of WSC content in grass in g/kg DM on the urinary N excretion in g/kg DMI (Δ; dashed line) and MUN in mg/dL (○; solid line) in 2000 and 2001.

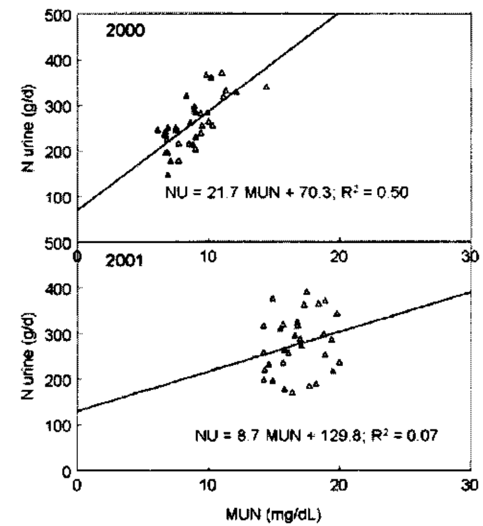

Figure 4 Relationships between Urinary N excretion (in g/d) with milk urea N (MUN; in mg/dL) in 2000 and 2001.

Conclusions

In general, small and sometimes significant differences in chemical composition among cultivars were found in their chemical composition, but these were only consistent for the WSC content in the two consecutive years. These small differences among cultivars did not result in significant differences in DMI and MP in dairy cows fed indoors. Urinary N excretion was more closely related to N content in the grass and NI, than to WSC content. Besides, these relationships were primarily associated with the three growing periods of the grass and individual cow differences rather than with cultivar differences. Based on the results of the six cultivars used in these two experiments it was not possible to assign traits for breeding of new cultivars, to increase the DMI, digestibility or MP or to improve the N utilisation by dairy cows.

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Chapter 5

Intake, milk production and N utilisation by dairy cows grazing perennial ryegrass cultivars

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Submitted to Animal Science

Abstract

The aim of this study was to evaluate the effect of sward characteristics and chemical composition of four diploid perennial ryegrass cultivars on the grazing behaviour, dry matter intake (DMI), milk production (MP) and its composition and finally the N utilisation by twelve grazing dairy cows. These effects were measured during four two-week periods in two consecutive summers, according to a 4 × 4 Latin square design with three replicate cows. In the first year, one cultivar had a lower DMI compared to the other cultivars, and this was associated with a lower herbage allowance (HA) and sward surface height (SSH), a lower water-soluble carbohydrates (WSC) content, and a more severe infection with crown rust. With a similar difference in WSC content among cultivars in the second year, no differences among cultivars in the herbage DMI were found. In the second year, differences in crown rust infection were smaller and the infection was less severe. No relationships between HA and SSH with grazing behaviour and DMI were found. Differences among cultivars in cell wall content were small and weakly related with differences in herbage DMI. In the first year, the lower DMI of one cultivar was associated with a lower milk production (MP), whereas in the second year MP did not differ. Only small differences in milk urea N (MUN) content were found, and in both years the N secretion in milk as proportion of N intake did not differ among cultivars.

Keywords: perennial ryegrass, cultivar, grazing, intake, N utilisation.

Introduction

In North-West Europe, around 20 to 25 % of the land area is grassland and a substantial proportion is intensively managed and provides feed for dairy cows. In this region with a temperate climate, these intensively managed grasslands are predominantly pure grass swards and perennial ryegrass (Lolium perenne L.) is the most abundant grass species with a high yield and nutritive value.

Dry matter intake (DMI) is the main determinant of milk production (MP) and milk fat and protein yield (e.g. Hristov et al., 2004). The conversion of feed into milk is more efficient at higher MP levels (Tamminga, 1996) and therefore dairy cows have been continuously selected for this trait. However, the increase in feed intake was less than half the amount needed to cover the increased requirements for MP (Veerkamp, 1998) and it was shown that cows with a high proportion of forages in their diet (Veerkamp et al., 1994) or grazing intensively managed grassland had lower MP than potentially possible (Kolver and Muller, 1998; Peyraud et al., 2004). The lower MP of grazing dairy cows in comparison with cows fed a nutrient balanced diet, could partly (61 %) be attributed to a lower DMI and partly to an imbalance between absorbed nutrients (Kolver and Muller, 1998). Feed intake behaviour of grazing dairy cows is a complex processes and may depend on sward characteristics (Orr et al., 2004), the palatability of the diet and the post-ingestive feedback (Forbes and Provenza, 2000). The DMI of grazing cows is in a linear or curvilinear way, positively related with tiller density, herbage mass (HM), sward surface height (SSH) and herbage allowance (HA) (Peyraud et al., 2004; Chilibroste et al., 2005), due to an increased ease of prehension. However, pasture utilisation and herbage DMI by dairy cows are inversely related, and in practice an optimum in grazing systems between both has to be found. Furthermore, palatability of the herbage may be affected by diseases like crown rust or by the chemical composition of the herbage, and this may play a role in the diet selection and the DMI of grazing animals. Physical fill of herbage in the rumen might be one of the many factors that are involved in intake regulation of grazing animals (Chilibroste et al., 2005).

Another concern of grassland based dairy farming is to improve the low N utilisation and to reduce N losses to the environment. Intensive use of fertilizer N and selection by the cows of young and leafy grass not only results in a high digestibility and energy content but also in a high crude protein (CP) content of the ingested grass. The CP is rapidly degraded in the rumen, and often the N level exceeds the level required for the optimal utilisation of carbohydrates available for microbial growth. Almost 80 % of this N in excess in the rumen is, after conversion in the liver to urea, excreted in urine (Van Vuuren, 1993). In grazing dairy cows, the supply of metabolizable energy was found to be first-limiting for MP (Kolver and Muller, 1998). This implies that absorbed protein will be in excess of requirements for maintenance and milk production, and this may result in N losses in the intermediate metabolism (Hof et al., 1994).

There are many ways to increase the energy intake and N utilisation by grazing cows and one way is the inclusion of selection criteria for these traits in grass breeding programmes (Beever and Reynolds, 1994). In North-West Europe, grassland is usually renewed every 3 to 15 yr and it is sown with a mixture, containing mostly a high proportion of cultivars of perennial ryegrass and in addition small proportions of other grass species and clover. Differences among cultivars in their water-soluble carbohydrates (WSC) content have been shown to be consistent and heritable (Humphreys, 1989) and to increase the in vitro dry matter digestibility (Radojevic et al., 1994). Two ryegrass cultivars differing in WSC content showed an increased digestible DMI, MP and N utilisation with an increased WSC content (Miller et al., 2001). Miller et al. (2001) speculated that the efficiency of microbial protein synthesis was increased due to the higher WSC content, and this was confirmed by infusing sugars to perennial ryegrass in an in vitro fermentation system (Lee et al., 2003), but could not be demonstrated in the rumen of steers (Lee et al., 2002[b]). However, in both studies a lower NH_3-N concentration in the rumen was found with increased WSC content in grass and this may have resulted in the higher N utilisation. These studies indicated that there are differences among cultivars of perennial ryegrass in nutritive quality and performance of dairy cows.

The aim of our study was to evaluate the effect of sward characteristics and chemical composition of four diploid perennial ryegrass cultivars on the grazing behaviour, DMI, MP and milk composition and finally the N utilisation by grazing dairy cows.

Materials and methods

Cows, cultivars and experimental design

In July and August 2002 and 2003, two grazing experiments were conducted with twelve dairy cows. Prior to the experiments cows grazed a perennial ryegrass sward for three weeks. Both experiments lasted eight weeks, divided in four periods of two weeks each. In 2002, the cows were in their second to fourth lactation, 67 ± 4.2 DIM at the start of the experiment, BW was 528 ± 7.1 kg and MP was 32.7 ± 0.9 kg/d in the adaptation period. The cows used in 2003 were in their second to fifth lactation, 79 ± 3.7 DIM at the start of the experiment, BW was 549 ± 6.7 kg and MP was 30.7 ± 0.9 kg/d in the adaptation period. Cows were blocked in three groups of four animals, according to their MP in the adaptation period.

The cows grazed four diploid perennial ryegrass cultivars (cultivars 1 to 4) and these were selected from zero-grazing experiments conducted in the two previous years. In these experiments cultivars showed differences in their chemical composition with the largest and consistent difference in WSC content (Smit et al., 2005). The four cultivars were sown on two paddocks in Wageningen, The Netherlands, according to a randomized complete block design with three replicates. Each paddock was divided into twelve strips of 22 x 84 m, four adjacent strips were blocked and sown at random with the four cultivars. At all times, the cutting and fertilising regime was similar for all cultivars. In 2002, the grass was cut in spring to promote tillering and sward establishment prior to the experiment in summer 2002. In autumn 2002, paddocks were grazed by sheep and in spring 2003 the paddocks were cut twice for silage making prior to the experiment. In both experiments, cows grazed in periods 1 and 3 on one paddock and in periods 2 and 4 on the other paddock. Prior to the experiment and after the first grazing event, the sward was cut at 6 cm above ground level and herbage was removed. After cutting, 75 kg N was applied and herbage was allowed approximately 25 d of re-growth, to obtain a HM of 2000 kg DM/ha. The pasture was irrigated in periods with low rainfall.

The twelve cows were assigned to the four cultivars according to a 4 x 4 Latin square design with three replicates. Therefore, first each group of four cows was assigned to one block on each paddock with the four cultivars sown in four strips. Then each cow within a group was assigned to one strip with a cultivar for a 14-d period and in each period the cow was assigned to another cultivar. A one-d rotational grazing system was applied and cows were moved daily at 12.00 h to a new plot. Mobile fences were established every 6 m across the twelve strips on a paddock to obtain daily plots of 132 m^2 per cow. With a HM of approximately 2000 kg DM/ha, this would result in a HA of 25 kg DM/cow per d, enough to ensure an ad libitum intake of fresh grass. Cows were supplemented with 2.7 kg DM/d concentrate feed in two equal portions at milking. The ingredients and the chemical composition of the concentrate are presented in Table 1. Cows were milked twice a day at 6.00 and 16.00 h. At each milking the MP per cow was recorded and two milk samples were taken. These samples were pooled to one sample from the evening and consecutive morning (1:1), one sample was analyzed for milk fat and protein content (ISO 9622) and the other for milk urea N (MUN) content with the pH difference method (ISO 14637) by the Melkcontrolestation, Zutphen, The Netherlands. Fat and protein corrected milk (FPCM) was calculated as: $(0.337 + 0.116 \times \text{fat}\ \% + 0.06 \times \text{protein}\ \%) \times \text{MP}$.

Sampling and Measurements

On days 11 to 14 in each period, the HM in each plot was measured. Therefore, an area of 5% of the total daily plot was mown with an Agria mower at a stubble height of approx. 4 cm, the cut herbage was collected, weighed and sampled for DM in duplicate. The DM was determined in a stove at 70 °C overnight. The HM and HA were calculated from the DM weight of herbage, the cut area and the plot size. The SSH was measured on the same days with a falling plate meter ('t Mannetje and Jones, 2000).

During days 8 to 13 in each period, samples of the herbage grazed by the dairy cows were taken by hand-plucking. To obtain a representative sample of what the cows ingested, grazing behaviour of the cows and the herbage removed were observed closely and mimicked by taking samples next to the grazed herbage at the same depth in the sward canopy. Each day at three times (13.30 h, 20.00 h and the next morning at 7.00 h) samples were taken and these times were close to the observed main grazing bouts of the cows. Samples were stored in the freezer at -20 °C pending further analyses. After cows were moved to a new plot at noon, on d 8 to 13 in each period all dung patches on a plot were sampled and these faeces samples were also stored in the freezer at -20 °C.

Table 1 Ingredients and chemical composition of concentrate feed.

Ingredients		***Chemical composition***	
Beet pulp (%)	20.0	DM (g/kg fresh)	869.4
Maize (%)	24.0	Ash (g/kg DM)	74.8
Barley (%)	19.3	CP (g/kg DM)	133.8
Soya hulls (%)	10.5	WSC (g/kg DM)	101.6
Coconut peels (%)	10.0	NDF (g/kg DM)	318.5
Beet molasses (%)	8.0	ADF (g/kg DM)	184.0
Argentinean Soya (%)	7.5	Fat (g/kg DM)[1]	30.4
Premix Vitamin-mineral (%)	0.7	Starch (g/kg DM)[1]	280.4
		Resistant starch (g/kg DM)[1]	84.3
		VEM (unit/kg DM)[2]	1120
		DVE (g/kg DM)[3]	109
		OEB (g/kg DM)[4]	-22

[1] Calculated by feed manufacturer based on ingredient composition

[2] VEM is feed unit lactation, which contains 6.9 kJ net energy for lactation.

[3] DVE is the sum of digestible feed and microbial true protein available in the small intestine corrected for endogenous protein losses.

[4] OEB is the rumen degradable protein balance.

Analyses

The hand-plucked herbage samples were dried in a stove at 60 °C for 48 h, ground to pass a 1 mm sieve (Peppink Hammermill), and analyzed with Near Infrared Reflectance Spectroscopy (NIRS) for organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent lignin (ADL), water-soluble carbohydrates (WSC), and in vitro organic matter digestibility (OMD) based on pepsin-HCL digestibility, using prediction lines developed by Centre de Recherches agronomiques de Gembloux , Belgium. The validation of NIRS to predict the chemical composition is described by Smit (2005). After NIRS analysis, the samples of each strip with a cultivar grazed by a cow in a period were pooled on an equal DM weight to one sample per cow per period. The daily faeces samples were thawn overnight and these daily samples were pooled to one sample per cow per period. These samples were freeze-dried and ground to pass a 1 mm sieve (Retsch ZM 100). The concentrate feed was sampled daily throughout the experiment and a pooled sample was dried at 60 °C overnight in a stove and ground to pass a 1 mm sieve (Retsch, ZM 100). The pooled herbage and faeces samples and the concentrate feed sample were analyzed for the n-alkanes (C_{27} to C_{35}) content as described by Mayes et al. (1986), but with slight modifications: direct saponification, 5 times extraction and duplicate analyses in a Gas Chromatograph. The procedure is described in detail by Smit et al. (2005).

Herbage DMI and grazing behaviour

To the concentrate feed C_{32} n-alkane was added. The cows received the concentrate feed twice daily and consumed the concentrate feed almost always completely. In case of refusals, these were collected, weighed and subtracted from the amount given to the cow. The C_{32} to C_{33} n-alkane ratio was used to estimate the herbage DMI per cow per period with the equation as described by Mayes et al. (1986), using the dosed amount of C_{32} with the concentrate feed and the contents of C_{32} and C_{33} in the herbage and faeces. As there was no C_{33} found in the concentrate, no correction was applied.

On days 11 and 13 of each period, the grazing behaviour was measured with four cows, blocked in one group, using IGER grazing behaviour recorders as described by Rutter et al. (1997). Recordings were analysed using Graze™ (Rutter, 2000) to determine the periods of eating, total eating time and number of bites. The intake rate (g DM/bite) and bite mass (mg DM/bite) were calculated from intake (g DM/d) and eating time (min/d) or number of bites (/d), respectively.

Calculations and statistical analyses

The efficiency of N utilisation was calculated from the N secretion in milk (protein yield/ 6.38) divided by the N intake (N content in grass (N= CP/ 6.25) x DMI). The OEB (rumen degradable protein balance) content of the grass was calculated as described by Tamminga et al. (1994), using regression equations for fresh grass from CVB (1999).

Both years were analysed separately, because maturity of the sward, growing conditions of the herbage differed between years and twelve different cows were used. The chemical composition of the herbage was analysed according to a two-way ANOVA with cultivar and period as main factors. The animal data were analyzed according to a 4 × 4 Latin square design, with the mean effects of four cultivars measured over four periods with three cows as replicates within each treatment. The grazing behaviour was analyzed with four cows and no replicates. The GLM procedure of SAS 8 was used with cultivar, period and cow as independent variables in a main factorial model. In 2002, two observations and in 2003 five observations were omitted from analyses due to illness of the cows. When a significant effect was found for a dependent variable, the Tukey-test (α = 0.05) was used to separate the least square means.

Table 2 The chemical composition, organic matter digestibility (OMD) and rumen degradable protein balance (OEB) of four cultivars in 2002 and 2003.

	Cultivars[1]						Significance[2]		
Factor[3]	1	2	3	4	mean	s.e.	C	P	C × P
2002									
DM (g/kg product)	179^{a}	171^{b}	180^{a}	173^{b}	176	1.8	***	***	***
OM (g/kg DM)	905.7^{a}	903.9^{ab}	898.1^{c}	902.6^{b}	902.7	1.0	***	***	
CP (g/kg DM)	182.9^{a}	174.6^{c}	182.0^{ab}	178.6^{bc}	179.4	1.4	***	***	**
WSC (g/kg DM)	148.8^{a}	132.3^{b}	109.1^{c}	143.8^{a}	133.2	2.0	***	***	
NDF (g/kg DM)	458.2^{b}	482.1^{a}	477.1^{a}	474.3^{a}	472.9	2.4	***	***	
ADF (g/kg DM)	272.3^{c}	290.5^{a}	289.6^{a}	284.1^{b}	284.2	1.7	***	***	
ADL (g/kg DM)	26.3^{b}	26.1^{b}	28.4^{a}	24.3^{c}	26.3	0.3	***	***	***
ADL/NDF (g/kg NDF)	57.3^{a}	53.9^{b}	59.0^{a}	50.9^{c}	55.3	0.7	***	***	***
OMD (g/kg DM)	911^{a}	886^{c}	879^{d}	897^{b}	893	2.8	***	***	***
OEB (g/kg DM)	19.7^{a}	13.9^{b}	21.1^{a}	18.6^{a}	18.3	1.1	**	***	***
2003									
DM (g/kg product)	219	216	222	216	218	4.2		***	
OM (g/kg DM)	919.4^{a}	915.5^{b}	914.1^{b}	915.3^{b}	916.2	1.0	**	***	
CP (g/kg DM)	207.3^{a}	195.0^{b}	205.8^{a}	196.3^{b}	201.1	2.3	**	***	
WSC (g/kg DM)	112.1^{b}	107.9^{b}	88.9^{c}	126.1^{a}	108.0	3.1	***	***	
NDF (g/kg DM)	471.3^{c}	489.6^{a}	483.6^{b}	473.4^{bc}	479.5	4.4	***	***	**
ADF (g/kg DM)	260.6^{b}	273.7^{a}	270.7^{ab}	264.9^{b}	267.3	2.3	**	***	
ADL (g/kg DM)	32.6^{a}	31.6^{a}	33.4^{a}	29.8^{b}	32.3	0.4	***	***	
ADL/NDF (g/kg NDF)	69.2^{a}	64.6^{b}	69.0^{a}	63.0^{b}	66.4	0.8	***	***	**
OMD (g/kg DM)	856^{a}	833^{c}	841^{bc}	854^{b}	846	3.9	***	***	
OEB (g/kg DM)	38.2^{a}	30.1^{b}	37.6^{a}	29.3^{b}	33.8	1.9	**	***	

1 Means of cultivar within row with different superscripts differ significantly ($P < 0.05$).

2 Significance of the main effects cultivar (C) and period (P) and their interaction (C × P) with * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

3 OMD = digestibility of OM estimated with NIRS; OEB = rumen degradable protein balance.

Results

Chemical composition of grass

The chemical composition of four cultivars in both years is presented in Table 2. In both years the largest difference among cultivars was found in the WSC content with a more than 30 g/kg DM higher WSC content of cultivar 4 than cultivar 3. The ranking was not completely consistent across years, with cultivar 1 having the highest WSC content in 2002, but an intermediate in 2003. The differences in CP content were smaller with around 10 g/kg and 5.5 % relative of the mean. In both years cultivars 1 and 3 had the highest and cultivars 2 and 4 the lowest CP content. The differences among cultivars in OEB content were similar as in CP content. The DVE content was in both years highest for cultivar 1 and lowest for cultivar 2. The relative difference of the NDF content was 4 %, with in both years the lowest NDF content of cultivar 1 and the highest NDF content of cultivar 2. Although the NDF content of cultivar 4 was high in 2002 and intermediate in 2003, the ADL content of cultivar 4 was in both years the lowest (24 to 30 g/kg DM).

These results showed that in both years the ranking of the cultivars in their chemical composition was fairly consistent. Comparing the chemical composition of both years, the CP content was more than 20 g/kg DM lower in 2002 than in 2003, whereas the opposite was found for the WSC content. This inverse relation between CP and WSC content is well known but does not seem to play a role within a cultivar. Cultivar 1 had in both years the highest CP content with also the highest WSC content in 2002 and an intermediate WSC content in 2003. The summer of 2003 was very warm with only a low amount of rainfall and this reduced the herbage growth rate. To stimulate the growth rate, the N fertilization was increased from 50 kg N per cut up to 90 kg N per cut in periods 2 and 3 and this increased the mean CP content in these periods. An inverse relationship between HA and CP content was observed in these periods.

Herbage mass, SSH, DMI and grazing behaviour

In 2002, the mean HA (above 4 cm) ranged from 25.1 to 61.1 kg DM/cow per day and a slightly lower and narrower range of HA with 20.4 to 46.1 kg DM/cow per day was found in 2003 (Table 3). In 2002, a higher ($P < 0.001$) HA was found for the cultivars 1 and 4 than cultivars 2 and 3 whereas in 2003 the HA of cultivar 4 was almost 5 kg DM/cow per d higher ($P < 0.01$) than cultivars 1 and 3. The SSH before grazing was on average above 16 cm in 2002 and 14 cm in 2003, and the SSH of cultivar 4 was higher ($P < 0.001$) than the other cultivars in both years.

In 2002, the cows consumed on average 2.4 kg DM/d more grass when grazing cultivar 4 than cultivar 3 ($P = 0.03$) and the DMI of the other two cultivars were in between. The time spent grazing ranged from 469 to 540 min/d and was slightly lower ($P = 0.19$) for cultivars 2 and 3 than for cultivars 1 and 4. The number of bites, total grazing jaw movements (TGJM) and the bite rate did not differ among cultivars. The bite mass of cultivar 3 was lower than cultivar 2 ($P = 0.04$), but did not differ from cultivar 4. Similar as in 2002, cultivar 4 had the highest herbage DMI in 2003, but the difference with the other cultivars was smaller (0.9-1.4 kg DM/d), and therefore no significant ($P = 0.53$) differences in DMI among cultivars was found. Moreover, the grazing behaviour did not differ among cultivars.

Milk production, milk composition and nitrogen utilisation

The MP and milk composition by twelve dairy cows in 2002 and 2003 are presented in Table 4. In 2002, cultivar 4 had a higher ($P < 0.05$) MP, milk fat and milk protein production and FPCM compared to cultivar 3. The milk fat and milk protein content varied only slightly among cultivars. The MUN content of cultivar 3 was slightly higher (up to 1.6 mg/dL) than the other cultivars, though this was not significant ($P > 0.17$). In 2003, the MP, FPCM and milk fat and protein yield and protein content did not differ among cultivars (Table 3). There was a small difference among cultivars in the fat content with cultivar 1 being 1.3 g/kg higher than cultivars 2 and 4. The MUN content was, similar to 2002, higher with cultivar 3 than cultivar 4. The utilization of N by the dairy cows in both years is also presented in Table 3. Although the herbage DMI differed ($P = 0.03$), there was only a tendency ($P = 0.06$) of a lower N intake of cultivar 3 than cultivar 4 due to the difference in CP content between the two cultivars. The N secretion in milk was also lower ($P = 0.01$) by dairy cows grazing cultivar 3 than cultivar 4. Therefore, the efficiency of N utilisation was not affected ($P = 0.90$) by cultivar and the mean was 0.25 ± 0.01. There were smaller differences in N intake and N secretion in milk in 2003 than in 2002 and no effects ($P > 0.18$) of cultivar were found. However, the mean N intake was higher and the N in milk was lower in 2003 than in 2002 and this resulted in a lower efficiency of N utilisation of 0.20 ± 0.01 in 2003. The higher N efficiency in 2002 than in 2003 was associated with a lower MUN content of almost 2 mg/dL.

Discussion

Herbage allowance, grazing behaviour and herbage DMI

The main aim of the two experiments was to evaluate the effect of four perennial ryegrass cultivars on the herbage DMI and MP of grazing dairy cows. With grazing dairy cows the herbage DMI depends much more on interactions between the sward canopy and the amount and nutritive quality of the ingested herbage than under zero-grazing conditions. Grazing dairy cows have often a lower MP than potentially possible and this is partly related to constraints in the herbage DMI (Peyraud et al., 2004). Among the main constraints in DMI

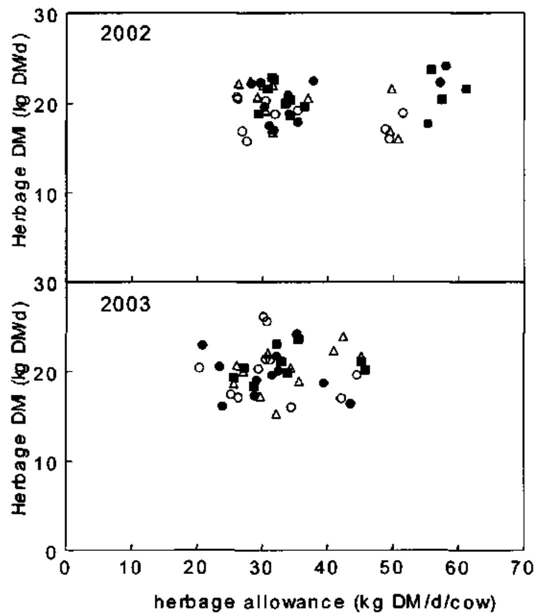

Figure 1 The relationship between herbage DMI and herbage allowance of the four cultivars (1 = ●, 2 = Δ; 3 = ■, 4 = ○) in 2002 and 2003.

under grazing conditions are HM, HA and SSH. The HA is the product of the HM and the area offered to the cow. Many authors have shown that there is a positive linear to curvilinear effect of HA on DMI, with strong almost linear effects at low levels of HA (up to 35 kg/d) and a diminishing effect on the DMI at higher levels (e.g. Meijs, 1981; Wade, 1991, Peyraud et al., 2004). The SSH is determined by the height of the sward canopy and the HM, and both have an effect on the ease of grazing the sward canopy (Wade, 1991; Laca and Ungar, 1992; Chilibroste et al., 2000). In our study, the SSH before grazing was on average above 16 cm in 2002 and 14 cm in 2003 and the mean HA was above 30 kg DM/d per cow in both years. At these relatively high levels of SSH and HA the plateau of the curvilinear relation was most likely reached and only small effects of cultivar on the grazing behaviour and DMI can be expected. Under rotational grazing, the optimum SSH was found to be 9-10 cm and DMI rapidly decreased when SSH was below 7 cm (Peyraud et al., 2004). Under continuous stocking, a decrease in SSH from 9 to 5 cm resulted in an increase in grazing time and a reduction in bite mass (Gibb et al., 1999). Although grazing time and bite rate in our experiment with strip grazing was similar compared to 9 cm SSH continuous stocking, the bite mass was higher in our experiment (613 and 630 mg DM/bite) than at a lower SSH (466 mg/bite), as reported by Gibb et al. (1999). Under strip grazing with a SSH of 16 cm, a similar

Table 3 Herbage allowance (HA) and sward surface height (SSH) of four cultivars of perennial ryegrass, the effect of these cultivars on the herbage dry matter intake (DMI) of twelve dairy cows and the grazing behaviour measured with four dairy cows.

	Cultivars[1]						Significance[2]		
Factor[3]	1	2	3	4	mean	s.e.	C	P	A
2002									
HA (kg DM/cow/d)	38.5^{a}	35.4^{b}	33.9^{b}	39.1^{a}	36.7	0.6	***	***	-
SSH (cm)	17.8^{ab}	17.4^{b}	16.4^{c}	18.0^{a}	17.4	0.21	***	***	-
Herbage DMI (kg/d)	17.5^{ab}	17.5^{ab}	15.6^{b}	18.0^{a}	17.1	0.6	*	*	
DMIMBW (kg/$BW^{0.75}$/d)	157.0	158.4	141.7	161.9	155.3	5.3	§	**	
Grazing time (min/d)	529	469	490	540	511	19.4		§	
Bites (x 1000/d)	28.7	26.3	29.1	31.1	29.0	1.9			
TGJM (x 1000/d)	36.5	33.1	35.9	37.8	36.1	2.0			
Bite rate (bites/min)	54.3	56.7	59.8	57.6	57.0	2.4			
Bite mass (mg DM/bite)	652^{ab}	701^{a}	539^{b}	587^{ab}	613	26	*		
Intake rate (g DM /min)	35.1	39.8	32.1	33.4	34.6	2.1			
2003									
HA (kg DM/cow/d)	30.2^{b}	33.8^{ab}	30.9^{b}	35.1^{a}	32.5	1.0	**	***	-
SSH (cm)	14.3^{b}	15.6^{a}	14.6^{b}	15.9^{a}	15.1	0.43	***	***	-
Herbage DMI (kg/d)	17.0	17.3	17.5	18.4	17.5	0.7			*
DMIMBW (kg/$BW^{0.75}$/d)	150.6	152.7	154.6	162.9	154.8	5.9		§	§
Grazing time (min/d)	532	535	526	547	535	22.5		*	
Bites (x 1000/d)	30.5	29.6	29.3	31.5	30.2	2.1		*	
TGJM[1] (x 1000/d)	38.1	37.7	36.9	39.6	38.1	4.0		*	
Bite rate (bites/min)	53.4	51.6	52.7	55.2	53.2	0.8	§	*	
Bite mass (mg DM/bite)	627	614	684	597	630	50		§	
Intake rate (g DM/min)	34.9	33.6	37.6	34.2	35.1	2.0			

1 Means of cultivar within row with different superscripts differ significantly ($P < 0.05$).

2 Significance of the main effects cultivar (C), period (P) and animal (A) with * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$, and § approaching significance ($P < 0.10$).

3 HA = herbage allowance, SSH = sward surface height, herbage DMI = herbage dry matter intake, DMIMBW = DMI per kg metabolic body weight, TGJM = total grazing jaw movements.

bite mass was found than in our experiment, however intake rate was higher and the eating time (461 min/d) was shorter than in our experiment (Orr et al., 2001). Moreover, the bite rate in that experiment was high (> 70 bites/min) in comparison with our data (53 and 57 bites/min) and data reported by Delagarde et al., (1997) (54-56 bites/min). In our experiment, the grazing behaviour was not related to HA and SSH. In 2002, the herbage DMI was 2.4 kg DM/d higher (P = 0.03) when cows grazed cultivar 4 than cultivar 3 and a smaller and not significant difference (P = 0.53) of 0.9 kg DM/d was found in 2003. The higher (P < 0.01) HA and SSH of cultivar 4 than cultivar 3 in both years corresponded with the difference in DMI, but using all data within each year no relationships were found between the HA or SSH with DMI. This absence of a relationship between HA and DMI is illustrated in Figure 1. Moreover, the strip grazing system in our experiment was one day and there was only one cow on a plot in contrast with the curvilinear relationship reported in the literature that was obtained with groups of dairy cows with three to five days of rotational grazing.

Palatability, grazing behaviour and herbage DMI

The grazing behaviour and the DMI may be influenced by differences among cultivars in their palatability (i.e. taste, smell and sight). Although palatability by dairy cows is difficult to perceive, it is well known that cows tend to avoid herbage that is infected with the fungus *Puccinia coronata* f.spp *lolii* (crown rust). In this study, the herbage was also infected with crown rust and the method to determine severity of infection and results are described by Smit (2005). Summarised, in 2002 cultivar 3 became severely infected with crown rust with more than 40 % of the leaves severely infected, whereas less than 15 % of the leaves of cultivars 1 and 4 were severely infected. Visual observations showed that the cows tended to avoid grazing (i.e. more moving around with the muzzle before taking a bite) the severely infected herbage and this may have resulted in the lower herbage DMI of cows grazing cultivar 3 in 2002. This avoidance may be related to the changed colour of the grass (brown) and an increased bitter taste associated with fungi, although reports about the effect of crown rust on palatability are lacking. However, the grazing behaviour recorded with the four cows did not show significant differences among cultivars in time spent grazing, number of bites, TGJM and in bite rate. The incidence of crown rust infection varies from year to year (Kimbeng, 1999) and this was also found in our experiments. In 2003, the overall infection was less severe with 75 ± 4.5 % of the leaves without any crown rust spot. Similarly as in 2002, cultivar 3 had a higher percentage (21 %) of severely infected leaves than cultivar 1 (8 %), cultivar 2 (12 %) and 4 (5 %), but in this year the DMI of cultivar 3 was not significantly lower than cultivar 4.

In both years the largest difference among cultivars was found in the WSC content with more than 30 g/kg DM difference and this may have affected the DMI. Cows showed a preference for a higher non-structural carbohydrate content among eight cultivars of tall fescue (Mayland et al., 2000) and among perennial ryegrass cultivars (including the four cultivars used in these two experiments) for a higher WSC content (Smit, 2005). Dairy cows are able to differentiate between the primary tastes and seem to prefer a sweet taste and this can explain the showed preference. However, the olfactory and gustatory response of cows is perceived at a molecular level instead of the proximate composition as WSC and therefore interpretation should be done with caution (Arnold, 1981). The higher intake of cultivar 4 in both years may be related with a higher palatability due to the higher WSC content of this cultivar in both years. However, no differences in DMI were found with zero grazing dairy cows fed grass differing in WSC and CP content but fairly similar NDF content (Valk et al., 2000) and with ryegrass cultivars differing in WSC content and fairly similar CP and NDF contents (Taweel, 2004).

Post-ingestive feedback on herbage DMI

The post-ingestive feedback on feed intake is an integration of signals from receptors that respond to distension of the rumen, ruminal pH and osmolality and metabolized nutrients most likely by additivity of signals (Forbes, 1996; Chilibroste et al. 2005). These signals are interrelated with palatability (Villalba and Provenza, 2000), but the post-ingestive feedback is the main factor controlling intake (Forbes, 1996; Faverdin, 1999; Forbes and Provenza, 2000). The physical distension depends on the weight and volume of the digesta and its degradation and passage kinetics, with the reticulo-rumen being the main compartment where distension occurs.

The volume of ingested grass is high due to the low DM content of grass and therefore the distension of the rumen will be higher. A reduction of internal water by drying grass (218 vs 302 g/kg product), increased DMI, but did not affect fresh grass weight in the rumen (Estrada et al., 2004). Moreover, Van Vuuren (1993) concluded that distension was not a limitation for grass intake. Therefore, the small differences in DM content among cultivars had most likely no effect on DMI. The digestibility of the diet and the rate of fermentation and passage depend for a large part on the NDF content, but also on other factors as particle size and chewing activity (Mertens, 1994; Allen, 1996). The predicted OMD with NIRS showed a high mean of 893 g/kg DM in 2002 and 846 g/kg DM in 2003. The largest difference in OMD was found between cultivar 1 and 3, with 33 g/kg DM in 2002. However, ranking of cultivars in their digestibility differed between years and digestibility was not related with DMI. This absence of a relation is in agreement with the observation of a diminishing effect on the DMI with an increasing digestibility (Allen, 1996) and the absence of a relation between DMI and high DM digestibility (more than 700 g/kg DM) as found with zero-grazing experiments with dairy cows fed perennial ryegrass (Valk et al., 2000; Chapter 3). Miller et al. (2001) reported a higher digestible DMI of a cultivar with an increased WSC content (39 g/kg DM) compared to a control. However, this difference in DMI was associated with a lower NDF content and higher digestibility (710 vs 640 g/kg) of the cultivar with an increased WSC content. In 2002, there were weak negative relationships between the NDF, ADF and ADL contents in grass with the DMI (R^2 = 0.20, 0.25 and 0.35, respectively). The ADL in the NDF was lowest in cultivar 4 and highest in cultivar 3 and this was associated with the highest and lowest DMI, but the overall relationship was weak (R^2 = 0.30). Taweel (2004) showed that there were no significant differences among cultivars in ruminating behaviour and rumen fill in 2002. Moreover, the rumen fill of continuously stocked dairy cows measured four times on a day, showed that the rumen fill was highest in the evening and thus may have limited the intake, but that during the day the rumen fill remained below 80 % of the rumen fill in the evening (Taweel et al., 2004). The metabolic control of the feed intake depends on the absorbed nutrients from the rumen and small intestine and because this was not measured in our experiments this effect will not be discussed.

Milk production and composition

The main determinant of MP is the digestible DMI and this was also observed among cultivar means in these two experiments. Miller et al. (2001) observed a similar increase in MP with an increase in digestible DMI between two perennial ryegrass cultivars differing in their WSC content. Lee et al. (2002[b]) found higher ($P < 0.05$) propionate and butyrate proportions and a lower ($P < 0.05$) acetate proportion when steers received grass with an increased WSC content compared to a control. A higher propionate production may have stimulated MP and to a lesser extent milk protein yield (Thomas and Martin, 1988). In 2002, cultivars 1 and 4 with a higher ($P < 0.001$) WSC content had a higher MP and milk protein and fat yield. However in 2003, no differences among cultivars were found in MP and milk protein and fat yield among these cultivars, whereas there was a similar difference in WSC content among cultivars as in 2002. In a stall feeding experiment with these cultivars, similar differences in WSC content among cultivars were found, but there were only minor effects on the proportions of volatile fatty acids in the rumen liquid (Taweel, 2004).

Milk urea N and N utilisation

In the liver, urea N is synthesized from NH_3 that originates from two sources: the surplus NH_3 not incorporated in microbial protein in the rumen, and NH_3 from deamination of amino acids in the intermediate protein metabolism. Urea equilibrates in aqueous solutions and therefore milk urea N (MUN) content is highly correlated with blood urea N content as reported by many authors (listed by Hof et al., 1997). The NH_3 in the blood is passively diffused from the rumen and depends on the ruminal-NH_3 concentration (Lobley et al., 2000). The OEB content of the grass reflects the difference between the potential microbial protein synthesis based on degraded feed CP and that based on energy available for microbial fermentation (Tamminga et al., 1994). The higher OEB content of cultivars 1 and 3 in 2003, but not in 2002, was

Table 4 Milk production, milk composition and N utilisation of four perennial ryegrass cultivars of twelve dairy cows in 2002 and 2003.

	Cultivars[1]						Significance[2]		
Factor[3]	1	2	3	4	mean	s.e.	C	P	A
2002									
Milk production (kg/d)	28.1^{a}	27.0^{ab}	26.0^{b}	28.8^{a}	27.5	0.5	***	***	***
FPCM (kg/d)	27.4^{a}	26.2^{ab}	25.4^{b}	27.8^{a}	26.7	0.4	***	***	***
Milk fat (g/d)	1092.9^{a}	1047.3^{ab}	1020.8^{b}	1105.7^{a}	1067.7	14.5	***	***	***
Milk protein (g/d)	877.7^{ab}	819.3^{bc}	790.1^{c}	884.1^{a}	843.2	15.5	***	***	***
Milk fat (g/kg)	39.0	38.8	39.4	38.4	38.9	0.4			***
Milk protein (g/kg)	31.3	30.4	30.4	30.8	30.7	0.2	*	*	***
MUN (mg/dL)	14.0	14.5	15.6	14.4	14.3	0.6		***	**
N intake (g/d)	511.9	481.5	448.2	516.0	490.2	18.0	§	**	
N milk (g/d)	137.1^{ab}	128.2^{bc}	123.7^{c}	139.0^{a}	132.0	2.4	***	***	***
N utilisation	0.25	0.24	0.24	0.25	0.25	0.01		***	*
2003									
Milk production (kg/d)	25.8	25.8	25.2	25.7	25.3	0.3		***	***
FPCM (kg/d)	24.4	24.5	24.7	23.7	24.1	0.4		***	***
Milk fat (g/d)	996.7	974.6	932.4	933.1	952.7	21.1	§	***	***
Milk protein (g/d)	789.5	783.9	741.9	764.3	761.1	17.1		***	***
Milk fat (g/kg)	39.2^{a}	37.9^{b}	38.6^{ab}	37.9^{b}	38.8	0.3	**	***	**
Milk protein (g/kg)	31.0	30.5	30.7	30.7	30.7	0.2		***	**
MUN (mg/dL)	16.0^{ab}	15.0^{b}	16.5^{a}	14.9^{b}	16.0	0.3	*	***	*
N intake (g/d)	563.0	544.8	573.5	578.0	654.2	21.2		***	*
N milk (g/d)	123.7	122.9	116.9	119.8	121.1	2.7		***	***
N utilisation	0.20	0.21	0.19	0.19	0.20	0.01		***	*

1 Means of cultivar within row with different superscripts differ significantly ($P < 0.05$).

2 Significance of the main effects cultivar (C), period (P) and animal (A) with * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$, and § approaching significance ($P < 0.10$).

3 FPCM = fat and protein corrected milk, MUN = milk urea N, N utilisation = efficiency of the secretion of N in milk as proportion of N intake.

associated with a higher MUN content than cultivars 2 and 4. However, cultivars 1 and 3 had a similar CP and OEB content, but differed in their WSC and NDF content. Boudon and Peyraud, (2001) reported that after ingestive mastication of perennial ryegrass, free sugars (60.8 %) and non protein nitrogen (57.7 %) were released easily from plant cells, fructans (42 %) were released intermediate, and a smaller proportion of the chlorophyll N (28 %) and protein N (22 %) were released. After 1.5 h in the rumen more than 90 % of the WSC was released compared with 40 to 50 % of chlorophyll N (Boudon and Peyraud, 2001). Theoretically, the WSC may supply energy to rumen microbes to balance the supply of N, mainly from non protein nitrogen within a meal, and the supply of N from degraded protein within a meal and from previous meals. In vitro measurements indicated a higher efficiency of microbial protein synthesis, with addition of different levels of glucose and inulin (80:20) to grass as substrate (Lee et al., 2003). In vivo, with steers fed grass differing in WSC content, however, the efficiency of microbial protein synthesis and flow of N to the duodenum as proportion of N intake were not affected by difference in WSC content (83 g/kg DM) (Lee et al., 2002[b]). Moreover, supplementing grazing dairy cows with non-structural carbohydrates at time of grazing or four hours after grazing, did not affect the mean ruminal-NH_3, blood urea N content and N secretion in milk per day (Kolver et al., 1998). The recycling of urea within the body to the rumen may reduce the effect within a day of imbalances in the energy and N supply to rumen microbes. Although urea recycled to the rumen as proportion of total urea synthesis decreases with an increase in CP content (Kennedy and Milligan, 1980), the absolute amount recycled remained similar with increased CP contents (Marini and Van Amburgh, 2003). Other confounding factors of importance are energy supply to rumen microbes from structural carbohydrates, depending on NDF content and fractional rate of degradation (Van Vuuren et al., 1992), and the surplus of absorbed protein resulting in metabolic N losses (Hof et al., 1994). Therefore, in our experiment, the cultivars with an increased WSC content did not result in a consistently lower MUN content neither in a higher proportion of N intake secreted in milk.

Conclusions

The mean HA was approximately 2 times the herbage DMI, and both the HA and SSH were not directly related with differences in grazing behaviour and DMI In 2002, the herbage DMI was reduced of one cultivar (3), that was severely infected with crown rust, that may have reduced palatability and hence DMI. In 2003, the crown rust infection was less severe, differences among cultivars were smaller and this was associated with no differences in herbage DMI. In both years, the four cultivars differed in their chemical composition, and the differences in decreasing order were: WSC > CP > NDF ($P < 0.001$). The reduced DMI of one cultivar in 2002 was associated with a lower WSC content, but with a similar difference in WSC content among cultivars in 2003, no differences in DMI were found. The lower DMI of one cultivar was associated with a lower MP and milk fat and protein yield. Only small differences in MUN content among cultivars were observed, and the proportion of N secreted in milk was very similar among cultivars in both years.

Chapter 6

General Discussion

General Discussion

Herbage quality is determined by genetic, environmental and management factors and their interactions. In this study, the genetic component was represented by eight perennial ryegrass cultivars (*Lolium perenne* L.), chosen for their expected contrasting characteristics, i.e., digestibility, water soluble carbohydrate (WSC) content, and also earliness (Smit, 2005). The environmental component was mainly present in the form of the four different years, with associated different weather conditions, as well as the seasonal weather fluctuations within years. Besides, different paddocks were used, but all were located closely together on a clay soil. One of the main management factors was N fertilization level which was always similar for all cultivars harvested during a period, and defoliation. In 2000 and 2001 a cutting treatment was imposed, and all cultivars in a replicate block were always cut on the same moment. In 2002 and 2003 a grazing treatment was imposed, again with a similar treatment for all cultivars in a replicate block. The experimental design aimed to study primarily the genetic component, i.e., differences among cultivars, while other factors were kept constant.
Cultivars were cut or grazed at a target yield, estimated by sward surface height, and the defoliation decision was based on the average yield estimate of the cultivars. The days of regrowth were always the same for cultivars within a replicate plot. This implies that the slower growing cultivars were harvested at a lower actual yield than the faster growing ones. In this thesis, mainly mean annual values are presented of all cultivars, whereas they also differ within a period. Actual dry matter yield and chemical composition of the herbage differed among years (Table 1) and this was associated with different amounts of N applied, with fluctuating weather conditions, and other circumstances (e.g., a longer winter grazing period by sheep than anticipated and hence a delayed heading in spring, due to veterinary regulations around the Foot and Mouth disease occurrence in The Netherlands in 2001).

Effect of N fertilisation on herbage yield

The annual fertilizer applied differed between the experimental years with approximately 300 kg N/ha per yr in 2000, 400 kg N/ha per yr in 2001, and around 220 kg N/ha per yr in the two years of grazing experiments. The herbage yield and chemical composition were not determined of cuts made before and after the months preceding (April till June) and following (September till November) the experiments, and therefore the annual N fertilisation and the proportion of N recovered in the herbage could not be determined. The amount of N fertilizer applied per period differed between years, as shown in Table 1. In some weeks the growth rate was low, therefore the N fertilisation level had to be increased to stimulate growth in order to obtain the target DM yield of 2000 kg DM/ha. In 2000, the mean N fertilizer level per period averaged 42 kg/ha and was lower than in the other years, when it ranged between 68 and 75 kg/ha per period. In 2000 and 2003, within each experiment there was almost no difference between the periods as indicated by the small SD (Table 1). In 2001 and 2002, the N fertilisation level varied between the periods as shown by the higher SD in these two years. The mean DM yield was slightly higher than 2000 kg DM/ha/period (Table 1) and differences among cultivars were found as shown in Table 2 in Chapter 3 and Table 2 in Chapter 5. In general, N fertilisation has two main effects: firstly it stimulates growth of the herbage and secondly it influences the crude protein (CP = N × 6.25) content of the herbage (Peyraud and Astigaragga, 1998). In our experiments the first effect was difficult to determine as N fertilisation was adjusted to (expected) herbage growth. Moreover, herbage growth depended on growing days and the growing conditions such as rainfall, temperature and irradiation and these effects were confounded with fertilizer N applied. Deenen (1994) reported that an optimum DM yield of grassland in the Netherlands was reached at a fertilisation level of 390 to 445 kg N/ha per yr under cutting, depending on the soil type, and of 220 to 308 kg N/ha per yr under grazing. However, with increasing N fertilisation levels the proportion of N recovered in the herbage decreased linearly. In agreement, the mean N recovery was higher in 2000 than in the other three years (Table 1). In the stall feeding experiment, cultivars 1 to 6 were sown on one paddock and cultivars 7 and 8 on an adjacent paddock. Although the N fertilisation level was almost the same on both paddocks, the mean CP content of the herbage of cultivars 7 and 8 was higher than of cultivars 1 to 6 (Chapter 2 and 3). This may partly be attributed to different growing conditions (precipitation, temperature, radiation) in the different periods and partly to a higher N supply from the soil, although soil analyses prior to sowing did not indicate a difference in N supply between paddocks. In the stall feeding experiment, no significant differences were found in the recovery of N among cultivars 1 to 6 and very small differences were found between cultivars 7 and 8 (difference in 2001: 0.03 kg N/ kg N fertilizer, P = 0.04). In the first year of the grazing experiment, N recovery of cultivars 2 and 3 was lower than cultivars 1 and 4 and this was mainly attributed to differences in DM yield. The second year no differences (P = 0.24) in N recovery among cultivars were found. The N recovery could not be corrected for the herbage growth, and thus N yield, when no N fertilisation was applied, because this was not measured. Therefore, the total response of N fertilisation was presented, and this differs from the marginal response of N fertilisation on N recovery by the herbage. Moreover, to determine the opportunities for increasing the N recovery by cultivar selection for this trait, these measurements should be conducted in a more controlled environment, with N fertilisation levels as treatments.

Effect of N fertilisation on chemical composition and nutritive value

The fertilizer N was positively related with the CP content of the herbage (r = 0.56 and r = 0.67; P < 0.001 in the stall-feeding and grazing experiment, respectively). In 2000, with almost the same fertilizer level the CP content differed between the six cultivars grown on one paddock compared to the two cultivars grown on another paddock as discussed in Chapter

3. The mean CP content of the six cultivars was 158.2 ± 1.5 g/kg DM, whereas the mean CP content of the other two cultivars (7 and 8) was 196.4 ± 0.1 g/kg DM in 2000. This difference between paddocks was not found in the other years. In the other three years the N fertilisation level per period was higher, and the mean CP content ranged from 179.4 to 201.7 g/kg DM. The increase of CP content with N fertilisation and the mean CP contents at these N levels were in agreement with data reviewed by Peyraud and Astigaragga (1998) and in the experiment described by Valk et al. (2000).

In the grazing experiment, the animals grazed twice on each paddock and therefore the N excreted with urine and faeces in the first period may theoretically have supplied N to the herbage in the second period. However, this was probably of minor importance. The recovery of dung N in herbage N in a pot experiment with perennial ryegrass after 16 weeks of growth was only 7-10 % (Jorgensen and Jensen, 1997), and in grazing experiments at a fertilisation level of 250 kg N/ha/yr, the mean N recovery after 10 months of dung N and urine N was only 8 % and 16 %, respectively (Deenen, 1994). Moreover, in our experiment, the dung N was partly removed by cutting and removing the sward residue after the first grazing period, which was done to obtain a homogeneous sward for the second grazing period. Despite this, the sward was more patchy, with a higher variation in sward surface height (SSH) within a plot (Mean ± SD: first two periods: 16.5 ±1.2 and 14.8 ± 1.2 cm, last two periods: 17.8 ± 2.0 and 15.4 ± 1.6 cm in 2002 and 2003, respectively) and colour differences were observed which may have been related with urine patches. With a similar N fertilisation (74 ± 1 kg N/ha/period) in 2003, the mean CP content in the last two periods was 220.1 g/kg DM and in the first two periods 183.5 g/kg DM and this may partly be attributed to the uptake of urine N by the herbage.

The N fertilisation level was negatively correlated with WSC content ($r = -0.67$ and $r = -0.87$ in the stall feeding and grazing experiment, respectively). In the grass an inverse relationship between CP and WSC content was found, and this effect can be attributed to a decrease in the utilization of carbon chains for protein synthesis and for production of the energy required for the nitrates reduction step before protein synthesis (Peyraud and Astigaragga, 1998). Moreover, this inverse relationship was higher for grass species with a high WSC content (Wilman and Wright, 1978), and this may explain the higher decrease in WSC content per unit increase in CP content in the overall linear relationship in our experiments (WSC (g/kg DM) = 382.1 (± 22.0) -1.38 (± 0.12) CP (g/kg DM); $R^2 = 0.51$; RMSE = 32.8 g/kg DM) than reported by Peyraud and Astigarra (1998) and Valk et al. (2000). However, the regression equations varied between years, with higher slopes in 2000 and 2002 when the CP content was lower and WSC content higher than in 2001 and 2003 (Figure 1 and Table 1). Although within a year no significant quadratic relationships were found, using the complete data set a significant quadratic relationship was determined: WSC = 935.7 (±144.7) -7.34 (±1.55) CP + 0.0158 (±0.004) CP^2; $R^2 = 0.55$, $P < 0.01$, RMSE = 31.4 g/kg DM. Within cultivars the inverse relationship between WSC and CP was almost absent (Chapter 3 and 5). Smith et al. (2002) also reported that the increased WSC content of perennial ryegrass cultivars with a consistently higher WSC content than controls was not associated with a lower CP content, but coincided with a lower neutral detergent fibre (NDF) content in the shoot.

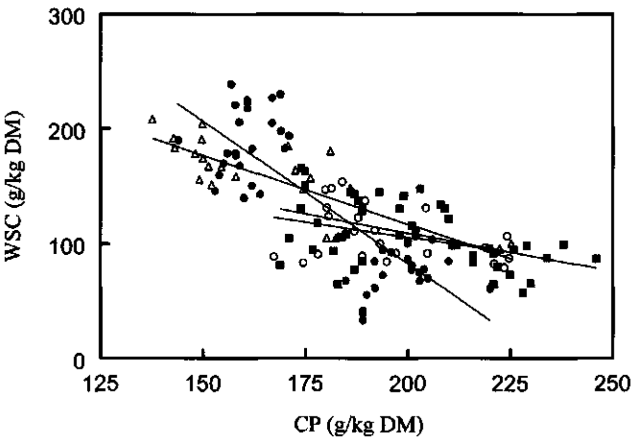

Figure 1 Relationships between WSC and CP content in grass in 2000 (Δ), 2001 (○), 2002 (●), and 2003 (■).

The nutritive value of the herbage in the four years was calculated based on the chemical composition determined with near infrared reflectance spectroscopy (NIRS) in the Barenbrug laboratory. The accuracy of the prediction of CP and WSC was high ($R^2 > 0.95$) and good for the NDF content ($R^2 = 0.65$-0.70), although the NIRS predicted higher NDF values than the wet-chemistry (see Smit (2005) for detailed discussion). The Net Energy for Lactation (NEL) was calculated according to Van Es (1978), and the DVE (intestinal digestible protein) and OEB (rumen degradable protein) were calculated according to Tamminga et al. (1994) and in these calculations the regression equations for fresh grass were used (CVB, 1999) (Table 1). The NEL was lower in the stall feeding experiment than in the grazing experiment and this was mainly related with the higher ash content and thus lower OM content in the stall feeding experiment. Probably, the higher ash content was partly caused by contamination with sand due to cutting the sward at approx. 6 cm and loading the grass in a wagon in the stall feeding experiment in comparison with the hand-plucked grass samples in the grazing experiment. Small differences were found in the DVE between years and these were similar to the CP content. The mean OEB content was lower in 2000 and in 2002 than in 2001 and 2003.

Table 1 Mean and standard deviation (SD) of grassland data, herbage composition and nutritive value of in the two years of stall feeding and in the two years of grazing experiments.

	Stall feeding				Grazing			
	2000 (n = 24)		2001 (n = 24)		2002 (n = 46)		2003 (n = 43)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Grassland								
N fertilizer (kg N/ha/period)	42	4	75	19	68	12	74	1
DM yield (kg DM/ha/period)	2124	160.0	2192	402.0	2138	454.5	2251	400.6
N yield (kg N/ha/period)	57.0	9.1	68.1	10.7	61.4	13.7	72.2	12.5
N recovery[1] (kg/kg)	1.34	0.13	0.95	0.21	0.91	0.17	0.98	0.18
Herbage composition[2]								
OM (g/kg DM)	877.7	5.4	881.1	8.3	902.7	5.4	916.3	4.0
CP (g/kg DM)	184.5	24.6	196.9	13.9	179.4	20.6	201.7	21.5
WSC (g/kg DM)	130.4	36.1	99.4	19.7	133.2	62.6	109.0	28.3
NDF (g/kg DM)	464.6	20.5	482.2	24.7	473.4	37.8	479.8	14.7
ADL (g/kg DM)	26.5	1.7	24.0	1.6	26.3	4.4	32.0	2.7
CF (g/kg DM)	260.8	13.6	266.0	13.7	259.0	17.3	256.0	13.1
OK (g/kg DM)	392.3	27.1	378.2	18.3	424.3	34.2	418.6	12.8
ADL/NDF (g/kg)	57.2	3.4	49.8	3.0	55.3	6.2	66.8	5.9
Nutritive value[3]								
NEL (MJ/kg DM)	5.88	0.22	5.89	0.16	6.14	0.15	6.34	0.17
DVE (g/kg DM)	82.9	6.0	85.4	3.4	86.3	3.1	92.8	4.9
OEB (g/kg DM)	27.3	20.4	36.8	11.4	18.5	17.5	33.8	16.3
DVE/NEL (g/MJ)	14.1	0.6	14.5	0.3	14.1	0.5	14.6	0.5
OEB/NEL (g/MJ)	4.6	3.3	6.2	1.9	3.0	2.9	5.3	2.4

1 N recovery = proportion of N fertilizer (kg N/ha/period) recovered in the herbage (kg N/ha/period).

2 Chemical composition predicted by near infrared reflectance spectroscopy. OM = organic matter, CP = crude protein, WSC = water soluble carbohydrate, NDF = neutral detergent fibre, ADL = acid detergent lignin, CF = crude fibre, OK (other carbohydrates) = OM - CP – CF - 40 (= fat). ADL/NDF = ADL in the NDF.

3 NEL = net energy for lactation, DVE = intestinal digestible protein (undegraded feed and microbial protein – metabolic faecal protein losses), OEB = rumen degraded protein balance.

Herbage intake

The main aim of the experiments described in this thesis was to determine if there was an effect of perennial ryegrass cultivar on dry matter intake (DMI), milk production (MP) and milk composition by stall-fed and grazing dairy cows. Cows were used after the peak of the lactation, to avoid the higher occurrence of metabolic disorders with cows in early lactation due to a decreased intake (Ingvartsen and Andersen, 2000) and we expected that after the peak of lactation the dry matter intake pushes the milk production (Tamminga and Hof, 2000). Therefore, the chemical composition of the grass and the digestible DMI determine the nutrients available for milk production and for milk fat and milk protein production.
In the stall-feeding experiment, there was less interaction between the animals and sward characteristics than in the grazing experiment and during stall feeding the feed residues were low to avoid selection within the offered grass. Therefore, the effects of sward characteristics was expected to be more indirect, for example increased NDF and ADL contents of the grass were associated with an increased proportion of stems in the sward. The daily individual intake was measured accurately in the stall feeding experiment, whereas it was estimated with the *n*-alkane marker technique in the grazing experiment (see Dove and Mayes, 1996 for detailed description of this technique). Smit et al. (2005) described the accuracy of the latter method compared with determination of the herbage mass before and after grazing, and with the estimated intake based on energy (NEL) requirements. Based on these comparisons, it was concluded that the *n*-alkane method gave a reliable estimate of the intake in the two years of grazing. In the experiments, the individual intake was measured in a two-week period, where in the second week the mean individual DMI and MP were measured. Although the adaptation period of one week was short, we expected to be able to measure effects of the diet on DMI and MP, because the relative changes in diet were small.
The regulation of intake is a complex process and depends on the palatability of the feed and the post-ingestive feedback (Forbes and Provenza, 2000). To understand the complex process, often the short term and long term regulation are distinguished and it is recognised that effects of the short term regulation, as palatability, influences the DMI over longer periods (Allen, 1996; Forbes and Provenza, 2000). The assessment of palatability (aversions or preference) is measured by the intake of an animal when the animal is given a choice to select in the diet and/or by an effect on meal size and frequency (Kyriazakis and Emmans, 1992; Forbes, 1995; Tolkamp et al., 1998). The WSC content of herbage has been associated with diet selection by ruminants in stall feeding and grazing trials (Orr et al., 1997; Ciavarella et al., 1998; Fisher et al., 1999; Leury et al., 2002). The preference of dairy cows grazing tall fescue cultivars was associated with a higher WSC content (Mayland et al., 2000) and a similar association was found with the cultivars used in our experiment (Smit, 2005). However, the preference for WSC as measured by a higher intake when given the opportunity to select among cultivars, did not result in a higher DMI of these cultivars when measured during a two-week period in

which only one cultivar was fed or grazed. The two cultivars with an increased WSC content did not affect the DMI in the stall-feeding experiment (Chapter 3) and in the second year of the grazing experiment (Chapter 5). Moreover, dividing the grass into two categories with a low or a high WSC content did not indicate an effect on DMI (Taweel, 2004). In the grazing experiment in 2002, two cultivars became heavily infested with crown rust (*Puccinia coronata* f.sp. *lolii*) and these cultivars had both a lower WSC content than the other two cultivars. The effect on palatability of the two cultivars with an increased WSC content was, therefore, confounded with a lower infestation of crown rust. In practice it is known that grass heavily infested with crown rust reduces the DMI of grazing animals, and thus the aversion for crown rust may have reduced the DMI of the two heavily infested cultivars. In the stall feeding experiment, almost no crown rust infestation occurred. In the second year of grazing the infestation was less severe and the difference among cultivars was less. The increased WSC content of grass in our experiments, therefore, was not consistently associated with a higher mean DMI measured in a two-week period. Crown rust resistance has been an important breeding goal in grass (Kimbeng, 1999) and our data of the grazing experiment showed the importance of a continuation in increasing the crown rust resistance of perennial ryegrass.

The post-ingestive feedback depends on many factors that influence the DMI. The DMI of forages, that have a relatively high cell wall (NDF) content and that are bulky, are assumed to have a limitation due to the physical fill of the feed in mainly the reticulo-rumen (Van Soest, 1994; Mertens, 1995; Allen 1996). The physical properties of the feed are associated with the cell wall content (NDF) and its lignification (acid detergent lignin = ADL). The fill in the rumen depends on the balance between feed intake and the sum of degradation in the rumen and passage from the rumen to the abomasum. The differences in NDF and ADL content among cultivars were relatively small and therefore no differences in the physical fill were expected to influence DMI (Chapter 3 and 5). Moreover, the fractional rate of degradation of NDF in the rumen, the undegradable fraction of NDF (Chapter 2) and the fractional rumen clearance of NDF (Taweel et al., 2005) varied only slightly among cultivars. Other factors including an increased proportion of stems in the sward due to a delayed heading date as discussed in Chapter 3, and the maturity of the sward and/or fertilisation level affected the fractional rate of degradation (Van Vuuren et al., 1991; Steg et al., 1994) and the rumen fill (Chilibroste et al., 2000) to a higher extent than the cultivar. Based on a compilation of rumen fill data with diets of grass and grass silage, Van Vuuren (1993) concluded that rumen fill was not limiting the DMI of fresh grass. Moreover, the rumen fill of grazing dairy cows varies during the day and rumen fill may influence the DMI during the evening grazing bout, but this effect was most likely small on the grazing bouts during the day (morning and afternoon) as eating was stopped before maximal rumen fill was reached (Taweel et al., 2004;

Chilibroste et al., 2000). Furthermore, the distension of the rumen wall by the volume of the fresh grass may play a role. The internal water of fresh grass was associated with the distension and influenced DMI (Estrada-Cabrera et al., 2004), although in comparing the volume of fresh grass and grass silage in the rumen no clear relationship was found (Van Vuuren, 1993). The DM content of the cultivars was very similar in our experiments (Chapter 5), therefore the volume of the grass was not an important factor on cultivar differences in DMI. The daily DMI is determined by the number of bites, the mass per bite and the time spent eating. Therefore, eating behaviour determines the feed intake. Under grazing conditions, the grazing time, bite rate and bite size to eat the large amount of fresh grass may also impose constraints on DMI (Chilibroste et al., 1997; Kolver, 2003). Unfortunately, in the stall feeding experiments the eating behaviour was not measured to make a comparison with the grazing experiment. In the two years of grazing, the time spent grazing did not differ significantly among cultivars and the time spent grazing was almost similar in both years with 511 ± 19 and 535 ± 23 min (Chapter 5). The cows spent almost 9 h per day grazing and this is similar as reported by Delagarde et al. (1997) with strip grazing and by Gibb et al. (1999) with continuous stocking at a high SSH (9 cm). However, at lower SSH (5 cm and 7 cm), the cows increased the grazing time and Rook (2000) reported up to 12 h per day. In the eating behaviour meals can be distinguished and the daily meal pattern and the meal frequency can be determined (Tolkamp et al., 2000). Comparing a diet with a low concentrate to forage (C:F) ratio in comparison with a high C:F ratio, the DMI was lower on the low C:F ratio diet than on the high C:F diet (Tolkamp et al., 2002). However, the meal pattern did not differ between these treatments, whereas the DMI per meal and the intake rate were reduced on the low C:F ratio (Tolkamp et al., 2002). With dairy cows continuously stocked, a higher intake rate was observed in the evening grazing bout than in grazing bouts during the day (Taweel et al., 2004). Dairy cows therefore seem to optimise their eating/ grazing behaviour, but this does not seem to be a constraint on DMI. Moreover, Chilibroste et al. (2000) and Taweel et al. (2004) showed that the end of a grazing bout could not be attributed to rumen fill nor rumen fermentation end products. In general, the feed intake seems to be regulated by an integration of various factors of the metabolic system and many of these factors are additive (Forbes, 1996; Forbes and Provenza, 2000). In our experiments, no significant cultivar differences were found in the DMI by dairy cows in three out of the four years. Therefore, it can be concluded that the effect of cultivar on these factors was too small to find differences in DMI.

Table 2 Mean and standard deviation (SD) of animal data in the two years of stall feeding and in the two years of grazing experiments by twelve dairy cows.

	Stall feeding				Grazing			
	2000 (n = 72)		2001 (n = 72)		2002 (n = 46)		2003 (n = 43)	
Animal data[1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight (kg)	608	42	535	43	534	27	549	42
DIM (d)	157	45	163	46	88	21	102	23
DMI (kg/d)	20.7	1.6	16.9	1.4	19.9	2.3	20.2	2.6
Herbage DMI (kg/d)	16.6	1.7	14.4	1.4	17.2	2.3	17.5	2.6
Concentrate DMI (kg/d)	4.1	-	2.5	-	2.7	-	2.7	-
*d*DM (%)	82.8	3.7	79.8	4.3	-	-	-	-
Milk production (kg/d)	27.2	4.4	23.4	3.9	27.5	3.4	25.7	2.5
FPCM (kg/d)	27.2	4.1	23.0	3.4	26.7	3.3	24.8	2.5
Milk fat (g/kg)	39.8	4.8	39.8	3.4	38.9	3.1	38.4	4.5
Milk protein (g/kg)	33.9	2.0	31.5	2.1	30.7	1.4	30.7	1.5
Milk fat (g/d)	1077.2	182.9	924.5	139.7	1067.1	146.9	963.4	132.1
Milk protein (g/d)	919.3	132.4	731.1	99.6	842.4	100.0	772.8	93.1

[1] DIM = days in milk, DMI = dry matter intake, *d*DM = dry matter digestibility, FPCM = fat and protein corrected milk.

Milk production and composition

The main determinant of milk production is DMI. In the two years of stall feeding experiments and the second year of grazing experiments, no significant differences in DMI and hence in MP among cultivars were found. These findings are in agreement with the results of stall feeding experiments with fresh grass from pasture with different N fertilisation levels (Van Vuuren, 1992; Peyraud et al., 1997; Valk et al., 2000). As mentioned before, the effect of fertilizer N has more effect on WSC and CP content of grass than on the NDF content. In the experiment of Van Vuuren et al. (1992) a difference in CP of 30-40 g/kg DM was found between treatments, but when NDF content did not differ the DMI was not affected. In studies in France with 0 or 80 kg N fertilisation per cut, differences in CP of 40 g/kg in DM and in WSC content of 65 g/kg DM were observed but even then DMI was similar between treatments (Peyraud et al., 1997). With three levels of N fertilisation (150, 300 and 450 kg N/ha per yr) no effect on DMI was found in spring, but in autumn a lower DMI was observed on the 150 kg N/ha per yr than on the 300 and 450 kg N levels (Valk et al., 2000). However, almost no differences in DMI and MP were observed between the two higher N fertilisation levels, although the mean CP content differed 40 g/kg DM and the mean WSC content 20 g/kg DM (Valk et al., 2000). It can be concluded that in the stall feeding experiment, the differences in chemical composition among cultivars were too small to find a significant effect on the DMI and hence on the MP. In the grazing experiment, in the first year the lower DMI of one cultivar was also associated with a lower MP, whereas in the second year no differences in DMI and MP were observed (Chapter 5).

In the stall feeding experiment, the MP was well correlated with DMI (r = 0.75) whereas the DM digestibility (*d*DM) was less clearly correlated with DMI (r = 0.52) and MP (r = 0.37) (Table 3). This was in agreement with the results of Valk et al. (2000) who did not find a relationship between DMI and *d*DM at similar digestibilities as in our experiment. The correlation coefficients of intake with CP, WSC and NDF were lower than with the DMI and are therefore not reported in Table 3. The mean milk protein contents were low in our experiments (Table 2), with 30.7 to 33.9 g/kg milk. However, the DVE intake was above the requirements for milk protein production (19.5 to 53.2 %, Table 4). The supply of amino acids for milk production was therefore most likely not limiting milk protein synthesis. There was a positive correlation between DMI and milk protein production (r = 0.81) and this correlation was similar for DVE intake with milk protein production. Milk protein production depends on energy supply as well as protein supply and therefore the ratio between the availability of DVE and NEL for production (intake – maintenance requirements) is an important factor influencing the milk protein production and the efficiency of milk protein synthesis (Hof et al., 1994). The mean ratio between DVE and NEL available for milk protein synthesis ranged in both years between 18.4 and 19.8 g/MJ and the mean efficiency of DVE available for milk protein synthesis was between 43.6 to 54.1 % (Table 4). This mean efficiency corresponded quite well with the efficiencies determined with the regression equation (Milk protein eff. = 113.5 -3.11 DVEc/NELc) reported by Hof et al. (1994) (viz. mean efficiency between 51.9 and 56.3 %). However, in the stall feeding experiment this relationship was poor (Milk protein eff. = 91.0 -2.0 DVEc/NELc, R^2 = 0.10) and it was absent in the grazing experiment (P = 0.83).

The difference in milk protein efficiency between 2000 and 2001 was similar to the difference in N secreted in milk as proportion of N intake (Table 5). Besides dietary factors, the low milk protein content could be attributed to genetic animal factors as the milk protein content was relatively low in the pre-experimental periods (Chapter 3).

Table 3 Correlation coefficients between dry matter intake (DMI), DM digestibility (*d*DM), milk production (MP), fat and protein corrected milk (FPCM) and milk fat and protein production in the stall feeding experiment.

	DMI	*d*DM	MP	FPCM	fat production	protein production
DMI	1					
*d*DM	0.52	1				
MP	0.75	0.37	1			
FPCM	0.72	0.39	0.95	1		
Fat production	0.62	0.35	0.83	0.95	1	
Protein production	0.81	0.43	0.92	0.93	0.82	1

All correlation coefficients have a significance of $P < 0.001$.

In 2001, the mean NEL balance was zero and on average met the daily animal energy requirements, whereas in the other years the mean NEL balance was positive. The mean daily body weight gain was almost negligible in 2001, whereas in 2000 the animals used the energy to gain body weight (0.46 kg/d). In the grazing experiment, the net energy requirements for maintenance were increased with 20 % according to the CVB, 2000. This additional energy is required for the higher energy expenditure for walking and grazing (Van Es, 1978). Moreover, based on feeding trials in metabolism cages and therefore not including the energy costs for walking and grazing, a 10 % increase of the maintenance requirements was proposed to compensate increased energy costs in the metabolism (oxygen consumption in the gastro-intestinal tract, synthesis of urea and excretion in urine) of grazing animals (Bruinenberg et al., 2002). Increasing the maintenance requirements with 10 % in the stall feeding experiment resulted in a lower positive NEL balance in 2000 and a negative NEL balance in 2001. In the grazing experiment the NEL balance was in both years positive, although in 2003 the mean body weight gain was slightly negative. In both summers, the mean daily temperature reached almost 25 °C with a maximum temperature of 32.8 °C, measured in the shadow (Taweel, 2004). Although the calculated NEL balance was positive in these periods, the animals lost body weight and this may be attributed to increased energy costs by an increased respiration rate to actively maintain body temperature (Fox and Tylutki, 1998). However, the grazing time was reduced at higher temperatures (Taweel, 2004) and the energy costs for grazing were therefore most likely lower. The results on the NEL should be interpreted with care, as the variation among animals was high as indicated by the high SD, the DMI by grazing animals was estimated, and the measurements for body weight were only done once every two weeks.

Seasonal effect on MP

The stall feeding experiments lasted 12 weeks and the grazing experiments 8 weeks. In the stall-feeding experiments the cows were in mid-lactation (DIM was 157 ± 45 in 2000 and 163 ± 46 (mean ± SD)), whereas in the grazing experiments the cows were in early to mid-lactation with 88 ± 21 and 102 ± 23 DIM. In all experiments, with the progress in DIM the MP per cow decreased. In the stall feeding, the decrease was on average 62 g/d (R^2 = 0.38) and in the grazing experiment the decrease was on average 93 g/d (R^2 = 0.46). On a weekly basis these figures can be recalculated to decreases of 1.7 and 2.5% respectively, which can be considered as a normal persistency. This effect may have consequences when the relationships between N intake and N secreted with milk will be determined. However, the relationships between DMI and DIM in the experiments were weak and a large variation between individual animals was found. Moreover, the protein yield was poorly correlated with DIM (R^2 = 0.24), the decrease was 2.2 and 1.6 g milk protein per DIM in the stall feeding and the grazing experiment, respectively. This decrease was small and therefore DIM did not influence the relationships determined between N intake and N secreted in milk.

Table 4 The net energy for lactation (NEL), intestinal digestible protein (DVE) and rumen degradable protein balance (OEB) intake, and as percentage of requirements for milk synthesis and the body weight (BW) change in the stall feeding and grazing experiment.

	Stall feeding				Grazing			
	2000 (n= 72)		2001 (n= 72)		2002 (n= 46)		2003 (n= 43)	
Factor[1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NEL intake (MJ/d)	129.0	11.8	104.0	8.9	125.5	15.4	131.5	16.7
DVE intake (g/d)	1819.6	195.5	1499.7	135.5	1763.8	209.1	1910.9	256.6
NEL balance (MJ/d)	7.8	10.5	0.0	7.9	2.9	12.6	15.5	17.2
OEB (g/d)	390.9	362.9	489.3	176.1	248.3	295.6	534.1	302.0
NELr (% requirements)	107.0	9.8	100.6	7.5	102.5	10.2	113.8	15.3
DVEr (% requirements)	117.8	16.1	122.6	10.8	124.7	13.8	148.4	27.2
DVEc/ NELc (g/MJ)	18.4	0.9	19.5	0.5	19.3	1.4	19.8	1.0
Milk protein eff. (%)	54.1	6.5	52.5	4.6	51.2	5.9	43.6	7.5
BW change (kg/d)	0.46	1.06	0.08	1.15	0.30	0.83	-0.03	0.98

[1] NEL = net energy for lactation, DVE = intestinal digestible protein, OEB = rumen degradable protein balance, NELr = NEL intake/ NEL requirements × 100, DVEr = DVE intake/ DVE requirements × 100; NEL available for milk synthesis (NELc) = NEL intake – NEL maintenance; DVE available for milk synthesis (DVEc) = DVE intake - DVE maintenance, Milk protein eff. = milk protein/ DVEc × 100, BW = body weight.

Nitrogen utilisation by grass fed dairy cows

The efficiency of conversion of N into products on a dairy farm is low and ranges from 0.12 to 0.26 of N input (Castillo et al, 2000) and there are substantial N losses that have a negative impact on the environment (Tamminga, 1992). In the soil-plant-animal conversions on a dairy farm, the conversion of N intake into milk and meat by the animal has the lowest efficiency (Van Keulen et al., 2000). In particular the efficiency of N utilisation of grass-fed or grazing dairy cows is low. Fresh grass from intensively fertilized grassland has a relatively high CP content in comparison with the energy available for microbial protein synthesis. The CP in grass is rapidly degraded by microbes in the rumen and the N is converted into ammonia (NH_3). In the liver, urea is synthesized from NH_3 that originates on the one hand from surplus NH_3 not incorporated in microbial protein in the rumen and on the other hand from losses in the amino acids metabolism in tissues (maintenance) and mammary gland (milk protein production). Urea equilibrates in aqueous solutions through diffusion, therefore milk urea N (MUN) content is highly correlated with blood urea N content as reported by many authors (listed by Hof et al., 1997). A proportion of the urea is recycled within the body to the gastro intestinal tract depending on dietary (Kennedy and Milligan, 1980) and animal factors (Brun-Bellut, 1996). The fate of urea-N is that a small amount is excreted in the faeces, a small amount is secreted in milk, whereas the majority is excreted in urine.

The mean (± SD) of MUN and partitioning of N (in g/d or as proportion of N intake) of the stall-feeding and the grazing experiment are presented in Table 5. In the stall feeding experiment, the intake was measured directly and accurately and the faecal excretion of N was determined, whereas in the grazing experiment N intake was estimated indirectly and faecal N excretion was not determined. Therefore, in the stall feeding experiment the urinary N excretion could be determined as N intake- (N milk + N faeces), assuming no body tissue mobilisation or deposition. In the grazing experiment, the excretion of N with faeces and urine was estimated by subtracting N secreted in milk from N intake. Due to the lower accuracy of the intake estimates in the grazing experiment, relationships including an intake or urinary excretion are presented based only on the stall feeding experiment. Relationships between diet composition and milk components are, however, based on the four-year data.

With data of the six cultivars in the stall feeding experiment, the urinary N excretion was highly related with the N intake, but there were small differences between the two years in the intercept and in the regression coefficient (Chapter 4). Using all data of each year including cultivars 7 and 8 that were fed in three periods, the difference between years in the regression coefficients was smaller (0.791 vs 0.839 in 2000 and 2001, respectively) and the intercept was similar. The relationships determined with a fixed model overestimated the regression coefficient and therefore the procedure MIXED in SAS was used with year as random factor, as described in detail by St.-Pierre (2001). The root mean square error (RMSE) and the determination coefficient (R^2) were determined by simple regression of the predicted values with the observed values. The overall relationship in both years was N urine (g/d) = -147.5 (± 14.6) + 0.812 (± 0.036) N intake (g/d); R^2 = 0.88, RMSE= 24.1 g/d; Figure 2). This means that within the range of N intakes, 81.2 % of the additionally ingested N will be excreted in the urine. In the overall relationship as well as within a year, the N excretion did not increase exponentially as observed by Castillo et al. (2000) and Kebreab et al. (2001) with N intakes above 400 g/d. The high N excretion with the urine implies that the response of N secretion in milk on N intake was low, with a slope of 0.125 (± 0.024) in 2000 and 0.167 (± 0.027) in 2001 and the higher proportion of N secreted in milk in 2000 than in 2001 was reflected by the higher intercept (75.5 vs 35.1 g/d in 2000 and 2001, respectively). The correlations within a year were not high with R^2 = 0.29 and 0.35 in 2000 and 2001, respectively. The overall relationship was N milk (g/d) = 60.8 (± 9.8) + 0.133 (± 0.025) N intake (g/d), R^2 = 0.55, P_{slope} = 0.12, RMSE = 16.1g/d. The mean response on N intake was 0.133, although this was not significantly different from zero (P_{slope} = 0.12). The N excreted in urine originates from losses in the digestive tract, as indicated by the positive mean OEB content (Table 1) and OEB balance (Table 4), from the surplus of available N as indicated by the 20 to 50 % higher DVE intake than required for milk protein synthesis and the low milk protein synthesis efficiency (Table 4), and from inevitable N losses in the protein metabolism in the animal. With decreasing intakes, the proportion of N secreted in milk increases with a maximum of 0.34 g N/g N intake. The N intake is the summation of DMI and the N content in the grass. The proportion secreted in milk was more related with the N content of the diet (r = -0.65) than with the N intake (r = -0.24). The correlation between faecal N excretion an N intake was poor in 2000 (R^2 = 0.08, P = 0.01) and absent in 2001 (P = 0.88) (Chapter 4).

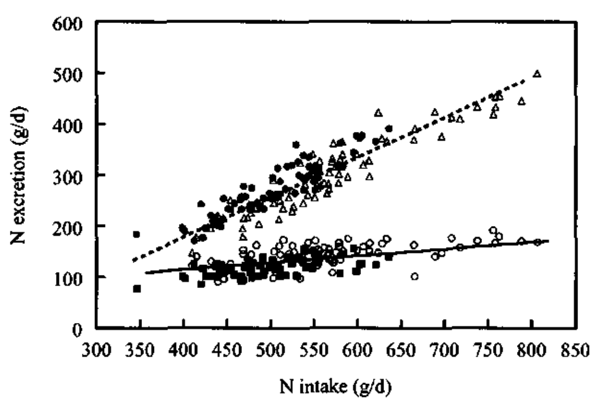

Figure 2 Relationship between N excreted in urine (dotted line; Δ = 2000, • = 2001) and milk (solid line; ○ = 2000, ■ = 2001) with N intake: N urine (g/d)= -147.5 (± 14.6) +0.812 (± 0.036) N intake (g/d); R^2 = 0.88, RMSE= 24.1 g/d; N milk (g/d)= 60.8 (± 9.8) + 0.133 (± 0.025) N intake (g/d), R^2 = 0.55, P_{slope} = 0.12, RMSE = 16.1 g/d.

Table 5 Mean and standard deviation (SD) of N intake, N secretion in milk and excretion in faeces and urine (in g/d and as proportion of N intake) by the dairy cows in two years of stall feeding and in two years of grazing experiments. Excretion in urine in stall feeding experiment, and excretion in faeces plus urine in grazing experiment both calculated by difference assuming zero N balance in body.

	Stall feeding				Grazing			
	2000 (n= 72)		2001(n= 72)		2002 (n= 46)		2003 (n= 43)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MUN[1] (mg/dL)	11.7	4.1	17.2	1.8	14.5	4.1	15.6	3.1
N intake (g/d)	573.0	92.0	501.6	58.0	548.0	76.3	623.3	103.4
N milk (g/d)	147.3	21.5	118.6	16.3	132.0	15.7	121.1	14.6
N faeces (g/d)	120.9	25.1	111.2	18.2				
N urine (g/d)	304.8	77.4	271.8	53.3				
N faeces and urine (g/d)	425.7	82.5	382.9	50.1	415.9	74.1	502.2	105.1
N milk (g/g N intake)	0.260	0.039	0.237	0.027	0.244	0.037	0.200	0.040
N faeces (g/g N intake)	0.214	0.049	0.225	0.045				
N urine (g/g N intake)	0.525	0.064	0.538	0.055				
N faeces and urine (g/g N intake)	0.740	0.039	0.763	0.027	0.756	0.037	0.800	0.040

[1] MUN = milk urea N content

Rumen Fermentation

Grass has a relatively high CP content that is readily degraded in the rumen and this may result in a surplus of N in comparison with energy available for rumen microbial protein synthesis. Rumen incubations with grass harvested in 2000 indicated an almost 0.02/h higher fractional degradation rate of CP than of OM. Moreover, the fractional degradation rate increased with the CP content and this was associated with a higher effective degradation of CP as well as OM in the rumen (Chapter 2). This effect was observed mainly due to differences between periods and not due to cultivar differences. Van Vuuren (1993) showed that at CP contents in grass above 155 g/kg DM, 79 % of extra CP is lost in the rumen due to a limited supply of energy. A major determinant of rumen microbial protein synthesis is the availability of energy-yielding substrates (Dijkstra et al., 1998) and in grass the structural (NDF) and non-structural carbohydrates (WSC) can be distinguished. The supply of energy from structural carbohydrates depends on the cell wall content (NDF) and the rate and extent of degradation in the rumen. In our experiments only small differences in the cell wall content and the rate and extent of degradation were found (Chapter 2) and there were no relationships observed between the fractional rate of NDF degradation with rumen parameters (Taweel, 2004). The WSC is released easily from grass with 60 % after ingestive mastication and 90 % within 1.5 h after ingestion (Boudon and Peyraud, 2001). The non protein nitrogen was released at a similar level as WSC, whereas protein-N and chlorophyll-N were released less easily (Boudon and Peyraud, 2001). Theoretically, the WSC may supply energy to balance the supply of N from non protein nitrogen within a meal and the supply of N from degraded protein from previous meals. In vitro measurements indicated a higher efficiency of microbial protein synthesis with increasing levels of WSC (Lee et al., 2003), but efficiency of microbial protein synthesis and flow of N to the duodenum as proportion of N intake measured in vivo was not affected by grass differing in WSC content (Lee et al., 2002[b]). The higher WSC content of two cultivars (1 and 4) than the other four cultivars did not result in a significantly lower mean NH_3-N concentration in the rumen liquid (Taweel, 2004) or in a higher utilisation of N for milk production in both years of the stall feeding experiment with data of the six cultivars (Chapter 4). Moreover, the MUN content was not consistently lower of the cultivars with a high WSC content in the stall feeding, nor in the grazing experiments (Chapters 4 and 5). The MUN content was also more related with the CP content than with the WSC content within each year (Chapter 4). As discussed in both Chapter 4 and 5, the considered increase in efficiency of microbial protein synthesis and hence N utilisation due to synchronisation of energy and N supply to rumen microbes with increased WSC contents in grass is debatable as shown by the absence of an effect of this synchronisation (Herrera-Saldana et al., 1990, Kolver et al., 1998). The recycling of urea within the body to the rumen may reduce the effect of imbalances in this energy and N supply within a day. Although urea recycled to the rumen as proportion of total urea synthesis decreases with an increase in CP content (Kennedy and Milligan, 1980), the absolute amount recycled remained similar with increased CP contents (Marini and Van Amburgh, 2003). The difference in CP and OEB content in grass between the years in the stall feeding experiment was reflected in both the mean NH_3–N concentration (109 vs 216 mg/L in 2000 and 2001, respectively) (Taweel, 2004) and mean MUN content (Table 5). The interpretation of the MUN content is discussed in the next paragraph.

Milk urea N content

The MUN content in milk reflects the blood urea N content as urea equilibrates in aqueous solutions, although the slope differed between data compilations and varied from 0.62 to 1.0 (Broderick and Clayton, 1997; Kauffmann and St. Pierre, 1997). The MUN content is an integration of the blood urea N content over the course of the day. The MUN content may depend on milking frequency and may differ between morning and afternoon sampling (Broderick and Clayton, 1997). The samples analysed in our experiments were based on a

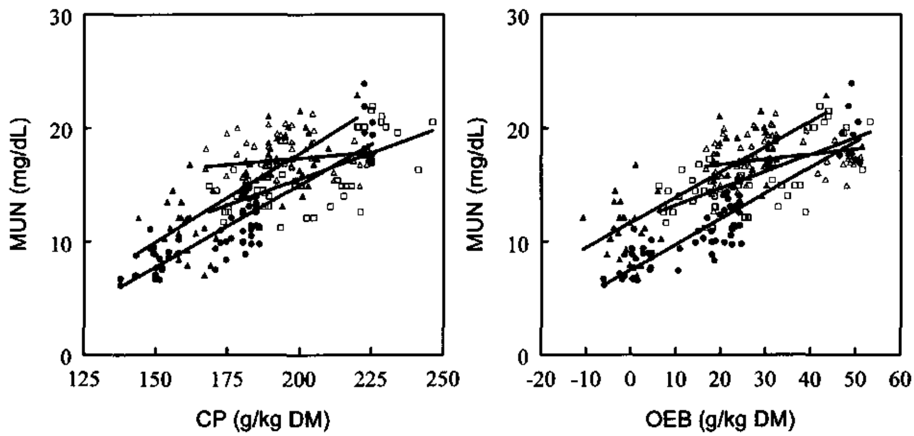

Figure 3 Relationship between MUN content with CP content and OEB content of the diet in (● = 2000, = 2001, ▲= 2002, □ = 2003).

pooled milk sample of the afternoon and morning milking and represented the MUN content over the whole day. The best single dietary factor predicting MUN is the CP content of the diet (R^2 > 0.77) (Broderick and Clayton, 1997; Broderick, 2003; Nousiainen et al., 2004). With our data, the CP content in the diet was also well related with MUN. Using a fixed model the regression was: MUN (mg/dL) = -12.9 (±1.7) + 0.15 (±0.009) CPdiet (g/kg DM); R^2 = 0.53, RMSE = 2.74 mg/dL). Using a mixed model, the slope decreased to 0.124 and this regression equation (MUN (mg/dL) = -7.3 (±7.0) + 0.124 (±0.035) CPdiet (g/kg DM); R^2 = 0.74, RMSE = 2.0 mg/dL) had a slope that was lower than the slopes of 0.17 in equations determined with fresh grass by Valk (2004) and with grass silage by Nousiainen et al. (2004). Slightly more of the variation in MUN (54 %) could be explained by the OEB content in the diet (MUN (mg/dL) = 10.4 (±0.3) + 0.194 (±0.012) OEB diet (g/kg DM), R^2 = 0.54, RMSE = 2.7 mg/dL) than based on the CP content. Including year as a random factor, the relationship was MUN = 11.7 (±1.7) + 0.160 (±0.041) OEB diet (g/kg DM), R^2 = 0.75, RMSE = 2.0 mg/dL. Fitting a curvilinear relationship did not result in a substantial improvement of the fits, neither did the balance between the CP content or OEB content in the diet with the net energy (NELc) available for milk synthesis.

At an OEB of zero, the MUN content was 10.4 mg/dL, and similar to those reported by Hof et al. (1997) (10.3 mg/dL) and Valk (2004) (11.0 mg/dL), whereas the slope was in between the values reported by Hof et al. (1997) (0.17 OEB (g/VEM) and Valk (2004) (0.25 OEB (g/kg DM)). However, the effect of year was high as indicated by the different lines fitted trough the data of each year. Similar to the regression as presented in Chapter 4, a high relationship was found in 2000, whereas this relationship was absent in 2001 (R^2 = 0.04, P = 0.06). Slopes and intercepts also differed between the two years of the grazing experiments (Figure 3). The difference between years can be attributed to the CP content of the grass. In 2000 and 2002, the mean CP content was 177.3 and 179.4 g/kg DM, whereas in 2001 the mean CP content was 193.9 and in 2003 the mean was above 200 g/kg DM (Table 1). These CP contents were above the datasets used by Broderick and Clayton (1997), Broderick (2003) and Nousiainen et al. (2004). Moreover, Broderick (2003) reported that dietary CP content was curvilinear related with the MUN content and this was also observed by Nousiainen et al. (2004) with high CP contents in leguminous forages.

Hof et al. (1997) did not find a significant relationship between MUN content and N losses associated with the absorbed protein (DVE) metabolism, although these losses accounted for 47 to 100 % of the urinary N losses. Nousiainen et al. (2004) opposed these findings by showing high relationships between MUN content and OEB content (g/kg DM) and DVE intake (g/d) and between MUN secretion (mg/d) with OEB and (excess) DVE intake (g/d). Our data are in contrast with these findings. For example, cultivar 3 in 2002 had lower OEB and DVE balances but a higher MUN content than the other cultivars (Chapter 5). Probably, the differences in regression equations between MUN and OEB content can partly be explained by differences in the DVE in CP content, the efficiency of milk protein synthesis and the relative contribution of the different N sources for urea synthesis.

Moreover, MUN content has not only been well correlated to the N content in the diet, but also to daily urinary N excretion (Jonker et al., 1998; Kauffmann et al., 1998; Broderick, 2003; Nousiainen et al., 2004; Valk, 2002). According to Jonker et al. (1998), the physiological basis of this close relationship depends on a constant blood urea pool size and blood flow through the kidney where the urea is actively and proportionally removed from the blood and excreted in urine. In 2000 a close relationship was found with urinary N excretion (N urine (g/d) = 115.8 (±14.2) + 16.1 (±1.14) MUN (g/d), R^2 = 0.74, RMSE = 39.9 g/d), whereas this relationship was poor and not significant in 2001 (N urine (g/d) = 117.9 (±59.2) + 9.0 (±3.42) MUN (g/d), R^2 = 0.09, RMSE = 51.2 g/d). More important, at a lower (30 g/d) mean urinary N excretion in 2001 than in 2000, the MUN content was on average 5.5 mg/dL higher in 2001 than in 2000. The slope of the regression in 2000 was high in comparison with other authors and also varied among these authors (Jonker et al., 1998; Kauffmann et al., 1998; Broderick, 2003; Nousiainen et al., 2004; Valk, 2002), and this has a large effect on the prediction of the urinary N excretion per animal per day. Similar as the N content in the diet, the N utilisation (g N milk/g N intake) decreased with an increase in MUN content (r = -0.57) and therefore MUN is an indicator of N utilisation. Besides dietary factors, animal factors as parity, milk production, days in milk (Jonker et al., 1998), and body weight (Kauffman and St.-Pierre, 2001) may influence the MUN content and these may also explain part of the observed variation between years.

In conclusion, the strength of the relationship between MUN and dietary components varied strongly between years and, moreover, the regression equations differed substantially. Therefore, the use of MUN, given the high CP contents in our experiments, did not accurately predict the urinary N excretion. However, a high MUN is an indicator of high N losses in the protein metabolism.

Conclusions

The effect of perennial ryegrass cultivars on sward and plant characteristics, the chemical composition and the nutritive value of the grass was low and small differences among cultivars were observed. Therefore, the DMI, digestibility, rumen degradation, MP and milk composition of dairy cows stall-fed fresh grass were almost not affected by the cultivar in the diet. In the grazing experiment, there was more interaction between the animal than in the stall feeding experiment. In the first year of the grazing experiment, one cultivar with a lower resistance against crown rust became heavily infected and this was associated with a reduced DMI, most likely by the negative effect on the palatability of the grass. Therefore, increasing the crown rust resistance of grass cultivars remains an important aspect in grass breeding programmes. In the second year no differences in DMI and MP among cultivars were found. Two cultivars had a consistently higher WSC content than the other cultivars, but this did not affect the DMI, MP and N utilisation of dairy cows. In the stall feeding experiment, the MUN content seemed to be lower with increased WSC content, but this was not found in the grazing experiment. The grass was highly digestible (around 80 %) and only very small differences in cell wall content (NDF and ADL) and in the fractional rate of degradation in the rumen among cultivars were found. The digestibility was only weakly related with the DMI and MP and therefore further improvement in digestibility by selection among cultivars seemed not to be beneficial.

The effect of growing conditions, management and its interaction had larger effects on the sward and plant characteristics, the chemical composition and the nutritive value than the cultivar did. Mainly N fertilisation level between years and between cuts or grazing periods within a year influenced the CP content and inversely the WSC content of the grass. Approximately 80 % of the extra ingested N was excreted in the urine and this was positively related with CP content. The N utilisation by the grass-fed dairy cows rarely exceeded the 0.30 g N milk/g N intake and decreased with an increase in CP content. Increasing the N utilisation of dairy cows fed diets comprising a substantial proportion of fresh grass can be achieved by decreasing the CP content in the grass to around 150 to 160 g/kg DM.

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Authors and affiliations

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Summary

Samenvatting

Summary

Introduction

In The Netherlands, intensively managed grasslands consist predominantly of pure grass swards. Perennial ryegrass (*Lolium perenne* L.) is the most abundant grass species, and has a high yield and nutritive value. Grassland is usually renewed every 3 to 15 yr and it is sown with a mixture, containing mostly a high proportion of cultivars of perennial ryegrass and in addition small proportions of other grass species and clover.

Grass is one of the main roughages in the diet of high productive dairy cows. The dry matter intake (DMI) is the main determinant of milk production (MP). However, many studies reported that dairy cows with a high proportion of grass in their diet, or solely grazing intensively managed grassland, had a lower MP in comparison to cows fed a nutrient balanced diet. This lower MP could partly be attributed to a lower DMI and partly to an imbalance between absorbed nutrients. In grazing dairy cows, the supply of metabolizable energy was found to be the main limitation for MP. Moreover, intensive use of fertilizer N and selection by grazing cows of young and leafy grass, result in the intake of grass with a high digestibility and energy content, but also with a high crude protein (CP) content. The CP of grass is rapidly degradable in the rumen, and often the N level exceeds the level required for the optimal utilisation of carbohydrates available for microbial growth. Almost 80 % of this N in excess in the rumen is, after conversion in the liver to urea, excreted in urine. The low utilisation of N by grass-fed or grazing dairy cows results in high N losses that have a negative impact on the environment.

There are many ways to increase the energy intake and to improve the N utilisation by grass-fed and grazing dairy cows. One way could be by including selection criteria for these traits in grass breeding programmes. Therefore, the aim of the research described in this thesis was to evaluate the effects of perennial ryegrass cultivars on 1) degradation of herbage in the rumen of dairy cows, 2) the DMI, digestibility and MP by dairy cows, and 3) the N utilisation by dairy cows. These effects were then related to sward characteristics and chemical composition of these cultivars, in order to identify new selection criteria that may be used in grass breeding programmes.

Experiments

During two summers, an experiment was conducted in which eight cultivars were fed to twelve dairy cows housed indoors. Two cultivars were sown on one paddock, whereas six cultivars were sown on an adjacent paddock. The two cultivars were fed during four periods of two weeks each. In between these four periods, the six cultivars were fed during three periods of two weeks each. Daily the grass was harvested, the DM yield was measured, and

grass was sampled to determine different plant fractions and the chemical composition. In the first year, samples were taken to evaluate the effects of sward characteristics and chemical composition of eight diploid perennial ryegrass cultivars during the growing season on degradation characteristics in the rumen of dairy cows (Chapter 2). Degradation characteristics were determined by incubation of grass samples in nylon bags for different durations in the rumen of grazing dairy cows. The fractional degradation rate and the effective degradation in the rumen were determined. In the two years of stall feeding, the DMI, digestibility, MP (Chapter 3) and the N utilisation (Chapter 4) were determined. From the eight cultivars used in the stall feeding experiment, the four cultivars with the largest differences in chemical composition were selected for further experiments. During two summers, the dry matter intake, grazing behaviour, and milk production of these four cultivars by twelve grazing dairy cows were measured (Chapter 5). The intake was estimated with the *n*-alkanes marker technique and the grazing behaviour was determined with grazing behaviour recorders. Furthermore, milk fat, milk protein and milk urea N (MUN) content were determined in both experiments. In the stall feeding experiment, the N intake and the N excretion in milk and faeces were determined and the N excretion in urine was calculated as the complement. In both experiments, N utilisation was expressed as N excreted in milk as proportion of N intake.

Results

Intake, digestibility and milk production

The intake may be influenced by the palatability of the diet. Cows seem to have a preference for sugar. Therefore, the hypothesis was that cows have a higher DMI of cultivars with a higher water soluble carbohydrate (WSC) content. Grass cultivars differed consistently in their WSC content. However, the DMI of grass cultivars with a higher WSC did not differ from the other cultivars in the two years stall-feeding experiment (Chapter 3) and in the second year of the grazing experiment (Chapter 5). In the first year of the grazing experiment, differences in WSC content were confounded with crown rust infestation and the aversion of crown rust may have decreased DMI of two cultivars with a lower WSC content (Chapter 5). However, the grazing behaviour was very similar among cultivars.

The digestibility was only determined in the stall feeding experiment. In both years the DM digestibility was high (> 77 %), with very small differences (< 0.5 %) among cultivars in the first year. These cultivars also hardly differed in the fractional degradation rate of the the neutral detergent fibre (NDF) (Chapter 2). Larger differences in digestibility among cultivars were found in the second year (up to 4 %). This was associated with a delayed heading date, resulting in larger differences in the proportion of leaf blades in the sward and in the NDF content among cultivars (Chapter 3).

The digestible DMI determines the amount of absorbed nutrients that are available for milk production. During three years, no significant differences among cultivars were found in the DMI and hence in the milk production. In the first year of the grazing experiment, the higher DMI of two cultivars was associated with a higher milk production of these two cultivars (Chapter 5).

N utilisation

In the dairy cow, N losses occur in the rumen and in the intermediate protein metabolism. Rumen N losses can be minimized by optimising the ratio between protein and energy available for microbial fermentation and increasing the efficiency of microbial protein synthesis. The rumen incubations with the grass cultivars indicated an almost 0.02 /h higher fractional degradation rate of CP than of OM. Moreover, the fractional degradation rate increased with the CP content and this was associated with a higher effective degradation in the rumen of CP as well as OM (Chapter 2). A major determinant of rumen microbial protein synthesis is the availability of energy-yielding substrates for microbes. In grass, the structural (NDF) and non-structural carbohydrates (WSC) can be distinguished. In our experiments, however, only small differences in the NDF content and the rate and extent of degradation of NDF were found (Chapter 2). The calculated balance between effectively degraded protein (N) and the energy (OM or carbohydrates) was lower of the cultivars with an increased WSC content than the other cutivars (Chapter 2). However, in the stall feeding experiment and in the grazing experiment, cultivars contrasting in WSC content did not differ in the N utilisation by dairy cows (Chapter 4 and 5). Strong linear relationships were found between urinary N excretion with N intake, and with N content in grass, whereas only poor relationships with WSC content were found. These strong relationships were, however, more associated with differences among periods and cows than with cultivar differences (Chapter 4).

In the liver, urea N is synthesized from NH_3 that originates from two sources: the surplus NH_3 not incorporated in microbial protein in the rumen, and NH_3 from deamination of amino acids in the intermediate protein metabolism. Urea equilibrates in aqueous solutions and therefore the milk urea N (MUN) content is highly correlated with the blood urea N content. The MUN content was not consistently lower of the cultivars with a high WSC content in the stall feeding, nor in the grazing experiments (Chapters 4 and 5). In the general discussion, the relationships between MUN and dietary components were discussed in more detail. The MUN content was well related with the CP content and the rumen degradable protein balance (OEB) content in the grass, and only weak related with the WSC content in the grass. However, the strength of these relationships and the regression equations determined based on data of one year, differed substantially between years. In the first year of the stall feeding experiment, a close relationship was found between MUN content and the urinary N excretion, whereas in the second year this relationship was poor and not significant.

Moreover, the mean urinary N excretion was lower, whereas the MUN content was higher in the second year than the first year of the stall feeding experiment. Therefore, based on our results with measurements on individual dairy cows, MUN content seems not to be an accurate predictor of the daily urinary N excretion.

Main Conclusions

The effects of growing conditions, grassland management and their interactions on the sward characteristics, the chemical composition and the nutritive value of perennial ryegrass were larger than the effect of ryegrass cultivar.

Among the perennial ryegrass cultivars used in our experiment, the differences in rumen degradation characteristics were small. There was no significant effect of perennial ryegrass cultivar on intake, milk production and milk composition in three of the four years of experiments. In one year in the grazing experiment, the lower intake of two cultivars was associated with a lower WSC content and a more severe crown rust infestation than the other two cultivars.

The N utilisation by dairy cows rarely exceeded the 0.30 g N milk/g N intake and no significant differences among cultivars were found. Approximately 80 % of the extra ingested N was excreted in the urine, and this was positively related with the CP content in grass. Increasing the N utilisation of dairy cows, fed diets comprising a substantial proportion of fresh grass, can be achieved by decreasing the CP content in the grass to around 150 to 160 g/kg DM.

The strength of the relationships between MUN and the chemical composition of the grass and the regression equations determined based on data of one year, differed substantially between years. Moreover, our results suggest that, measurements on individual dairy cows, MUN content seems not to be a very accurate predictor of the urinary N excretion.

Implications

Dairy farmers should select a grass seed mixture for grassland renovation mainly based on the agronomical aspects. The growing conditions and the management of the farmer (i.e. fertilizer level, growing days, harvesting time) determine more the grassland production and the nutritive value of the grass than the effect of individual cultivars. However, these grass seed mixtures should contain ryegrass cultivars with a high resistance against crown rust.

Dairy farmers, advisors and researchers should not only focus on optimal DM yield of the grassland, but more on optimal N yield of the grassland and on increasing the N utilisation by dairy cows. When the diet contains a high proportion of fresh grass, the CP content of grass should be around 150 -160 g/kg DM. Therefore, the N fertilizer level should be reduced and the aim should be an optimal CP content in the grass.

Grass breeders should continue with increasing the DM yield, crown rust resistance and drought resistance of (perennial ryegrass) cultivars. Based on the results in our feeding experiments, no new selection criteria for grass breeders were identified.

Samenvatting

Introductie

In Nederland bestaat het gewas van intensief beheerd grasland voornamelijk uit raaigras. Engels raaigras (*Lolium perenne* L.) is de meest voorkomende grassoort, doordat het een hoge opbrengst en voedingswaarde heeft. Grasland wordt gewoonlijk elke 3 tot 15 jaar vernieuwd. Het wordt ingezaaid met een mengsel, dat meestal bestaat uit een groot aandeel Engels raaigrasrassen en daarnaast een klein aandeel andere grasrassen en klaver.

Gras is één van de belangrijkste ruwvoeders in het rantsoen van hoogproductieve melkkoeien. De drogestofopname (DS-opname) is het belangrijkste element voor de melkproductie. Uit onderzoek is gebleken dat melkkoeien met een groot aandeel gras in het ranstoen, òf die uitsluitend geweid worden op intensief beheerd grasland, een lagere melkproductie hadden in vergelijking met koeien die een uitgebalanceerd rantsoen werd gevoerd. Deze lagere melkproductie kan deels worden toegeschreven aan een lagere DS-opname en deels aan een onevenwichtige beschikbaarheid van geabsorbeerde nutriënten. Bij koeien die grazen in de wei was het aanbod van de metaboliseerbare energie de meest beperkende factor voor melkproductie. Een hoge stikstofbemesting en selectie van jong en bladrijk gras door koeien in de wei leidt bovendien tot een opname van gras met een hoge verteerbaarheid en energiegehalte, maar ook met een hoog ruw eiwit (RE) gehalte. Het RE van gras is snel afbreekbaar in de pens en dikwijls overschreidt het N gehalte het gewenste niveau voor de optimale benutting van koolhydraten die beschikbaar zijn voor bacteriële groei. Bijna 80 % van het overschot aan N in de pens wordt, na omzetting in ureum in de lever, uitgescheiden met de urine. De lage benutting van N door koeien die vers gras worden gevoerd of worden geweid resulteert in grote N-verliezen, die een negatieve invloed hebben op de omgeving.

Er zijn veel manieren om de energieopname te verhogen en de N-benutting te verbeteren van koeien die vers gras vreten of die worden geweid. Eén van die manieren kan zijn door selectie criteria voor deze kenmerken op te nemen in graszaadveredelingsprogramma's. Het doel van het onderzoek beschreven in dit proefschrift is daarom het evalueren van de effecten van Engels raaigrasrassen op 1) de afbraak van gras in de pens van melkkoeien, 2) de DS-opname, de verteerbaarheid en de melkproductie, en 3) de N-benutting van melkkoeien. Deze effecten werden daarna gerelateerd aan gewaskenmerken en aan de chemsiche samenstelling van deze rassen, om daarmee nieuwe selectie criteria vast te stellen die gebruikt zouden kunnen worden in graszaadveredelingsprogramma's.

Proeven

Gedurende twee zomers werd een proef uitgevoerd waarin acht Engels raaigrasrassen werden gevoerd aan twaalf koeien, die gehuisvest waren in een grupstal. Twee rassen waren ingezaaid op één perceel, terwijl de zes andere rassen op een ernaastgelegen perceel waren ingezaaid. De twee rassen werden gevoerd in vier periodes van elk twee weken en tussen iedere periode werden de andere zes rassen gevoerd in drie periodes van elk twee weken. Het gras werd dagelijks gemaaid, de drogestof opbrengst werd gemeten en een grasmonster werd genomen voor het bepalen van de verschillende plantfracties en voor de chemische samenstelling. Tijdens het eerste jaar werden er ook grasmonsters genomen om de effecten van gewaskenmerken en de chemische samenstelling van acht Engels raaigrasrassen gedurende het groeiseizoen op de afbraakkenmerken in de pens van melkoeien (hoofdstuk 2). Deze afkbraakkenmerken werden bepaald door grasmonsters in nylon zakjes te incuberen in de pens voor verschillende tijdsduren van grazende koeien. De fractionele afbraaksnelheid en de effectieve afbraak in de pens werden bepaald. Gedurende de twee jaar stalvoedering werden de opname, verteerbaarheid en melkproductie (hoofdstuk 3) en de N-benutting bepaald (hoofdstuk 4). Uit de acht rassen die op stal werden gevoerd werden vier rassen met de grootste verschillen in chemische samenstelling geselecteerd voor verder onderzoek. Gedurende twee zomers werden van vier rassen de DS-opname, het graasgedrag en de melkproductie door twaalf melkkoeien in de wei bepaald (hoofdstuk 5). De opname werd geschat met de *n*-alkaan methode en het graasgedrag werd bepaald met graasrecorders. Tevens werd het gehalte aan vet en eiwit in de melk en het melkureum N (MUN) in beide experimenten bepaald. In de stalvoederproef werden de N-opname en de N uitscheiding met melk en mest bepaald en de uitscheiding in de urine werd berekend door het verschil. In beide proeven werd de N-benutting uitgedrukt als de N uitgescheiden in de melk als proportie van de N-opname.

Resultaten

Opname, verteerbaarheid en melkproductie

De opname kan worden beïnvloed door de smakelijkheid van het rantsoen. Koeien lijken een voorkeur te hebben voor suiker. De hypothese was daarom dat koeien een hogere DS-opname hebben van grasrassen met een hogere wateroplosbare koolhydraten (WOK) gehalte. Grasrassen verschilden consequent in hun WOK gehalte. De DS-opname van grasrassen met een hogere WOK-gehalte was echter niet verschillend van de andere rassen in de twee jaar van de stalvoederproef (hoofdstuk 3) en in het tweede jaar van de beweidingsproef (hoofdstuk 5). In het eerste jaar van de beweidingsproef waren de verschillen in WOK-gehalte verstrengeld met kroonroestbesmetting. De afkeur van kroonroest zou tot een lager DS-opname van twee rassen met een lagere WOK-gehalte hebben kunnen geleid (hoofdstuk 5). Echter, het graasgedrag was vrijwel gelijk tussen de grasrassen.

De verteerbaarheid werd alleen bepaald in de stalvoederproef. In beide jaren was de DS-verteerbaarheid hoog (> 77 %) met kleine verschillen (< 0.5%) tussen de rassen in het eerste jaar. De rassen verschilden ook nauwelijks in de fractionele afbraaksnelheid van de neutral detergent fibre (NDF) (hoofdstuk 2). Grotere verschillen in verteerbaarheid tussen de rassen (tot 4%) werden gevonden in het tweede jaar. In dat jaar was de doorschietdatum vertraagd en dat resulteerde in grotere verschillen in het aandeel van bladeren in het gewas en in het NDF-gehalte tussen de grasrassen (hoofdstuk 3).

De verteerbare DS-opname bepaald de hoeveelheid geabsorbeerde nutriënten die beschikbaar zijn voor de melkproductie. Gedurende drie jaar werden geen significante verschillen tussen rassen gevonden in de DS-opname en de melkproductie. In het eerste jaar van de beweidingsproef resulteerde de hogere DS-opname van twee rassen ook in een hogere melkproductie van deze twee rassen (hoofdstuk 5).

N-benutting

In de melkkoe treden verliezen van N op in de pens en in de intermediaire eiwitstofwisseling. Verliezen van N in de pens kunnen worden beperkt door de verhouding tussen eiwit en energie die beschikbaar zijn voor microbiële fermentatie te optimaliseren en de efficientie van de microbiële eiwitsynthese te verhogen. De incubaties van de grasrassen in de pens toonden aan dat de fractionele afbraaksnelheid van RE bijna 0.02 /uur hoger was dan de afbraaksnelheid van de organische stof (OS). Bovendien nam de fractionele afbraaksnelheid toe met het RE-gehalte en dit was verbonden met een hogere effectieve afbraak in de pens van RE en ook van de OS. Een bepalende factor in de microbiële eiwitsynthese in de pens is de beschikbaarheid van energieleverende substraten voor bacteriën. In gras kunnen hiervoor de structurele (NDF) en de niet-structurele koolhydraten (WSC) worden onderscheiden. In onze proeven werden echter maar kleine verschillen gevonden in het NDF-gehalte en in de snelheid en omvang van de afbraak van NDF in de pens. De berekende verhouding tussen de effective afbraak van N en energie (OS of koolhydraten) was lager van de rassen met een hoger WOK-gehalte dan de andere rassen (hoofdstuk 2). In de stalvoederproef en in de beweidingsproef verschilden de rassen met een verschillend WOK-gehalte niet in de N-benutting door melkkoeien (hoofdstukken 4 en 5). Sterke lineaire verbanden werden gevonden tussen de N-uitscheiding in de urine met de N-opname en met het RE-gehalte van gras, terwijl deze verbanden zwak waren met het WOK-gehalte. Deze sterke verbanden waren echter meer verbonden met de verschillen tussen de periodes en tussen koeien, dan door verschillen tussen grasrassen (hoofdstuk 4).

Ureum-N wordt gesynthetiseerd in de lever uit NH_3, dat afkomstig is van twee bronnen: het teveel aan NH_3 dat niet geïncorpereerd wordt in microbiëel eiwit in de pens en NH_3 van de de-aminatie van aminozuren in de intermediaire eiwitstofwisseling. Ureum equilibreert in waterige oplossingen en daardaar is het melkureum-N (MUN) gehalte sterk gecorreleerd aan het bloodureum-N gehalte. Het MUN-gehalte was niet consequent lager van de rassen met een hoger WOK-gehalte, zowel in de stalvoederproef als in de beweidingsproef (hoofdstukken 4 en 5). De relaties tussen MUN en componenten in het gras zijn in meer detail besproken in de general discussion. Het MUN-gehalte was goed gerelateerd aan het RE-gehalte en aan onbestendige eiwit balans (OEB) gehalte, en alleen matig gerelateerd aan het WOK-gehalte in het gras. Echter, de sterkte van deze verbanden en de regressievergelijkingen bepaald met gegevens in één jaar verschilden behoorlijk tussen de jaren. In de eerste jaar van de stalvoederproef werd een sterk verband gevonden tussen het MUN-gehalte en de N-uitscheiding in de urine, terwijl in het tweede jaar dit verband zwak en niet significant was. Bovendien was de gemiddelde N-uitscheiding lager, terwijl het MUN-gehalte hoger was in het tweede jaar dan het eerste jaar. Gebaseerd op onze resultaten met metingen aan individuele melkkoeien, lijkt het MUN-gehalte geen betrouwbare voorspeller te zijn van de N-uitscheiding.

Voornaamste conclusies

De effecten van groeiomstandigheden, graslandbeheer en hun interactie op de gewaskenmerken, de chemische samenstelling en de voedingswaarde van Engels raaigrasrassen waren groter dan de effecten van de verschillende rassen van Engels raaigras.
De verschillen in afbraakkenmerken tussen de Engels raaigrasrassen gebruikt in onze proef waren klein. Er was geen significant effect van Engels raaigrasras op de opname, melkproductie en melksamenstelling in drie van de vier jaar van de voederproeven. In één jaar in de beweidingsproef was de lagere DS-opname van twee rassen verbonden met een lagere WOK-gehalte en een hogere kroonroestbesmetting dan de andere twee rassen.
De N-benutting door melkkoeien kwam zelden boven de 0.30 g N in melk/ g N-opname en er waren geen significante verschillen tussen rassen gevonden. Ongeveer 80 % van de extra opgenomen N werd uitgescheiden in de urine en dit was positief gerelateerd met het RE-gehalte in gras. Het verhogen van de N-benutting door melkkoeien, die een hoog aandeel vers gras in het rantsoen hebben, kan worden bereikt door het verlagen van het RE-gehalte in het gras tot ongeveer 150 tot 160 g/kg DS.
De sterkte van de verbanden tussen het MUN-gehalte en de chemische samenstelling van het gras en de regressievergelijkingen bepaald op basis van gegevens van één jaar in de proeven, verschilden behoorlijk tussen de jaren. Op basis van onze resultaten met metingen aan individuele melkkoeien, lijkt het MUN-gehalte bovendien geen betrouwbare voorspeller te zijn van de N-uitscheiding.

Implicaties

Melkveehouders zouden graszaadmensgels voor graslandvernieuwing hoofdzakelijk moeten selecteren op basis van agromomische aspecten. De groeicondities en het management van de veehouder (i.e. N-bemestingsniveau, aantal groeidagen, maaitijdstip) bepalen meer de graslandproductie en de voedingswaarde van het gras dan het effect van individuele grasrassen. Deze graszaadmengsels zouden echter wel Engels raaigrasrassen moeten bevatten die een hoge resistentie tegen kroonroest hebben.

De melkveehouders, adviseurs en onderzoekers zouden zich niet alleen moeten richten op een optimale DS-opbrengst van het grasland, maar meer op een optimale N-opbrengst van het grasland en een N-benutting door melkkoeien. Wanneer het rantsoen een hoog aandeel vers gras bevat, zou het RE-gehalte van het gras ongeveer 150-160 g/kg DM moeten zijn. Het N-bemestingsniveau zou daarom moeten worden verlaagd en de doelstelling zou een optimale RE-gehalte in het gras moeten zijn.

Graszaadveredelaars zouden moeten doorgaan met het verhogen van deDS-opbrengst, de kroonroestresistentie en de droogteresistentie van (Engels raaigras-) rassen. Op basis van de resultaten in onze voederproeven konden geen nieuwe selectiecriteria worden vastgesteld voor graszaadveredelaars.

Slotwoord

De eerste kennismaking met het diervoedingsonderzoek door Germ Hof en Wilbert Pellikaan tijdens mijn afstudeervak bij Diervoeding heb ik zeer positief ervaren. De mogelijkheid om direct na het afstuderen te beginnen als assistent in opleiding bij Diervoeding heb ik daarom ook met beide handen aangegrepen. Ik was zeer geïnteresseerd in het onderwerp en de werkzaamheden die bij de uitvoering van het onderzoek horen. Bij deze wil ik dan ook de leerstoelgroep Diervoeding bedanken voor het bieden van deze kans.

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Bart

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Passagekarakteristieken van de NDF-fractie afkomstig van mengvoedergrondstoffen door het maagdarmkanaal bij melkkoeien. Concept eindrapport, WVH-05-06.

Training and Supervision Plan			**Graduate School WIAS**
Name PhD student	Bart Tas		
Project title	Effect of perennial ryegrass cultivars on intake and milk production		
Group	Animal Nutrition		
Daily supervisor(s)	Dr. J. Dijkstra		
Supervisor(s)	Prof. dr. S. Tamminga, Dr. A. Elgersma		
Project term	from: 15-5-2000	until: 15-9-2004	
Submitted	date: 2-3-2005	first plan / midterm / **certificate**	

EDUCATION AND TRAINING (minimum 21 cp, maximum 42 cp)		
The Basic Package (minimum 2 cp)	year	cp*
WIAS Common Course (mandatory)	2001	2.0
Course on philosophy of science and/or ethics (mandatory)	2001	1.0
Subtotal Basic Package		**3.0**
Scientific Exposure (conferences, seminars and presentations, minimum 5 cp)	year	cp
International conferences *(minimum 2 cp)*		
European Grassland Federation, La Rochelle, France, 27-30 may 2002	2002	0.8
European Association of Animal Production, Cairo, Egypt, 30 August -2 September 2002	2002	0.8
European Grassland Federation, Luzern, Switzerland, 2004	2004	0.8
Seminars and workshops		
Nederlandstalige Voedingsonderzoekersdag (2000, 2003, 2004)	2000/03/04	0.6
WIAS Science Day (2001, 2002, 2003, 2004)	2001-2004	0.8
WIAS Seminar Plus "Potential Use of Stable Isotopes, related to Studies on Stress and Metabolic Adaptation"	2001	0.3
WIAS Seminar Plus "Herd dynamics of smallholder dairy in Kenya Highlands"	2003	0.3
Presentations *(minimum 4 original presentations of which at least 1 oral, 0.5 cp each)*		
European Grassland Federation, La Rochelle, France, 27-30 may 2002 (oral)	2002	0.5
European Association of Animal Production, Cairo, Egypt, 30 August -2 September 2002 (oral)	2002	0.5
WIAS Science day 2002, Wageningen (oral)	2002	0.5
Nederlandstalige Voedingsonderzoekersdag, België, 2003 (poster) and Wageningen, 2004 (oral)	2003/2004	1.0
European Grassland Federation, Luzern, Switzerland, 21-24 June 2004, (poster)	2004	0.5
International Symposium on the Nutrition of Herbivores, Mérida, Mexico 2004 (poster)	2004	0.5
Subtotal International Exposure		**7.9**
In-Depth Studies (minimum 4 cp)	year	cp
Disciplinary and interdisciplinary courses		
WIAS course "Stable Isotopes in studies of nutrient dynamics"	2001	0.6
VLAG course "Ecophysiology of the gastro-intestintinal tract"	2001	1.0
Advanced statistics courses		
WIAS advanced statistics course "Experimental Design"	2000	1.0
Undergraduate courses		
Nutrient dynamics and modelling	2003	2.0
Subtotal In-Depth Studies		**4.6**
Professional Skills Support Courses (minimum 2 cp)	year	cp
WIAS Course Techniques for Scientific Writing	2001	0.8
Use of Laboratory Animals (mandatory when working with animals)	2000	3.0
Subtotal Professional Skills Support Courses		**3.8**
Didactic Skills Training (optional)	year	cp
Lecturing		
Hoorcollege Economie studententen	2002	0.4
Hoorcollege Inter Specialisatie Blok (voeropname)	2002	0.6
Boerderijproject	2001/2002	1.0
Analyse Veeteelt Literatuur (2 years)	2001/2002	2.0
Supervising practicals and excursions		
Biologie dierlijke productie (4 years)	2000-2003	2.0
Supervising MSc theses *(maximum 1 cp per MSc student)*		
9 MSc students	2000-2004	9.0
4 BSc students	2001-2003	4.0
Subtotal Didactic Skills Training		**19.0**
Management Skills Training (optional)	year	cp
Membership of boards and committees		
WIAS Associated Ph.D. Students council (incl. AIO Raden Overleg, Landelijk AIO Overleg) (3 years)	2001-2003	3.0
Subtotal Management Skills Training		**3.0**
Education and Training Total (minimum 21 cp, maximum 42 cp)		**41.3**

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

Curriculum Vitae

Bart (Bartholomeus Martinus) Tas was born on the 14th of January 1976 in Callantsoog, The Netherlands. In 1994, he graduated from High School at the Etty Hillesum College in Den Helder. In the same year, he started with the study Animal Sciences at Wageningen Agricultural University. He conducted master theses in the specialisations Animal Health & Reproduction and Animal Nutrition. In 1999, he fulfilled a traineeship, observing homosexual behaviour of rams and stags, at the Veterinary Science, Massey University in Palmerston North, New Zealand. In spring 2000, he graduated in Animal Sciences and started as assistant-in-opleiding (AIO) at the Animal Nutrition Group of Wageningen University and Research Centre. From October 2004 until January 2005, he was appointed as ruminant nutrition researcher at the Animal Nutrition Group. Since Februari 2005, he works as a member policy staff livestock farming for the Dutch Organisation of Agriculture and Horticulture in The Hague.

Bart (Bartholomeus Martinus) Tas is geboren op 14 januari 1976 te Callantsoog. Hij is opgegroeid op het melkveebedrijf van zijn ouders. In 1994 behaalde hij zijn Atheneum-diploma aan het Etty Hillesum College in Den Helder. Hetzelfde jaar begon hij de studie Zoötechniek aan de Landbouwuniversiteit Wageningen. Zijn specialisaties waren Gezondheidsleer & Reproductie en Veevoeding. In 1999 liep hij stage aan de Veterinaire Faculteit van Massey University in Palmerston North, Nieuw-Zeeland, waar hij onderzoek deed naar homosexueel gedrag van rammen en hertenbokken. In het voorjaar van 2000 rondde hij zijn studie Dierwetenschappen af en begon hij als assistent-in-opleiding (aio) bij de Leerstoelgroep Diervoeding aan Wageningen Universiteit en Researchcenter (WUR). Bij diezelfde groep was hij van oktober 2004 tot januari 2005 werkzaam als onderzoeker rundveevoeding. Sinds februari 2005 werkt hij als beleidsmedewerker veehouderij bij LTO Nederland (Land- en tuinbouworganisatie Nederland) in Den Haag.

The research described in this thesis was conducted in close collaboration between the Animal Nutrition Group and the Crop and Weed Ecology Group of Wageningen University in The Netherlands.

The experiments were conducted at the experimental fields of "Unifarm" (Plant Sciences Group) and the dairy cows and housing facilities were used of "De Ossekampen" (Animal Sciences Group).

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