Perennial ryegrass for dairy cows:

Effects of cultivar on herbage intake during grazing

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<u>Abstract</u>

Smit. H.J., 2005. Perennial ryegrass for dairy cows: effects of cultivar on herbage intake during grazing. Perennial ryegrass (*Lolium perenne* L.) is the most important species for feeding dairy cows. The majority of the farmers in the Netherlands graze their dairy cows during summer. During grazing, the limited herbage intake is the main limitation of the dairy cows' production. This study examined opportunities for grass breeders to influence the quality of perennial ryegrass cultivars to improve the herbage intake of dairy cows.

Six cultivars of perennial ryegrass (Abergold, Respect, Agri, Herbie, Barezane, Barnhem) were intensively studied during two years (2000 and 2001) between June and September. There were differences between the cultivars for important characteristics influencing herbage intake, e.g. herbage yield, sward surface height and density. Furthermore, differences between cultivars were found for the proportions and the biomass of leaf, stem and pseudostem in the sward. The sward cutting and the n-alkanes technique, both methods to determine the herbage intake by grazing dairy cows, were compared with an average that was calculated from the milk production and the live weight of the dairy cows. The sward cutting technique gave very variable results that varied largely per cow and year, and did not match with the expected calculated intake. The n-alkanes method gave less variable results that were more related to the expected calculated intake values. Within the n-alkanes method, the ratio C_{32}:C_{33} gave the best results.

The effects of perennial ryegrass cultivars on the intake of grazing dairy cows were examined. In 2002 and 2003, cows grazed each of the four cultivars during four 2-week periods. Herbage intake was estimated using the C_{32}:C_{33}-alkanes method. In 2002, a clear difference was found between the cultivars, but in the second year no differences were found. A higher herbage intake was related with a higher mass of herbage and green leaf, a higher sward surface height, a lower infestation rate of the crown rust fungi and a lower lignin content in the herbage. The cultivars differed too little in the other quality parameters to induce effects in the cow. The aspects of selection and preference were examined in an experiment with the six earlier described cultivars. The experiment was conducted in three 4-day periods in spring, summer and autumn. Herbage intake was measured using the sward cutting method. The dairy cows very consistently preferred cultivars with a high water soluble carbohydrates concentration, a high digestibility, a low cell wall concentration and a low ash concentration. It was concluded that in the selection process the chemical quality of the herbage plays an important role.

Keywords: Perennial ryegrass, *Lolium perenne*, sward morphology, sward cutting, n-alkanes, herbage intake, selection, preference.

Who covers the sky with clouds.
Who prepares rain for the earth.
Who makes grass grow.
He provides food for the cattle.

Contents

Abbreviation key

ADL:	Acid Detergent Lignin
ASH:	Crude Ash
BD:	Bulk Density
BW:	Body Weight
CF:	Crude Fibre
CFAT:	Crude Fat
CP:	Crude Protein
CV:	Coefficient of Variation
D:	Days after 1st of April
DCP:	Digestible Crude Protein
DM:	Dry Matter
DMI:	Dry Matter Intake
DMY:	Dry Matter Yield
DOM:	Digestible Organic Matter
ETH:	Extended Tiller Height
FPCM:	Fat and Protein Corrected Milk
GE:	Gross Energy
GLM:	Green Leaf Mass
ME:	Metabolizable Energy
NDF:	Neutral Detergent Fibre
NE:	Net Energy
NFE:	Nitrogen Free Extract
NIRS:	Near Infrared Reflectance Spectroscopy
OM:	Organic Matter
SD:	Standard Deviation
SED:	Standard Error of Difference
SEM:	Standard Error of Mean
SSH:	Sward Surface Height
TD:	Tiller Density
TW:	Tiller Weight
WSC:	Water Soluble Carbohydrates

Chapter 1

General introduction:

Cultivars effects of perennial ryegrass on herbage intake by grazing dairy cows

General introduction

Grassland in the Netherlands

Grassland in the Netherlands consists of approximately 1 million hectares, which is half of the total agricultural area. Grazing areas in the Netherlands are mainly located in the North (Friesland), the West (Groene Hart) and some parts of the East (Overijssel, Achterhoek) of the country (Figure 1). The Dutch landscape is largely dominated by grasslands and these have significant economic value for the country. The main purpose of this grassland is feeding the 1.5 million dairy cows and 1.1 million young stock (CBS, 2004), which form a major part of the Dutch agriculture. Until now the majority of the farmers graze their dairy cows during summer, but there is tendency to summer stall feeding (van den Pol van Dasselaar *et al*., 2002), mainly because of decreasing faith in quality of grass feeding. Also other factors as nutrient legislation, increasing herd sizes and automatic milking systems play a role.

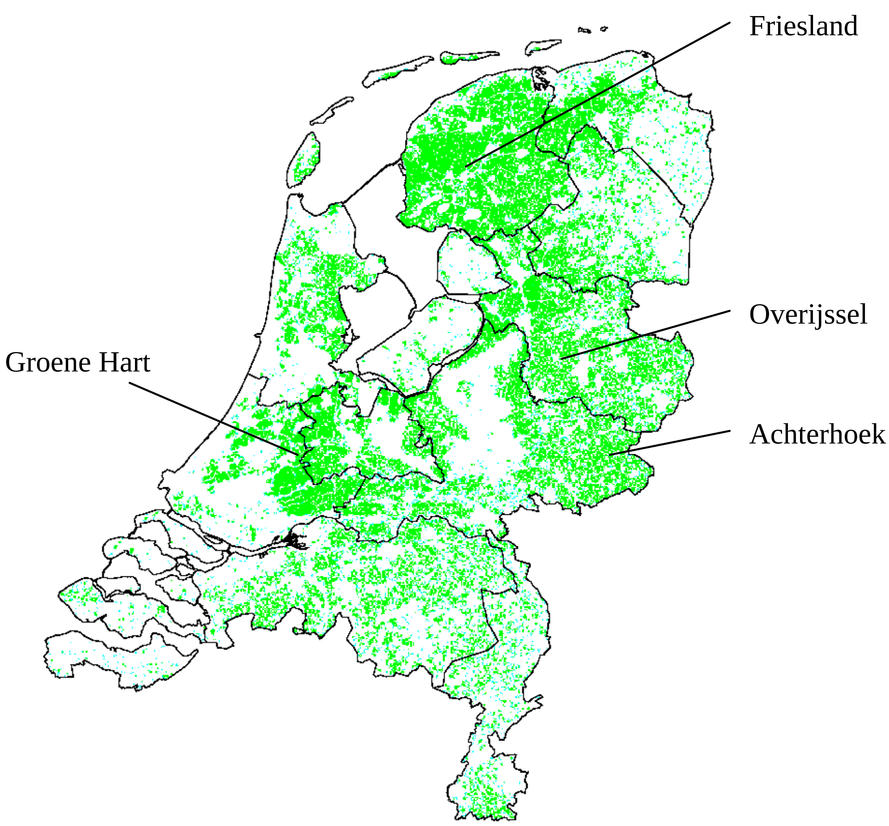

Figure 1 Grassland areas (grey) in the Netherlands. (Map kindly provided by Alterra, Wageningen University).

The average annual yield of these grasslands is around 8 to 9 tons ha^{-1} (Schils *et al.*, 2002). The Dutch climate and soil conditions are ideal for growing grass and in particular perennial ryegrass. Perennial ryegrass is the most abundant species in pastures of north-western Europe and is generally considered as a high quality fodder (Bonthuis *et al.*, 2004). An agronomical good sward should botanically consist of over 50% perennial ryegrass (de Vries *et al.*, 1942; Neuteboom, 1986; Asjiee, 1993).

Resowing

When the botanical composition is changing to less preferred species, e.g. couch grass (*Elymus repens* L.) or annual meadow grass (*Poa annua* L.), but also when land is needed for arable cropping, farmers decide to renovate their pastures. An estimated 125,000 ha of grassland is renovated in the Netherlands annually (Schils *et al.*, 2002), which is approximately 12% of the total Dutch grassland area (CBS, 2004). As shown in Table 1, sandy soils are renovated most regularly, whereas peat soils are on average only renovated once in thirty years.

Table 1 Grassland renovation in 1999 (ha × 1000), in relation to soil type (taken from Schils *et al.*, 2002) and the renovation rate (renovated area year^{-1}).

	Total grassland area	Renovated permanent grassland	Ley - arable	Renovation rate
Sand	450	55	26	0.18
Clay / Loam	400	12	29	0.10
Peat	175	3	3	0.03

Generally, mixtures of different grass species are sown, although also 100% perennial ryegrass is used, as blends of different cultivars. Varieties used in mixtures have to be approved grass varieties that appear on the Dutch recommended list of Cultivars for Agricultural Crops (Bonthuis *et al.*, 2004) or on the EU cultivar list. To be included in the list of cultivars, a cultivar must be registered and tested (Visscher, 1998). Until now, the list contains mainly yield related parameters, annual yield, yield of the first cut, crown rust resistance, winter hardiness, persistence and heading date (Bonthuis *et al.*, 2004). In recent decades, the annual genetic improvement for dry matter (DM) yield of perennial ryegrass was estimated at 0.5% (van Wijk and Reheul, 1991). This

was mainly due to increased persistence of the new varieties, resulting in an increase in DM yields in the third and fourth year after their establishment (van Wijk *et al.*, 1993). The farmer benefits from this improved yield by producing more forage on farm or by producing the same amount of forage at lower fertilizer rates. In this way, new cultivars contribute to a better use of nutrients (Visscher, 1998). Grass in the Netherlands is mainly used as feed in animal production systems and for dairy production in particular. One can question whether farmers benefit most from testing programmes that are focused on grass yield parameters only. Farmers benefit most from an efficient conversion from herbage into milk. Until now, the official Dutch testing programmes do not include parameters, such as *in vitro* digestibility, chemical composition or palatability. Other testing programmes (UK and Australia) include digestibility or select for grasses with specific contents, e.g. high sugar content.
In an assessment among the world's leading nutritionists and breeders (Smith *et al.*, 1997) of the relative importance of specific traits for genetic improvement of nutritive value: high digestibility, the high rate of digestion, increasing non-structural carbohydrates content and high palatability were listed number, 1 to 4.

General objective

The general objective of this study was to investigate whether there is scope for breeding diploid perennial ryegrasses for improved herbage intake. Therefore, the following sub-questions were asked:

- Is variability among diploid cultivars in sward structural and morphological characteristics large enough to be important for herbage intake? (Chapter 2)
- How is intake measured in a grazing situation? (Chapter 3)
- Are there differences among cultivars in herbage intake by dairy cows grazing a single cultivar? (Chapter 4)
- Are there differences due to selection among cultivars in herbage intake by dairy cows grazing all cultivars? (Chapter 5)

Variability among cultivars in sward structure and morphology important for herbage intake (Chapter 2)

Sward structure is an important quality aspect of grass with respect to intake and digestibility (Laca *et al.*, 1992; Laidlaw and Reed, 1993), two important factors in the conversion of herbage to milk in grazing systems. Sward structure includes herbage mass, sward surface height, bulk density, tiller density, morphological and botanical composition and textural characteristics such as shear and tensile strength (Laca *et al.*, 1992; Hazard *et al.*, 1998; O'Donovan, 2001; Barrett *et al.*, 2001). While measurement of sward characteristics is vital for the understanding of animal-sward interactions, the techniques to measure them are time-consuming and labour-intensive. Notwithstanding that, it is essential to give a full description of the sward to allow full interpretation of the intake data (Laidlaw and Reed, 1993).

Differences in sward structural characteristics between grass species are well recognized, but more recently differences have also been found among perennial ryegrass varieties (Hazard *et al.*, 1998; O'Donovan, 2001; Gilliland *et al.*, 2002). The aim of the experiment described in Chapter 2 was to assess the genetic variation among six diploid perennial ryegrass varieties for plant morphological traits which have been reported as important for grass intake, such as DM yield, sward surface height, bulk density, tiller density, proportion of green leaf, extended tiller height and sheath length.

Techniques to measure herbage intake of grazing dairy cows (Chapter 3)

To compare effects of cultivars on herbage intake by grazing animals, a good technique to estimate herbage intake during grazing needed to be developed. Several techniques were available.

The classical method to determine intake is the so-called sward-cutting method. A measured proportion of the area allotted to the animals is harvested and the total herbage offered to the animal can be calculated. The residual herbage after grazing is determined in a similar manner. The difference between these two herbage masses and a correction for the regrowth gives an estimate of the herbage consumed in the area grazed (Meijs, 1981; Macoon *et al.*, 2003). The sward cutting method can provide reliable estimates of intake when short grazing periods are applied and when a large part of the offered herbage is consumed (Walters and Evans, 1979; Meijs, 1981). However, this methodology has a large variation (Meijs, 1981) and is mainly used to determine herbage intake for groups of animals.

Recently, a new method for determining dry matter intake (DMI) in grazing animals has been developed, the so-called n-alkanes method (Dove and Mayes, 1991; 1996, Dillon, 1993). This marker-ratio method calculates DMI from a known dose of an external indigestible marker, the content of a naturally occurring indigestible marker in the herbage and the ratio of these two markers in the faeces. In Chapter 3, both methods are compared with a calculated herbage intake, estimated from the energy requirements (van Es, 1978).

Cultivar effects on herbage intake of grazing dairy cows (Chapter 4)

With the gained knowledge of chapter 2 and 3, four cultivars out of the eight were selected for a large experiment to investigate cultivar effects on herbage intake by grazing dairy cows. Cultivar effects on herbage intake have been found in sheep (Orr *et al.*, 2003; Hazard *et al.*, 1998) and beef cattle (Lee *et al.*, 2002; O'Riordan *et al.*, 1998). In dairy cows, differences have been found between diploid and tetraploid cultivars (Hageman *et al.*, 1992) showing that tetraploid cultivars have a high potential for grazing animals, such as a higher water soluble carbohydrates level, and a high green leaf mass (GLM). However, until now the majority of selected cultivars on the Dutch Recommend List (Bonthuis *et al.*, 2004) are diploid. Also, differences have been found between late and intermediate heading cultivars (Gowen *et al.*, 2003), showing that late heading cultivars had a better potential for high DMI by grazing dairy cattle in spring and summer. In contrast, no effects of cultivars on DMI by dairy cows were found by Miller *et al.* (2001) and Tas (2005). However, this might be related to the fact that these were stall-feeding experiments and there was no real interface between the animal and the standing crop.

The aims of this chapter were to determine cultivar differences in DMI of four diploid perennial ryegrass cultivars during grazing by highly productive dairy cows, and to relate this to sward structural and chemical characteristics.

Cultivar effects on selection by grazing dairy cows (Chapter 5)

Although large herbivores are generally considered bulk feeders, the literature is far from conclusive about what forage diet the animals prefer to eat (Parsons *et al.*, 1994). Preference is usually measured during a very short term interval (Laca *et al.*, 1992; Provenza *et al.*, 1996; Griffiths *et al.*, 2003). Preference behaviour among different forage species is a well-recognized and frequently studied concept (Heady, 1964; Belovsky, 1978; Stephens and Krebs, 1986; Rutter *et al.*, 2002). Species that are less abundant are generally highly selected or rejected, while abundant species, in most cases grasses, furnish the bulk of the diet (van Dyne and Heady, 1965). In modern agriculture, however, pastures have become completely dominated by grasses, sometimes by only one single species (e.g. perennial ryegrass. The effects of various cultivars of perennial ryegrass on preference behaviour of dairy cows during grazing were studied.

General discussion

The first part of the general discussion, the choice of cultivars that were used and the use of near infrared reflectance spectroscopy were critically evaluated. Furthermore the use of alkanes in preference tests was discussed.

In the second part of the general discussion, the results of four years of experiments were combined to evaluate the variation in the examined cultivars in plant-related traits as yield, sward structure and morphology, chemical properties, physical characteristics and disease resistance. Furthermore, the effect of perennial ryegrass cultivars on animal herbage intake and animal performance was discussed.

Chapter 2

Sward characteristics important for intake in six *Lolium perenne* varieties

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Abstract

An experiment was conducted to determine genetic variation among diploid perennial ryegrass (*Lolium perenne* L.) varieties for sward structural characteristics considered to be important for intake by cattle. Assessments were made between June and September in 2000 and 2001. Six varieties (Abergold, Respect, Agri, Herbie, Barezane and Barnhem) were subjected to a cutting trail where swards were cut after 3 to 4 weeks of regrowth during the growing season. The variables, measured in three 2-week periods, were herbage mass of dry matter (DM), sward surface height (SSH), bulk density, proportion of green leaf, tiller density, tiller weight, extended tiller height, length of sheath and length of leaf blade. Significant differences among varieties were found in both years for herbage mass of DM, SSH, bulk density, proportion of green leaf, tiller density, tiller weight and length of sheath. The results show that there is significant genetic variation among diploid perennial ryegrass varieties for sward characteristics important for intake during grazing.

Keywords: Perennial ryegrass, cultivars, sward morphology, herbage intake

Introduction

Dairy production in north-western Europe depends mainly on grass-fed systems, as grass provides a cheap fodder of high quality. Perennial ryegrass (*Lolium perenne* L.) is known to be highly productive and digestible, and is the most widely sown grass in western Europe (de Jong, 1991; Sheldrick, 2000). The annual genetic improvement for dry matter (DM) yield of perennial ryegrass was estimated to be proportionally 0.005 in the period from 1965 to 1990 (van Wijk and Reheul, 1991). This gain was mainly due to increased persistence of the new varieties, resulting in an increase in DM yields in the third and fourth year after establishment (van Wijk *et al.*, 1993). Yield of DM is an important characteristic in the Dutch Recommend List of Varieties. However, little attention has been given to characteristics that can promote herbage intake of grazed perennial ryegrass (Gilliland *et al.*, 2002), a factor that still limits the milk production and maintenance of liveweight of highly productive dairy cows (Chilibroste, 1999; Taweel, 2004).

Sward structure is important in influencing herbage intake (Laidlaw and Reed, 1993; Hazard *et al.*, 1998). Livestock respond to several sward characteristics, e.g. herbage mass, sward surface height (SSH), bulk density (BD), tiller density (TD) and canopy morphology (Laca *et al.*, 1992; Hazard *et al.*, 1998; O'Donovan, 2001; Barrett *et al.*, 2001). Differences in sward structural characteristics between species are well recognized, but more recently differences have also been reported within perennial ryegrass varieties (Hazard *et al.*, 1998; O'Donovan, 2001; Gilliland *et al.*, 2002). Ploidy has been shown to have an effect on herbage mass, extended tiller height (ETH) and BD (Gilliland *et al.*, 2002; O'Donovan, 2001). Stage of maturity has also been found to have an effect on sward structure, and Gilliland *et al.* (2002) found that late-heading varieties had a higher BD, but a lower SSH and ETH than early- and intermediate-heading varieties. O'Donovan (2001) found, in the first year, higher BD for late varieties compared to intermediate ones but no differences in the second year.

According to the self-thinning rule, dense swards are negatively associated with heavy tillers (Yoda *et al.*, 1963; Matthew *et al.*, 1995). Hazard and Ghesquière (1997) pointed out that varieties with large tillers and rapid leaf elongation would yield more under lenient defoliation, whereas varieties with smaller tillers, but with higher tiller densities would give a slightly higher yield under severe defoliation. In a two-year grazing experiment O'Donovan (2001) found large differences in tiller density (4411 vs 6634 tillers m^{-2}) between four

perennial ryegrass varieties. Diploid varieties had higher tiller densities than tetraploids.
The length of new leaves is influenced to a large extent by the length of the sheath tube through which the leaves emerge (Grant *et al.,* 1981). If the sheath tube is long, the leaves will also be relatively long and may require more time to appear (Chapman and Lemaire, 1993). Within perennial ryegrass a large amount of genetic variation in sheath (33-72 mm) and blade length (100-260 mm) has been found (van Loo, 1993). Jackson (1974) showed that perennial ryegrass was able to adapt its sheath length to cutting height. In an experiment with four cutting heights (3, 6, 9 or 12 cm), the average sheath length was 2.4, 4.3, 6.6 and 9.2 cm, respectively. Grass tillers had adapted their morphology to the mowing regime but showed similar green leaf mass (GLM).
The aim of this chapter was to assess the genetic variation among six diploid perennial ryegrass varieties for plant morphological traits which have been reported as important for herbage intake and to analyse the relationships between these traits.

Materials and methods

Site and experimental design

A series of plots were sown with perennial ryegrass (*Lolium perenne* L.) in October 1999. The plots were situated on a clay soil in a field at Wageningen University, The Netherlands (51°58’N / 5°40’E, 7 m.a.s.l.) which had previously been used for arable cropping. Soil analyses of the 0-25 cm layer were performed in March 2000. The pH (KCl) was 7.0, the organic matter content was 5.5 % and the percentage of soil particles < 2 µm was 41 %.
Six diploid perennial ryegrass varieties were used: three intermediate-heading types (Abergold, Respect and Agri) and three late-heading types (Herbie, Barezane and Barnhem). The heading date of the intermediate-heading varieties is in the period of 25-28 May and of the late-heading varieties in the period of 7-9 June (Ebskamp and Bonthuis, 2000). Varieties were sown in adjacent plots of 110 m x 2.2 m. The experimental design was a randomized block design with 21 replicate blocks. The experiment was divided into three 2-week periods (P1-3). In each period, 14 of the 21 blocks were used to cut grass daily at a target herbage mass of 2000 kg DM ha^{-1} to provide herbage for an intake experiment reported elsewhere (Tas, 2005). Only 14 out of the 21 blocks available were needed, as one block provided feed for one day. A

flexible system was used where herbage was cut daily from a block that had the target herbage mass. This herbage mass was estimated from an established relationship between sward surface height, measured with a falling plate meter, and herbage mass of DM (Elgersma and Schlepers, 1997). Excess neighbouring blocks were cut as well at the approximate target herbage mass, but no data were recorded. Due to changing growing conditions during the experiment, different blocks were used in the various periods.

Grassland management

Within each block, all varieties were always treated the same. Plots were fertilized in spring and after each cut (Table 1) according to requirements as indicated by soil analyses. In 2000, the spring growth (first cut) was cut on 3 May for silage. A second cut was taken at various dates, starting on 29 May to 14 June, to create variation in regrowth stage among blocks. The third cut was the first experimental cut (P1); P1 lasted from 23 June to 6 July, P2 lasted from 21 July to 3 August and P3 lasted from 18 August to 31 August. Weather conditions in 2000 were wetter than normal. During the winter 2000-2001 the paddocks were grazed by sheep. In 2001, spring growth was cut on 3 May for silage. A second cut was taken at various dates, starting on 5 June to 14 June, to create variation in regrowth stage among blocks. The third cut was the first experimental cut (P1).

Table 1 Nitrogen (N), phosphate (P_2O_5) and potash (K_2O) applications (kg ha^{-1}) to the experimental plots after each harvest in 2000 and 2001.

Cut	Date	Fertilizer			Date	Fertilizer		
		N	P_2O_5	K_2O		N	P_2O_5	K_2O
	2000				2001			
Spring	6 April	65	35	0	5 April	52	42	84
1	3 May	52	42	84	3 May	82	42	84
2	22 May – 8 June	45	0	0	6 June – 25 June	100	0	0
3	14 June – 3 July	47	36	72	3 July – 23 July	50	0	0
4	4 July – 23 July	40	32	64	27 July – 10 Aug.	75	60	120
5	24 July – 11 Aug.	40	32	64	13 Aug. – 1 Sept.	60	48	96
6	18 Aug. – 4 Sept.	30	0	0	2 Sept. – 7 Sept.	30	0	0

In 2000, the total applications of N, P_2O_5 and K_2O were 319, 177 and 284 kg ha^{-1}, respectively and, in 2001, 449, 192 and 384 kg ha^{-1} respectively.

Weather conditions in June 2001 were drier than normal and irrigation (15 mm) was needed to avoid drought stress. In the other months weather conditions were wetter than normal. Due to some difficulties in reaching the target herbage mass of 2000 kg DM ha^{-1} in July, the length of the first period needed to be adjusted, and P1 lasted 12 days rather than 14 days. P1 lasted from 2 July to 13 July, P2 lasted from 27 July to 9 August and P3 lasted from 24 August to 6 September. The two missing days were treated as missing values.

Grass samples

The SSH was measured daily with a falling plate meter (‘t Mannetje and Jones, 2002). Ten recordings per plot were made and the average was used in further calculations. Complete plots in one block were cut daily between 13.30 h and 14.30 h with a front-mower (Vicon CM230, Kverneland, Dronten) at a cutting height of 6 cm and herbage was collected with a pick-up wagon (K327, Deutz-Fahr, Lauingen). Fresh yield was determined after harvesting by weighing the total fresh yield in the pick-up wagon using a weighing bridge. Core samples of approximately 300 g fresh grass were taken of each harvest daily, weighed and dried at 70 ºC for 24 h to assess the DM content. Bulk density above 6 cm was estimated by dividing the herbage mass of DM by (SSH – 6 cm cutting height) and expressing BD as kg DM m^{-3}.

To measure canopy morphology a sample of several hand-plucks was taken randomly from the cut herbage every day. In 2000, the herbage was split into three fractions: leaf blade, stem plus pseudostem and dead material. Leaves, stems and pseudostems were separated at the ligule and all material without green colour was considered dead. In 2001, the herbage was split into four fractions: leaf blade, stem, pseudostem and dead material. Plant parts were dried at 70 ºC for 24 h to assess the DM content. Green leaf mass above 6 cm was calculated by multiplying the herbage mass of DM by the proportion of leaf blade.

Tiller density and tiller weight

In 2000, tiller density in each variety was determined twice in each period in the first and the second weeks. In each plot, all tillers within seven quadrats of 360 cm^2 (50 cm × 7.2 cm) were counted. During P1 and P2 the counting was done in the field, but from P3 and in 2001 the herbage in the quadrats was cut at ground level with a knife. The herbage was carefully put horizontally in a tray with minimal disturbance of the sward structure and transported to the

laboratory where all tillers were counted. In 2001, each variety was analyzed twice in each period. In each plot, three quadrats of the same size were cut.
During period 3 in 2000 and in all periods in 2001, after all tillers had been counted, the material was dried for 24 h at 70 °C to determine the average individual tiller weight.

Tiller and canopy morphology
In 2000 in P3 and in 2001 in P2 and P3, three sub-samples of 40 intact tillers were randomly taken from the herbage that was cut for the tiller density measurement. The length of the leaf sheath and of the youngest fully expanded leaf, as well as the extended height, was individually measured for all 40 tillers with a ruler.

Statistical analyses
The data were analyzed with the model:

$$Y_{ijkl} = \mu + P_i + D_j\,(P_i) + H_k + V_l(H_k) + P_i \times H_k + P_i \times V_l(H_k) + e_{ijk} \qquad (1)$$

Where, μ is general mean; P_i is period effect (j = 2, 4, or 6); D_j (P_i) is day within period effect (D_j = 1,…14); H_k is heading effect (intermediate or late); V_l (H_k) is variety effect; $P_i \times H_k$ is the interaction between period and heading type; $P_i \times V_l\ (H_k)$ is the interaction between period and variety within heading type and e_{ijkl} is the residual term. The harvested plot was the experimental unit. Data were analyzed by the SPSS statistical package (SPSS for Windows, Rel. 11.0.1, 2001. SPSS inc., Chicago).

Results

In both years, differences were found for herbage mass of DM between the two heading types (Table 2). However, the intermediate-heading varieties had a higher herbage mass of DM (P>0.001) in 2000, whereas in 2001 the late-heading cultivars were higher (P>0.001). In both years, variation was found among varieties within the late-heading group. Barnhem was the variety with the highest herbage mass of DM (P<0.001) in both years and Barezane was the lowest. In the intermediate-heading group, there was no difference between varieties.
Intermediate-heading varieties had the highest SSH in 2000 and late-heading varieties had the highest SSH in 2001, following the same pattern as the

herbage mass of DM. In 2000, the varieties Agri and Respect had the tallest swards in the intermediate-heading group and varieties Barezane and Barnhem had the lowest SSH in the late-heading group. In 2001, no differences (*P*>0.05) were found between varieties within heading groups.

In 2000, bulk density differed (*P*<0.001) between varieties, but not between heading types. In 2001, BD was much higher than in 2000 and the intermediate-heading varieties had higher (*P*<0.001) values than the late-heading varieties. In 2000, BD increased during the season whereas, in 2001, it decreased (data not shown).

In 2000, the GLM of the late-heading varieties was higher than of the intermediate-heading group. In 2001, the opposite was found but the variation within each group was high. Varieties Abergold and Barnhem had the highest GLM in their heading category in both years.

In 2000, the proportion of leaf blade was lowest in the intermediate-heading varieties, particularly in P1 (Figure 1A). The late-heading varieties, especially Barezane and Barnhem, had the highest proportion of leaf blade throughout the experiment. The proportion of stem plus pseudostem declined rapidly during the experiment and was largely complementary to the proportion of leaf blade. In 2001, the late-heading varieties had the lowest proportions of leaf blade (Table 2), which resulted from the large difference in P1 (Figure 1B). However, during the other two periods, the late-heading varieties had a slightly higher proportion of leaf blade. The proportion of pseudostem was low in P1 (0.015), but increased during the experiment (0.066 in P3). The intermediate-heading varieties had a higher proportion of pseudostem in all periods but the effect was largest in P3, mainly due to the variety Barezane, which always had the lowest (*P*<0.01) proportion of pseudostem. The proportion of dead material in 2000 was on average 0.014, and in 2001 it was 0.054.

A significant (*P*<0.01) and consistent difference in tiller density among varieties was found in both years (Table 3). Late-heading varieties had on average the highest TD. However, as an exception the late-heading variety Herbie had the lowest TD. The varieties Agri, Herbie and Respect always had a low TD, whereas varieties Abergold, Barezane and Barnhem always had a high TD. The varieties in the low-density group already exhibited low TD in June 2000 and, although the overall TD increased threefold, in September 2001 they still had the lowest density.

In 2000, the intermediate-heading group had heavier tillers (*P*<0.05) on average. No variation among varieties was observed. However, there was a tendency (*P*=0.082) for a lower tiller weight (TW) in varieties Respect, Agri

Table 2 The herbage mass of dry matter above 6 cm (kg ha^{-1}), sward surface height (SSH) (cm), bulk density (BD) (kg DM m^{-3}), green leaf mass (GLM) (kg ha^{-1}), and proportions of leaf blade, stem, pseudostem and dead material, of three intermediate- and three late-heading diploid perennial ryegrass varieties. Standard error of the mean (S.E.M.) are presented. Effects of period (P), heading type (H) and variety within heading (V(H)) and their interactions were tested.

Year	Variable	Heading Type						S.E.M.	Effects				
		Intermediate			Late								
		Abergold	Respect	Agri	Herbie	Barezane	Barnhem		P	H	V(H)	P×H	P×V(H)
2000	Herbage mass	2294	2222	2248	2157	2067	2250	27.3	***	***	**	NS	NS
	SSH	21.7	22.3	22.3	21.9	20.5	20.7	0.24	***	***	**	NS	***
	BD	1.46	1.36	1.37	1.36	1.42	1.52	0.02	***	NS	***	NS	***
	GLM	1832	1726	1693	1693	1743	1914	18.5	***	*	***	***	*
	Proportion of												
	Leaf blade	0.820	0.802	0.789	0.812	0.859	0.880	0.008	***	***	***	***	***
	Stem + pseudostem	0.167	0.182	0.200	0.174	0.125	0.107	0.009	***	***	***	***	***
	Dead	0.013	0.016	0.011	0.014	0.016	0.014	0.001	***	NS	**	NS	*
2001	Herbage mass	2240	2245	2312	2404	2393	2575	31.1	***	***	***	***	***
	SSH	18.8	19.3	19.7	21.7	21.7	21.5	0.21	**	***	NS	***	***
	BD	1.83	1.72	1.75	1.56	1.56	1.69	0.02	***	***	***	***	*
	GLM	1914	1853	1895	1756	1741	1873	25.7	***	***	**	*	***
	Proportion of												
	Leaf blade	0.841	0.827	0.824	0.768	0.753	0.756	0.010	***	***	**	***	***
	Stem + pseudostem	0.113	0.113	0.118	0.185	0.190	0.190	0.010	***	***	NS	***	**
	Stem	0.063	0.066	0.066	0.142	0.155	0.143	0.011	***	***	NS	***	*
	Pseudostem	0.051	0.047	0.052	0.042	0.035	0.047	0.002	***	***	**	NS	*
	Dead	0.046	0.060	0.058	0.048	0.058	0.053	0.002	***	NS	*	**	NS

***: $P<0.001$; **: $P<0.01$; *: $P<0.05$; NS: not significant.

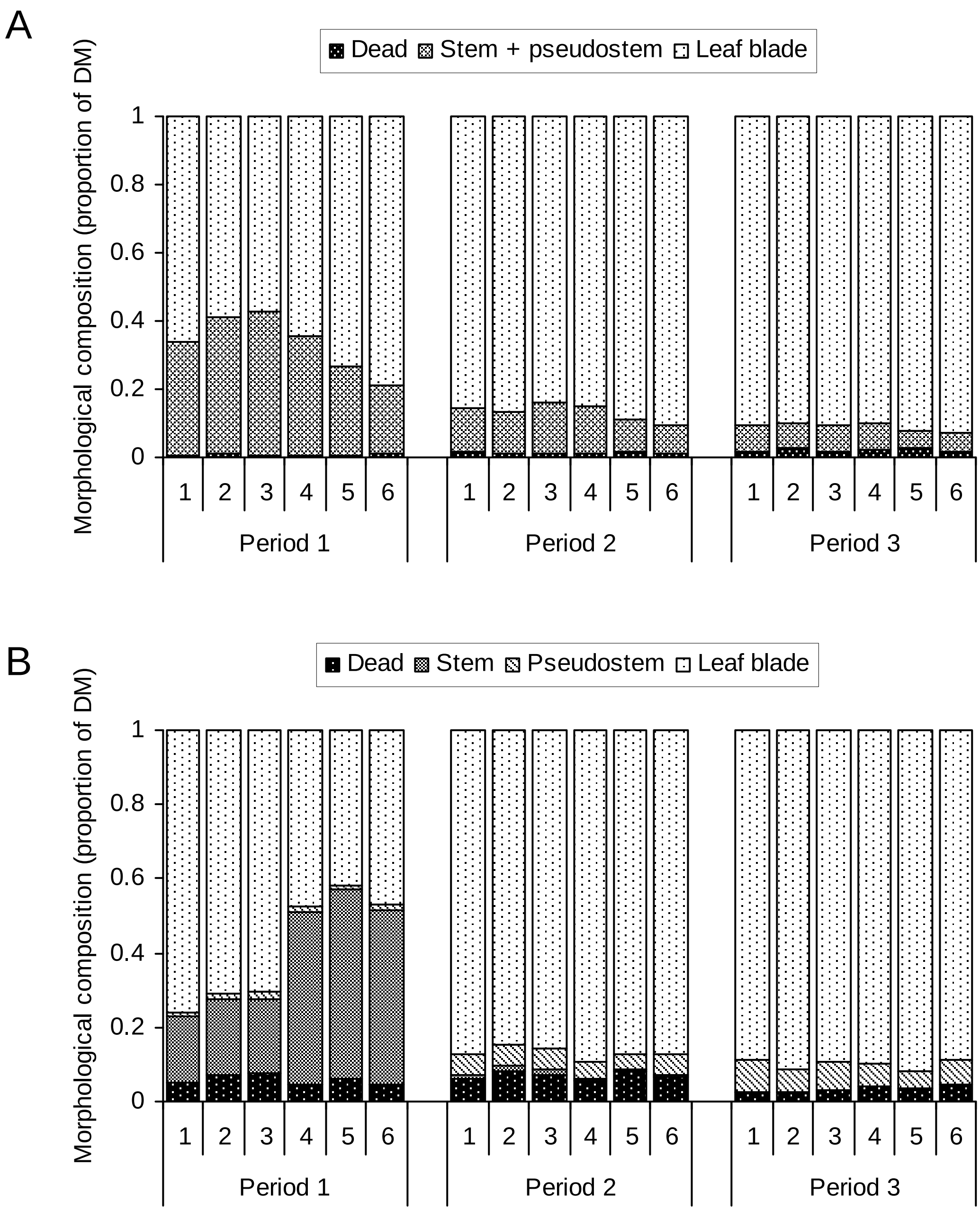

Figure 1 The morphological composition of six perennial ryegrass varieties, Abergold (1), Respect (2) and Agri (3) (intermediate-heading) and Herbie (4), Barezane (5) and Barnhem (6) (late-heading) in (A) 2000 and (B) 2001. The morphological composition in 2000 was dead material, stem + pseudostem and leaf blade, and in 2001 dead material, stem, pseudostem and leaf blade.

Table 3 Tiller density (tillers m^{-2}), tiller weight (mg) and extended tiller height (ETH) (mm) divided into leaf sheath and leaf blade length (mm), of three intermediate and three late-heading diploid perennial ryegrass varieties. Standard error of the mean (S.E.M.) are presented. Effects of period (P), heading type (H) and variety within heading (V(H)) and their interactions were tested.

Year	Variable	Heading type						S.E.M.	Effects				
		Intermediate			Late								
		Abergold	Respect	Agri	Herbie	Barezane	Barnhem		P	H	V(H)	P×H	P×V(H)
2000	Tiller density	6007	4413	4691	4063	5755	6166	360	***	**	***	NS	NS
	Tiller weight	45.5	52.0	51.6	49.9	43.2	44.3	1.2	-	*	NS	-	-
	ETH	273	309	300	299	246	283	9.3	-	NS	*	-	-
	Leaf sheath	55	64	62	58	49	58	2.1	-	*	*	-	-
	Leaf blade	218	245	238	241	197	225	7.3	-	NS	NS	-	-
2001	Tiller density	13297	12163	10872	11409	15344	14887	518	***	*	**	NS	NS
	Tiller weight	29.7	30.8	35.6	35.0	25.1	27.0	1.0	**	NS	**	NS	NS
	ETH	272	281	297	269	262	283	5.6	*	NS	NS	NS	NS
	Leaf sheath	58	59	66	57	56	62	1.5	*	NS	NS	NS	NS
	Leaf blade	214	221	230	213	206	221	4.3	**	NS	NS	NS	NS

***: $P<0.001$; **: $P<0.01$; *: $P<0.05$; NS: not significant; – : not possible to test.

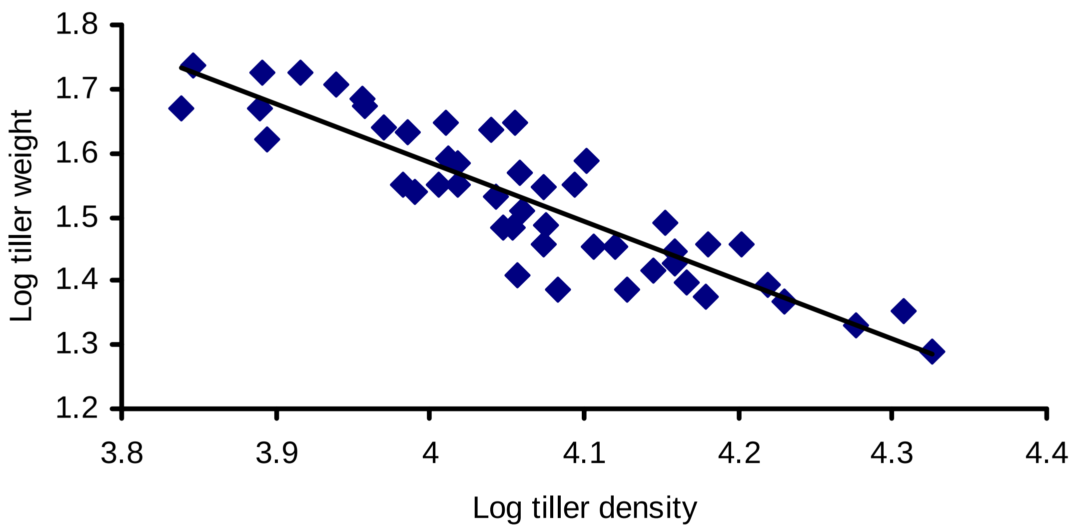

Figure 2 The relationship between tiller weight (y, log mg) and tiller density (x, log number of tillers). Y = –0.92X + 5.25 (R^2 = 0.788, n=48).

and Herbie. This finding was in line with expectations because these varieties had open swards. In 2001, varieties Agri and Herbie differed (P<0.01) from the other varieties in their group. There was a large difference between average TW in 2000 (47.7 mg) and 2001 (30.6 mg). The relationship between TW and TD is shown in Figure 2. TW was negatively correlated with TD (R^2 = 0.788, n=48).

Leaf sheath lengths and leaf blade lengths are also shown in Table 3. They were positively correlated (R^2 = 0.80). The leaf sheath length was on average 0.216 of the total tiller length. The leaf blade length was on average 0.784 of the total tiller length and there was little variation in this proportion during the year. The effect of variety on leaf sheath length was more pronounced. The variety Barezane had the lowest values for leaf sheath, leaf blade and total tiller length, but this was only significant (P<0.05) for leaf sheath length.

Discussion

Varietal effects on sward structure

Herbage mass of DM, sward surface height, bulk density and tiller density are sward structural characteristics known for their positive influence on rate of herbage intake by dairy cows (Forbes, 1986; Laca *et al.*, 1992; Hazard *et al.*, 2002). The combined use of these sward structure variables could ease the selection of varieties to increase herbage intake.

However, the relationship between sward structural variables was not consistent. In 2000, the intermediate-heading varieties had higher herbage masses, had a higher SSH, but no differences in BD were found. However, in 2001 the late-heading varieties had higher herbage masses, a higher SSH and a lower BD. The only consistent variable was TD. Throughout the entire experiment the late-heading varieties had the highest values. There was, however, also a large effect of variety within heading type ($P<0.01$) on TD in both years. This might explain the contrast with the results of O'Donovan (2001), who found a higher TD in the intermediate-heading diploid variety Portstewart than in the late-heading diploid variety Spelga. Laidlaw (2004) found no difference between intermediate-heading varieties and late-heading varieties, but did find differences on some sampling dates with early-heading varieties, which were not included in this study.

In both years, variation among varieties within a heading-date group was found for almost every sward structural characteristic, except for SSH in 2001. This variation was greatest among the late-heading varieties. This is in accordance with Gilliland *et al.* (2002) who also found the largest differences between varieties in the late-heading diploid group for sward structural characteristics (herbage mass, SSH and BD).

Varietal effects on sward morphology

Hazard *et al.* (1998) and Weddell *et al.* (1997) pointed out the importance of leafiness for intake by ruminants. In the present study, three variables relating to leafiness were examined: the proportion of leaf blade in herbage, GLM and length of leaf blade. Heading type had large effects on the leafiness variables, but there was also a clear interaction with year. Late-heading varieties had the highest GLM and proportion of leaf blade in 2000, but intermediate-heading varieties had the highest GLM and proportion of leaf blade in 2001. No effects of heading type and variety were found for length of leaf blades.

The effects of variety within heading type were pronounced and in both years differences were found among varieties for GLM and proportion of leaf blade. The average proportion of leaf blade found in this experiment (0.80) is comparable with the proportion found in Gilliland *et al.* (2002) (0.84). However, Gilliland *et al*. (2002) had cuts at very low herbage mass and their cuts were carried out at a cutting height of 3 cm. O'Donovan (2001) found much lower values (0.62-0.64). O'Donovan (2001) conducted a grazing experiment over the whole summer season, which included the reproductive period of the grasses and made cuts at a cutting height 4 cm. The current study

started at least 14 days after the heading date of the varieties used, so the very high proportion of stem was avoided. This was carried out in order to compare the varieties at the same vegetative stage and herbage quality.
The varieties differed significantly in TD. This was not related to herbage mass, but varieties with the highest TD also had the highest bulk density. A high density is important to increase rates of intake by grazing dairy cows (Laca *et al*., 1992). TD has been shown to be a very important variable for the prevention of weeds that can decrease the quality of the herbage significantly (Wilkins, 1997). Furthermore, TD was negatively related to sward surface height, especially in the vegetative stage. This relationship was stronger in 2000, the first growing year, than in 2001. A significant negative relation between TD and TW was found. The dense swards contained many light tillers and the more open swards contained heavier tillers. This is in accordance with the self-thinning rule proposed by Yoda *et al.* (1963). However, the correlation found in this study (-0.92) differed from the -3/2 boundary (Davies, 1988; Matthew *et al.,* 1995). This might be related to the fact that in July 2001 the densest swards were observed. The dense swards were probably due sheep grazing in the previous winter that had to continue for longer than anticipated due to the occurrence of Food and Mouth Disease. In the same period some tillers were reproductive and the average tiller weight was heavier than to be expected with pure vegetative sward.

The average length of leaf sheath in this experiment was very close to the fixed cutting height of 6 cm. Van Loo (1993) observed genetic variation in sheath length among perennial ryegrass varieties Wendy, Condesa and Barry. In the current study, the largest genetic variation in sheath length (from 4.9 to 6.4 cm) was found in 2000. The differences in leaf sheath length among the cultivars were not significant in 2001.
The proportional length of leaf sheaths in 2000 was approximately 0.20 of the ETH. In 2000 this was, however, only measured in one period. Grant *et al.* (1981) and Davies (1988) described how the length of the sheath tube can influence the length of new leaves. Parsons *et al.* (1991) reported slightly different values of 0.25 sheath and 0.75 blade. In 2001, the proportional length of leaf sheaths was 0.22 of the ETH. This slight increase in sheath length could also be seen in the proportion of green leaf, which decreased by 0.035 from 2000 to 2001. Due to the fixed cutting height of 6 cm, this small increase in sheath length could have had a relatively large effect on the proportion of leaf blade in the cut herbage.

Variety effects on yield-related parameters of perennial ryegrass are evaluated routinely in current breeding programmes all over Europe. However, the variety effects on other sward structural or canopy morphological characteristics are not considered in these programmes (Ebskamp and Bonthuis, 2000; Gilliland *et al.*, 2002). The current study shows that in the main grazing period in the Netherlands, diploid perennial ryegrass varieties differ substantially in sward structure. These differences could lead to different intake rates in dairy cows. However, in the animal experiment associated with this study (Tas, 2005), no differences among varieties for intake under stall-feeding conditions were found. The effects of sward structural and canopy morphological characteristics will probably be more pronounced with a true interface between the animal and the sward, and a grazing experiment should clarify this.

<u>Conclusion</u>

In conclusion, the six diploid perennial ryegrass varieties used in this study differed for important factors that affect intake, such as herbage mass, sward surface height, bulk density and tiller density. Furthermore, varietal effects were found for leafiness characteristics such as proportion of leaf blade and green leaf mass. Minor differences were found in extended tiller length, leaf blade and tiller weight. Because of these sward morphological differences, an effect could be expected on intake during grazing.

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Chapter 3

Comparison of techniques for estimating herbage intake by grazing dairy cows

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Abstract

For estimating herbage intake during grazing, the traditional sward cutting technique was compared in grazing experiments in 2002 and 2003 with the recently developed n-alkane technique and with the net energy method. The first method estimates herbage intake by the difference between the herbage mass before and after grazing and the regrowth between the two points in time. The second technique estimates herbage intake by the ratio of a dosed even-chain synthetic n-alkane (C_{32}) and a naturally occurring odd-chain n-alkane (C_{31} or C_{33}) in the herbage and faeces. The third technique calculated the intake from the animal's energy requirements for milk production and maintenance. The sward cutting technique estimated herbage intake with the highest coefficient of variation, and had different results in the two experimental years. The n-alkanes method gave less variable results, whereas the net energy method gave the least variable results. In 2002, the estimates of the alkane ratio C_{32}:C_{33} were best related with estimations of the net energy method. In 2003, the estimates of the alkane ratio C_{32}:C_{31} were best related. The estimate based on the alkane ratio C_{32}:C_{33} had a lower coefficient of variation than the one based on the alkane ratio C_{32}:C_{31}. Therefore, the C_{32}:C_{33} alkanes method was considered to be a better direct estimator for herbage intake by grazing lactating dairy cows.

Keywords: Dairy cow, herbage intake, sward cutting, n-alkanes.

Introduction

Limited herbage intake is considered to be one of the main constraints for ruminant production (milk, meat, wool) (Forbes, 1995). The measurement of dry matter intake (DMI) during grazing is, however, still not very accurate. The classical method to determine intake is the so-called sward cutting method. A measured proportion of the area allotted to the animals is harvested and the total herbage offered to the animal can be calculated. The residual herbage after grazing is determined in a similar manner. The difference between these two herbage masses and a correction for the regrowth gives an estimate of the herbage consumed in the area grazed (Meijs, 1981; Macoon *et al.*, 2003). The sward cutting method can provide reliable estimates of intake when short grazing periods are applied and when a large part of the offered herbage is consumed (Walters and Evans, 1979; Meijs, 1981). However, this method has large variation (Meijs *et al.*, 1982; Reeves *et al.*, 1996) and is mainly used to determine herbage intake for groups of animals.

In the late 1980s and early 1990s, a new method of for herbage intake was developed, the n-alkanes method (Mayes *et al.*, 1986; Dove and Mayes, 1991; Dillon, 1993). The n-alkanes are long chain (C_{25} to C_{35}) hydrocarbons, present in the cuticular wax of plants. In grassland species, the odd-numbered chain length alkanes (especially C_{29}, C_{31} and C_{33}) are present in much greater amounts than the even-numbered chain length (Tulloch, 1976; Dove and Mayes, 1991). Herbage intake could be estimated by using the n-alkanes as faecal markers. Animals are dosed with a synthetic even-numbered alkane and consume herbage with a certain content of naturally occurring odd-numbered alkane. Herbage intake can be calculated from the alkane dose, the alkane content in the herbage and ratio of the dosed and natural alkanes in the faeces. Although the faecal recovery of alkanes might not be complete, alkanes of adjacent chain length (e.g. C_{32} and C_{33}) have similar recoveries (Mayes *et al.*, 1986; Stakelum and Dillon, 1990) and it was shown that herbage intake of dairy cows could be estimated accurately (Dillon, 1993; Lippke, 2002).

The aims of this chapter were to measure DMI of grazing dairy cows using the two methods and compare their estimates. Furthermore, the estimates of the two methods were compared with a calculated DMI based on the net energy requirements for lactation and maintenance of the cows and the net energy content of the herbage.

Materials and methods

Experimental set-up

During the summers of 2002 and 2003, two grazing experiments with similar set-up were conducted. Twelve dairy cows were used in each experiment. Two paddocks were sown with four perennial ryegrass cultivars in a randomized block design with three replicates. Each paddock consisted of 12 strips of 22 m wide and 84 m long. The strips were divided into 14 plots of 22 × 6 meters (Figure 1). The experiment was designed as a strip grazing system, in which each cow was allowed to graze individually a plot during 24 hours. A mobile fencing system was used, and each cow was moved daily to a new plot at 12:00 h. In total each experiment consisted of four periods of 14 days.

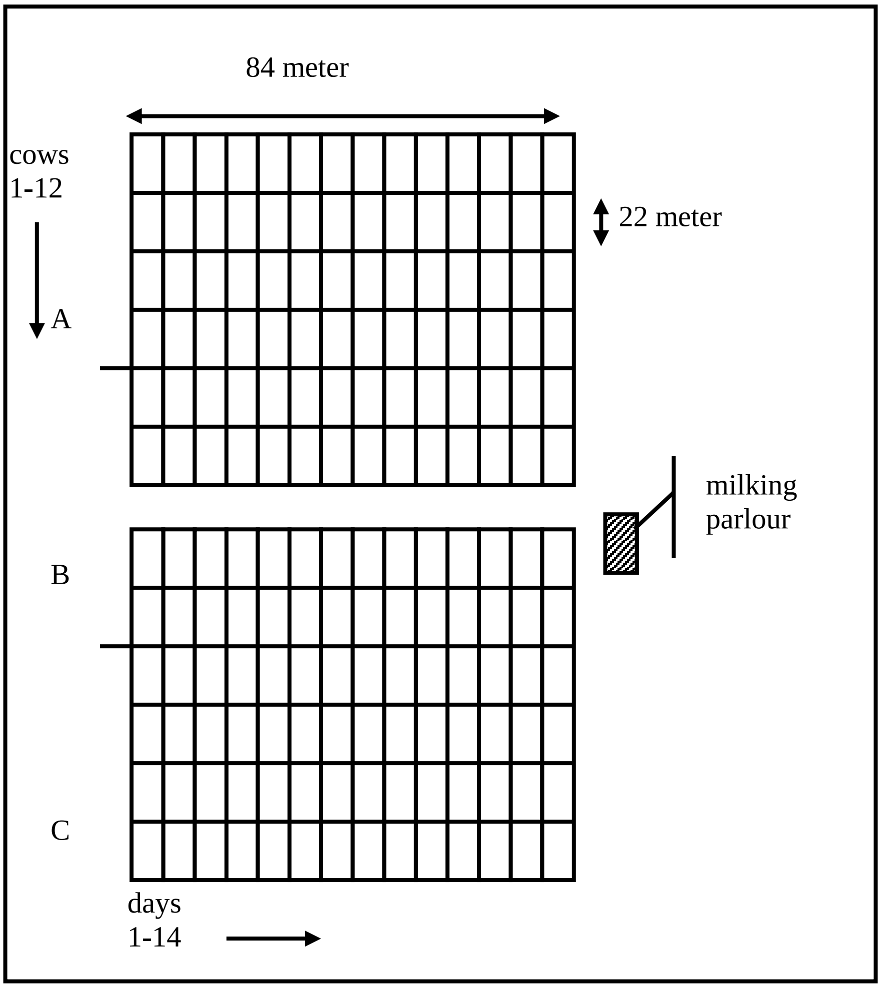

Figure 1 Set-up of the grazing experiment.

Animals

Twelve multiparous Holstein Friesian dairy cows were used. In 2002, cows were 67 ± 4.2 days in milk (DIM) and in 2003 cows were 114 ± 3.7 DIM. Body weight (BW) was recorded every week. The pre-experimental BW was 528 ± 2.0 kg and 549 ± 4.2 kg in 2002 and 2003, respectively. Animals were milked twice a day, at 6:00 h and 16:00 h. Milk yield was recorded after every milking and daily milk was analyzed for fat and protein content. Milk production is expressed as fat and protein corrected milk (FPCM). The Institutional Animal Care and Use Committee of Wageningen University approved the experiment.

Sward cutting method

Herbage allowance. On days 10, 11, 12 and 13 of each experimental period, fresh herbage yield, dry matter percentage and dry matter yield (DMY) were measured before the cows were allowed grazing (pre-grazing). Fresh herbage yield was measured by cutting at least 5% of the total area with a mowing machine (Agria 3200, cutter bar 1.25 m) at a stubble height of 4 cm. In 2002, in periods 1 and 2, in total 7 m^2 was cut in one strip and in periods 3 and 4, in total 14 m^2 was cut in two strips of 7 m^2 each. In 2003, in all periods in total 7 m^2 was cut in two strips of 3.5 m^2 each. The cut herbage was collected, weighed and sampled for dry matter determination. Duplicate core samples of approximately 200 g of fresh material were taken and dried at 70 °C for 24 hours.

Herbage residual. On days 11, 12, 13 and 14 of each period, the residual herbage (post-grazing) was measured as described for the herbage allowance, but now twice the amount of strips was cut (10% of the area) (Green, 1949; Meijs, 1981). Herbage samples were collected and processed as described for the herbage allowance.

Herbage accumulation. Herbage accumulation was calculated using the LINGRA model (Schapendonk *et al.*, 1998), which calculates daily regrowth of perennial ryegrass swards using the meteorological data (daily photosynthetic radiation (MJ m^{-2} d^{-1}) and temperature (°C)) of the Haarweg meteorological station, located 500 meters from the experimental fields. LINGRA successfully predicted growth and development of perennial ryegrass in a vegetative stage at the level of potential and water limited production (Barrett *et al.*, 2004). Herbage accumulation was incorporated in the herbage intake calculation using the Linehan equation (Linehan *et al*., 1952; Meijs, 1981).

Dry matter intake (DMI) (kg DM d^{-1}) was calculated as follows:

$$DMI = (allowance - residual) \times \frac{\log(allowance + \Delta\, regrowth) - \log(residual)}{\log(allowance) - \log(residual)} \qquad (1)$$

DMI was measured during 4 days; the mean of these 4 days was calculated and considered representative for the whole period. As 12 cows were measured during 4 periods, there were potentially 48 recordings in each year. However, in the third period of 2002 one cow was removed from the data set, because of health problems, so in total 47 recordings were made. In 2003 one cow was removed from the complete experiment, because of health problems and another cow in the last period, so 43 recordings remained.

n-Alkanes Method

Field procedure. Each cow received twice daily during milking 1.5 kg of concentrates containing an added C_{32}-alkane. To prepare the concentrates, the even-chain n-alkane C_{32} (Fernz Health & Science Ltd) was dissolved over cellulose powder (arbocel) (1:10 ratio) using a Rotavapor (Büchi) (90 ˚C), then the mix was cooled, sieved and added to the concentrates in the required quantity (0.3 g kg^{-1}) before pelleting in the feed mill. In 2002, 839.3 mg C_{32}-alkane was dosed daily to each cow and in 2003 709.9 mg. The alkane dosing started one week prior to the experiment and continued throughout the experiment to obtain a stable excretion pattern. The concentrates were fed in small portions to prevent spilling by the cows, care was taken that each cow consumed everything.

Faeces were sampled from each cow individually, by sampling each dung patch present in each plot with a spoon. Each day 12 faeces samples were gathered and stored in the freezer. Faeces samples were freeze-dried and milled to pass a 1 mm screen.

Grass samples, 100 g fresh material, were taken daily on days 8 to 14 of each experimental period at three time points (14:00, 20:00 and 7:00 h), which correspond to the main three grazing bouts of the animals (Taweel *et al.*, 2004). Every sample was taken by walking with the cows for five minutes and taking hand-plucked samples in every spot where the cow was grazing. Grass was oven-dried at 60 ˚C for 48 hours and milled to pass a 1 mm screen.

The daily samples of grass and faeces were pooled on equal dry weight basis for each cow in each period. Concentrates were pooled for each period. In total

48 grass samples, 48 faeces samples and 4 concentrate samples were collected each year, and were analyzed for n-alkanes. Because of health problems mentioned earlier, some cows were removed from the dataset. In 2002, one cow showed reluctance to consume the alkane-marked concentrates, therefore these data were also removed from the data-set.

Analysis. Samples were analyzed according to Mayes *et al*. (1986). Samples of grass (1 g), concentrates (0.25 g) and faeces (0.5 g) were weighed, in duplicate, into a screw cap (Schott) vial (25 ml). Vials were checked on leakage with chloroform before usage. Internal standard (250 µl) was added to the sample using a glass syringe with an adapter. Overnight these samples were saponified with 10 ml alcoholic KOH solution (1.5 M) in an oil bath at 90 ˚C. The alcoholic KOH solution was prepared daily to prevent discoloration (8.42 g/100 ml ethanol). After cooling, 8 ml heptane and 5 ml of demineralized water were added and the tubes were shaken vigorously. The samples were placed in a water bath at 45 ˚C for 5 minutes and centrifuged. The non aqueous top liquid layer was removed with a Pasteur pipette. Three further extractions of these samples were carried out by the addition of 5 ml heptane. The samples were evaporated to dryness and re-dissolved in 2 ml of heptane and applied to the top of a small column containing silica gel (3 cm) and glass-fibre (0.5 cm). This eluate was again evaporated to dryness and 250 µl heptane was added; 1 µl was injected in a gas chromatograph with N_2 as carrier gas, at a flow of 30 ml min^{-1}. Peak areas of n-alkanes were determined using Chrom Card Data System 2.2 (Thermo Finnigan).

The DMI was calculated with the following equation:

$$DMI = \frac{C_j}{\frac{F_j}{F_i} \times H_i - H_j} \qquad (2)$$

where F_i and F_j are faecal concentrations of odd numbered alkanes and the even numbered alkanes (mg kg^{-1} DM) respectively; H_i and H_j are the concentrations of the odd and even numbered alkanes in the grass (mg kg^{-1} DM); C_j is intake of dosed alkane in the concentrate (mg d^{-1}). In equation 2, it is assumed that even and odd numbered alkanes with nearly similar chain lengths have a similar faecal recovery, independent of their source (natural occurrence in grass and concentrate or dosed). In this experiment, C_{32} alkane was used to dose the animals, this was added to a concentrate with no natural occurring alkanes.

C_{32}:C_{31} and C_{32}:C_{33} alkane-combinations were examined as potential estimators for DMI.

Net energy method

Dry matter intake could also be calculated from the net energy requirements of the cows and the net energy content of the grass. Daily energy requirements for milk production and maintenance ($NE_{L,\ required}$) were calculated according to the standard energy system used in the Netherlands (van Es, 1978; CVB, 1999a), using the following equation:

$$NE_{L,required} = 6.9 \times \left[\left(42.4 \times BW^{0.75} + 442 \times FPCM\right) \times \left(1 + \left(FPCM - 15\right) \times 0.00165\right)\right] \quad (3)$$

where BW is the average body weight of the cow (kg) during the measurement period and FPCM is the fat and protein corrected milk (kg d^{-1}). Because the animals were grazing, an extra allowance of 20% of their maintenance requirements was assumed (van Es, 1978; CVB, 1999a). The energy value of the grass was calculated from the chemical composition of the grass, which was determined by near infrared reflectance spectroscopy (NIRS) in the samples taken for the alkane analysis and assumed to be representative of the quality of grass ingested by the cows. NIRS calibration equations were developed with more than 1000 fresh grass and hay samples by Centre de Recherches Agronomiques de Gembloux in Belgium (Biston *et al.*, 1998). Samples were analyzed for crude ash (ASH), crude protein (CP), crude fibre (CF) and water-soluble carbohydrates (WSC). Crude fat (CFAT) was assumed to be 40 g kg^{-1} DM (CVB, 1999b). Nitrogen free extract (NFE) was calculated by subtracting ASH, CP, CF and CFAT from 1000 g DM.

Digestible crude protein (DCP) and digestible organic matter (DOM) expressed as gram per kilogram DM were calculated using the following equations (van Es, 1978; CVB, 1999a):

$$DCP = \left(0.959 \times CP + 0.04 \times ASH - 40\right) - 0.1 \times \left(D - 105\right) \quad (4)$$

$$DOM = \left(1029 - 0.77 \times CF - 1.12 \times ASH - 0.3 \times D\right) \quad (5)$$

where, D is the days after 1st of April. The gross energy (GE), metabolizable energy (ME) and net energy (NE_L) per kg DM were calculated using equations 6, 7 and 8. The DMI was calculated using equation 9.

$$GE = 24.14 \times CP + 36.57 \times CFAT + 20.92 \times CF + 16.99 \times NFE - 0.63 \times WSC \quad (6)$$

$$ME = 14.2 \times DOM + 5.9 \times DCP \quad (7)$$

$$NE_L = 0.6 \times \left(1 + 0.004 \times \left(\frac{ME \times 100}{GE} - 57\right)\right) \times 0.9752 \times ME \quad (8)$$

$$DMI = \frac{NE_{L,required} - NE_{L,concentrate}}{NE_{L,herbage}} \quad (9)$$

Statistics

The DMI estimates of all methods were compared using a paired t-test. Each year was analyzed separately. Correlations were calculated using the two-tailed Pearson correlation coefficient.

Results

Sward cutting method

Table 1 shows the herbage allowance, residual and intake. In 2002, the herbage allowance was higher (P<0.05) than in 2003. Mean herbage allowance was on average almost 35 kg DM animal^{-1} d^{-1}. The herbage residual in 2002 was much higher (P<0.001) than in 2003. The herbage accumulation in 2002 was 2.2 kg and was higher (P<0.001) than in 2003, due to the hot and dry weather conditions in the latter year. The herbage intake in 2002 was lower (P<0.001) than in 2003. Contrary to what was expected due to ongoing of the lactation period, no decrease in DMI with time was observed.

Standard errors in 2002 for herbage allowance, residual and accumulation were higher than in 2003. The standard error for herbage intake, however, was slightly lower in 2002.

Table 1 Herbage allowance, residual, accumulation and intake (kg DM animal^{-1} day^{-1}) and standard error in 2002 and 2003.

Item	2002	2003
Allowance	36.4 ± 1.5	32.5 ± 1.0
Residual	21.8 ± 1.4	14.5 ± 1.0
Accumulation	2.2 ± 0.07	0.9 ± 0.06
Intake	16.2 ± 0.3	18.6 ± 0.4

n-Alkanes method

The results of the n-alkanes method are presented in Table 2. The level of natural occurring n-alkanes in the concentrates was below detection limits. The levels of odd numbered alkanes (C_{31} and C_{33}) in the herbage were considerably higher than that of the even numbered alkane (C_{32}), which was 12 mg kg^{-1} DM in both years. In contrast, the levels of C_{33} and especially C_{31} were much lower (P<0.001) in 2002 than in 2003.

The odd numbered alkane levels in the faeces were higher (P<0.001) in 2003 compared to 2002, but the levels of C_{32} in the faeces were higher (P<0.001) in 2002, due to the higher dose (839.3 vs. 709.9 mg d^{-1}) in 2002 than in 2003. The level of C_{31} in the faeces was much lower (P<0.001) in 2002 compared to 2003 (448.2 vs 695.9 mg kg^{-1} DM), the level of C_{33} was also lower (P<0.001) in 2002 compared to 2003, but the increase was less pronounced (402.4 vs 487.2 mg kg^{-1} DM). This is in line with the variation in the odd numbered alkane in the herbage.

Results of the DMI estimated by the n-alkanes method are presented in Table 3. The C_{31}-estimates for DMI were 18.2 kg DM d^{-1} in both years. The C_{33}-estimates were 17.2 and 17.5 kg DM d^{-1}, respectively, and did not differ among years (P = 0.46). In both years, the lowest DMI values (C_{32}:C_{31} and C_{32}:C_{33}) were observed in the last period. The standard errors of the C_{33}-estimates of DMI were smaller than in the C_{31}-estimates of DMI.

Net energy method

The net energy content (NE_L) of the grass was calculated with equations 4 to 8 and the chemical composition as presented in Table 4. NE_L of the grass was lower (P<0.001) in 2002 than in 2003, mainly because of the lower CP concentration (P<0.001) in the grass in 2002 compared to 2003 (180 vs 201 g kg^{-1} DM). The WSC concentration was higher (P<0.001) in 2002 compared to 2003 (134 vs 109 g kg^{-1} DM). The other chemical characteristics did not differ between years.

Table 2 Concentration of n-alkanes (mg kg^{-1} DM) and standard errors in herbage and faeces in 2002 and 2003.

n-Alkane	Herbage		Faeces	
	2002	2003	2002	2003
C_{31}	144.3 ± 2.6	230.7 ± 5.7	448.2 ± 10.1	695.9 ± 14.8
C_{32}	12.8 ± 0.6	12.4 ± 0.3	185.0 ± 3.2	158.3 ± 2.9
C_{33}	134.2 ± 2.4	164.9 ± 3.3	402.4 ± 10.6	487.2 ± 9.5

Table 3 Dry matter intake from herbage (kg) and standard errors estimated with the n-alkanes method using different ratios (C_{32}:C_{31} and C_{32}:C_{33}) in 2002 and 2003.

n-Alkane ratio	2002	2003
C_{32}:C_{31}	18.2 ± 0.5	18.2 ± 0.5
C_{32}:C_{33}	17.2 ± 0.3	17.5 ± 0.4

Table 4 Chemical composition (g kg^{-1} DM) and energy value (MJ kg^{-1} DM) and standard errors of herbage in 2002 and 2003.

Item (DM-basis)	2002	2003
ASH	97.6 ± 0.8	83.8 ± 0.6
WSC	133.6 ± 9.0	109.8 ± 4.3
CF	258.6 ± 2.5	255.7 ± 2.0
CP	179.6 ± 3.0	201.8 ± 3.2
CFAT	40.0[a]	40.0[a]
NFE	424.1 ± 4.9	418.8 ± 1.9
D	127	135
DCP	134.0 ± 2.7	153.9 ± 2.9
DOM	682.4 ± 2.5	697.9 ± 1.7
GE	18.3 ± 0.04	18.7 ± 0.02
ME	10.5 ± 0.03	10.8 ± 0.03
NE_L (MJ kg^{-1} DM)	6.1 ± 0.02	6.3 ± 0.02

[a] assumed value, no variation

ASH: crude ash. CFAT: crude fat. CF: crude fibre. D: days after 1st of April. DOM: digestible organic matter. DCP: digestible crude protein. GE: gross energy. ME: metabolic energy. NE: net energy. NFE: nitrogen free extract. WSC: water-soluble carbohydrates.

Table 5 Body weight (BW), fat and protein corrected milk production (FPCM[a]) and energy requirements ($NE_{L,\ required}$) and herbage intake (DMI) with standard errors of 12 dairy cows during four 14-d periods in 2002 and 2003.

Trait	2002	2003
BW, kg	534.0 ± 3.9	549.0 ± 6.4
FPCM, kg d^{-1}	26.7 ± 0.5	24.8 ± 0.3
$NE_{L,\ required}$, MJ d^{-1}	122.9 ± 1.6	117.3 ± 1.2
DMI, kg d^{-1}	16.8 ± 0.2	15.3 ± 0.2

[a] FPCM = ((0.337+ 0.116 Fat (%) + 0.06 Protein (%)) x Milk production (kg)

The energy requirements of the dairy cows used in this experiment were calculated according to equation 3, and are presented in Table 5. The BW of the dairy cows slightly increased during the experiment in 2002, and varied among periods in 2003. The milk production (FPCM) decreased during the grazing experiments in both years. The energy requirement ($NE_{L,required}$) decreased with stage of lactation. The DMI was calculated by subtracting 20.2 MJ from $NE_{L,required}$, because the animals consumed this amount of energy with the concentrates (3.0 kg). The corrected value was divided by the energy concentration in the grass (Table 4). DMI decreased during the experiment, except in the last period of 2003. This increase in DMI was due to an increased BW and therefore higher maintenance costs. DMI was higher in 2002 (16.8 kg DM d^{-1}) than in 2003 (15.3 kg DM d^{-1}), mainly due to lower milk production in 2003.

Comparison between methods

Figure 2 shows the relation between DMI measured by the sward-cutting method, the C_{32}:C_{33}-alkane method and net energy method, respectively. In both years, there was a relation (P<0.01) between sward-cutting and the alkanes methods, but the relation was rather weak (R<0.50) as shown in Table 6. The relation between estimates of the two alkanes methods was high (R>0.90). There was a relation (P<0.01) between the net energy method and the sward cutting method in 2002, but no relation was found in 2003. C_{32}:C_{31} showed in both years a relation, whereas C_{32}:C_{33} showed only a relation in 2002.

In 2002, the C_{32}:C_{31}-alkane method estimate was higher (P<0.01) than all other methods (Table 7). The DMI estimate of the sward cutting method was lowest, but not different from the net energy method. The C_{32}:C_{33}-alkane method was also not different from the net energy method in 2002. In 2003, the net energy method was lower (P<0.001) than that determined using the other methods. The DMI estimates of the sward cutting and the C_{32}:C_{31}-alkane methods were both higher (P<0.05) than the C_{32}:C_{33}-alkane method.

The sward cutting method showed a large difference between the two experimental years, 16.2 and 18.6 (kg DM d^{-1}) in 2002 and 2003, respectively. The two alkanes methods were not different (P>0.05) between the two experimental years. The net energy method differed (P<0.001) between years (16.8 and 15.3 kg DM d^{-1} in 2002 and 2003, respectively). Thus, while the sward-cutting method estimated the highest DMI in 2003, the net energy method's estimate was lowest in 2003.

The range of the DMI estimated by the C_{32}:C_{33} alkane and the sward cutting method were comparable, 9.0 and 10.8 (kg DM d^{-1}) in 2002 and 2003 respectively. However, the range of DMI estimated the C_{32}:C_{31} alkane method was much larger (Table 7).

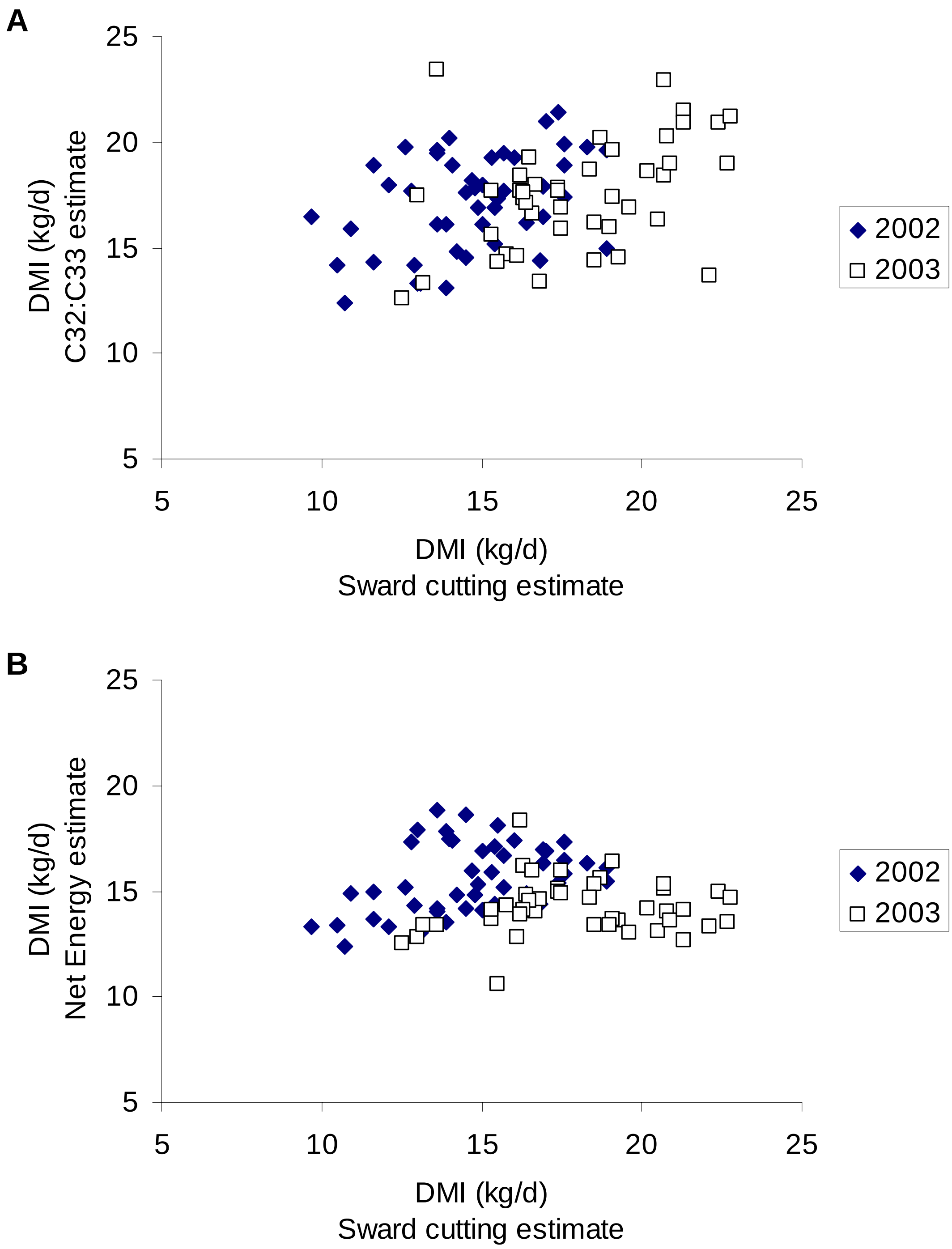

Figure 2 Relation between DMI from herbage estimated by the sward cutting method and C_{32}:C_{33} alkanes (A) and DMI from herbage estimated by the sward cutting method and net energy method (van Es, 1978) (B).

Table 6 Correlation coefficients of the relation between the sward cutting method, the n-alkanes method (C_{32}:C_{33} and C_{32}:C_{31}) and net energy method in 2002 and 2003.

	2002			2003		
Method	C_{32}:C_{33}	C_{32}:C_{31}	Net energy	C_{32}:C_{33}	C_{32}:C_{31}	Net energy
Sward cutting	0.433**	0.398**	0.434**	0.413**	0.504***	0.120
C_{32}:C_{33}		0.941***	0.503***		0.908***	0.241
C_{32}:C_{31}			0.392**			0.358*

NS: P>0.05, *: P<0.05, **: P<0.01: ***: P<0.001

Table 7 Descriptive statistics of all DMI methods in 2002 and 2003

Method	Mean	Min.	Max.	Range	SD	CV
2002						
Sward cutting	16.2[a]	11.0	20.9	9.9	2.36	14.7
C_{32}:C_{33}	17.2[b]	12.4	21.4	9.0	2.31	13.4
C_{32}:C_{31}	18.2[c]	12.5	26.8	14.3	3.27	18.0
Net energy	16.8[ab]	13.5	19.8	6.3	1.64	9.8
2003						
Sward cutting	18.6[c]	12.7	23.4	10.7	2.75	14.8
C_{32}:C_{33}	17.5[b]	12.6	23.4	10.8	2.58	14.7
C_{32}:C_{31}	18.2[c]	12.7	26.2	13.5	3.17	17.4
Net energy	15.3[a]	11.6	19.4	7.9	1.36	8.9

[a,b,c] Means within the same year with different superscripts differ significantly (P<0.05).

SD: Standard Deviation

CV: Coefficient of Variation

Discussion

A good method for any scientific research purpose should give values with a small variation and should be highly repeatable. Measuring herbage intake by grazing dairy cows is a complicated procedure because the herbage intake itself is very variable, too. Even in a controlled stall feeding experiment, a coefficient of variation of 10% in DMI was observed (Taweel, 2004).

Sward cutting method

The classical sward cutting method can give a good estimate of herbage intake by grazing animals (Walters and Evans, 1979; Meijs, 1981; Macoon *et al.*, 2003), but often a large variation in the estimation of herbage mass is found. Variation of both pre- and post-grazing measurements are added, hence the herbage intake values become even more variable. Some experiments described intake measurements in groups of cows (Walters and Evans, 1979; Meijs, 1981; Macoon *et al.*, 2003), but this experiment obtained dry matter intake measurements from individual animals that were kept in individual fields.

Walters and Evans (1979) pointed out that animals (especially sheep) may graze below stubble height of cutting, which would result in an underestimate of the herbage intake. However, in our experiment the sward surface height after grazing was always above the cutting height. Another point to consider is the herbage accumulation in the period between sward-cutting, which might result in an underestimate of the herbage intake. This herbage accumulation has a large influence over longer grazing periods (> 3 days) (Walters and Evans, 1979), but is of less importance in short grazing periods when a large difference exists between pre- and post herbage yield (Linehan *et al.*, 1952; Meijs, 1981). In this study, the herbage accumulation was not measured, because only one day elapsed between the pre- and post-grazing cut in this experiment and the decline in herbage yield was large (on average 1000 kg DM ha^{-1}). Nevertheless, the herbage accumulation, calculated from the LINGRA growth model (Schapendonk *et al.*, 1998), was significant (2.2 and 0.9 kg DM d^{-1} in 2002 and 2003, respectively). Using the Linehan equation, the herbage accumulation was responsible for 9% of the total herbage intake in 2002, but only for 3% in 2003. The herbage accumulation, also in short grazing periods should not be considered as negligible when growing conditions are favourable for grass growth. The sward cutting method is vulnerable to variation due to machine conditions, e.g. sharpness of the knives. Also changing operator during the measurement period could induce additional variation (Meijs, 1981). In this experiment, the machine was regularly cleaned and the same operator preformed the sampling throughout the experimental period.

The largest variation was due to spatial variation in the sward. The estimation of herbage yield, especially the post-grazing yields were very difficult to estimate. The residual herbage was very irregularly distributed over the field, especially when there was a large availability of herbage. This irregular distribution could be due to selection, as cows try to avoid faeces patches (Bosker *et al.*, 2002). However, when the available herbage was depleted, selection became less important and cows grazed close to dung patches. Furthermore, cows did not graze regularly in horizons (Wade *et al.*, 1989), but grazed deep in certain patches, while other patches were left untouched. Dung patches were avoided during harvesting the residual, because including dung would overestimate the post-grazing yield, but avoiding them could overestimate the intake.

Green (1949) argued that taking more samples per field would considerable reduce the variability of grazed residual herbage mass. Therefore, the number of samples taken from the residual was twice as many as that from the initial herbage mass. Meijs (1981) mentioned that locating the strips for sward-cutting next to each other would improve the measurement. Therefore, in this experiment, where possible, the two post-cut strips were placed adjacent to the pre-cut strips.

n-Alkanes method

The n-alkanes method has been shown to be a good estimator of DMI during grazing (Malossini *et al.*, 1994; Reeves *et al.*, 1996; Dove *et al.*, 2000). Nevertheless the n-alkanes method is also associated with sources of variation.

Diurnal patterns of n-alkanes excretion have always been a major concern for variation in marker studies (Dove and Mayes, 1991; Dove *et al.*, 2002; Lippke, 2002). The faecal concentrations of the natural odd alkanes tend to be relatively constant, but due to the dosing schedule diurnal variation in excretion of the dosed alkane can occur (Stakelum and Dillon, 1990; Dove and Mayes, 1991). The animals in the present experiment were dosed twice daily, which was an attempt to reduce the diurnal variation in alkane ratios in the excreted faeces (Stakelum and Dillon, 1990; Dove *et al.*, 2000). Besides, in this experiment, no rectal faeces samples at one time point were taken, but all dung patches left by the cow in each plot were sampled, which ensured a representative faecal sample over 24 hours. This way of sampling will not be applicable in other grazing studies, with several cows grazing in one group.

Also the sampling of herbage could be a source of variation. Animals can select for certain plant species or plant parts, which have a different n-alkane levels than the average field sample (Dove *et al.*, 1996). The paddocks used in this experiment were monocultures of perennial ryegrass, with a very low infestation level of weeds. To try to ensure that the herbage sample was not different from what the cow consumed, the sampling took place three times a day. In the used strip grazing system, cows grazed down the sward in one day, the three sampling points ensured that the herbage was representative to what the cow consumed (Taweel *et al.*, 2004). The herbage samples were oven-dried at 60 °C for 48 hours, this was done because of lack of freeze drying capacity to dry more than 900 samples each year. Freeze-drying of herbage and faeces is recommended (Dove and Mayes, 1991; Sandberg *et al.*, 2000). However, the drying method is thought not to affect the n-alkane concentration in herbage, especially perennial ryegrass (Dove and Mayes, 1991) and oven-drying herbage was done by others (Smith *et al.*, 2001a; Martins *et al.*, 2002). n-Alkanes concentration in the faeces has been found to be affected by the drying (Sandberg *et al.*, 2000) and therefore all faeces samples were freeze-dried.

Dosing of the synthetic alkane could be seen as a major source of variation of the alkanes method, the dosing should by very precise. In this case, the alkanes were dissolved, followed by mixing the solution with a pre-mix of cellulose that was subsequently mixed with the other ingredients of the concentrates, so that an equal distribution of the alkanes over the concentrates was ensured. The dosing of the alkane-marked concentrate to the animals might be largest source of variation. Care was taken that each cow consumed all the concentrates, nevertheless sometimes some concentrates could be spilled. Next to the synthetic C_{32} the animals were dosed with, there was also a small amount of natural C_{32} present in the herbage. The concentrations of odd n-alkanes exceeded this small amount by far, but in comparison with the daily dose it could be a considerable amount; e.g. in 2003, the C_{32} level in the herbage was 12.4 mg kg^{-1} DM (Table 2). The estimated herbage intake (C_{32}:C_{33}) was 17.5 kg DM (Table 3), resulting in a daily intake of natural C_{32} alkane of 217 mg, which was 31% of the synthetically dosed amount of C_{32} alkane. A change in the concentration of C_{32} in the herbage can have a large influence on the DMI (e.g. 1 mg change in the C_{32} concentration of the herbage gives an average change in DMI of 0.5 kg DM). This would suggest a need for a larger dose of synthetic C_{32} alkane than the 700-800 mg d^{-1} given in this experiment. The doses used by others in experiments with cattle were however in the same range (600-1000 mg) (Ohajuruka and Palmquist, 1991; Reeves *et al.*, 1996; Dillon, 1993; Malossini *et al.*, 1994). The average C_{32} alkane concentrations were 12.8 and 12.4 mg kg^{-1} DM in 2002 and 2003, respectively (Table 2). Lower values (ranging from 5-10 mg kg^{-1} DM) were found by others (Dove and Mayes, 1991; Dillon, 1993; Malossini *et al.*, 1994). In 2003, concentrations of all odd n-alkanes in the herbage were higher than values reported in the literature. This might be related to the high temperatures and drought stress. Plant waxes that contain alkanes play a major role in defense mechanisms against water losses (Kolattukudy, 1976; Tulloch, 1976). The values of C_{31} (144 vs. 230 mg kg^{-1} DM) were much more variable than the values of C_{33} (134 vs. 164 mg kg^{-1} DM).

Comparison between Methods

The n-alkanes method has been mainly validated under stall-feeding conditions. In a grazing situation, the sward-cutting method and the n-alkanes method have not been compared to our knowledge. Reeves *et al*. (1996) concluded that herbage intake estimates from the pre- and post-grazing mass, estimated with the rising plate meter were not acceptable, because of large errors in estimating tropical grass intake. In the present study, a large difference in herbage intake estimated with sward-cutting method was found between 2002 and 2003 (16.2 vs 18.6 kg DM, respectively). This difference was not expected, because a similar group of dairy cows was used in 2002 compared to 2003, in terms of milk production (26.7 and 24.8 kg FCPM) and body weight (534 and 549 kg). Based upon their milk production, cows required an even higher DMI in 2002 than in 2003, as can be seen in Table 5. The sward cutting method estimate was, however, lower in 2002. The cows did increase in body weight, the estimation by the sward cutting technique seemed to underestimate DMI in 2002.

The n-alkanes method gave similar estimates for DMI in both years, 18.2 and 18.2 kg DM and 17.2 and 17.5 kg DM for C_{32}:C_{31} and C_{32}:C_{33}, respectively. Although the mean of the C_{31}-estimate gave more constant results over years, its coefficient of variation was considerably higher than the C_{33}-estimate (Table 7), which is in line with results found by others (Mayes *et al*., 1986; Stakelum and Dillon, 1990; Reeves *et al*., 1996). However, in pastures with a high clover content the concentration of C_{33} is much lower (Dove *et al*., 1996; Lee and Nolan, 2003) and the C_{31}-estimate might be a better option. The n-alkanes could give direct and precise estimates of herbage intake from pasture, which overcame also the major problems in the Cr_2O_3 and net energy method (Malossini *et al*., 1996; Reeves *et al*., 1996; Dove *et al*., 2000), because the n-alkanes method is independent of the digestibility, while other methods are dependent on an *in vitro* digestibility value, which can differ among individual animals. The estimations by the n-alkanes techniques covered the energy requirements; especially in 2003, the cows ate over 2 kg DM more than needed according to their requirements.

Practical Considerations

There are also some practical considerations to choose for a certain method. The sward-cutting method was more labour intensive in the field than the n-alkanes method. The sward-cutting method gave fast results; 24 hours after the post-grazing cut, herbage intake could be calculated. The n-alkanes method was more time-consuming and it took more than a month before the data for herbage intake were available. Besides, the n-alkanes method needs expensive equipment for measuring and analysing, whereas the sward-cutting method is much cheaper in materials.

Conclusions

It was concluded that for herbage intake estimations of individual grazing animals the n-alkanes technique is the best technique to use. It is recommended to use the C_{32}:C_{33} alkane ratio in pastures dominated by perennial ryegrass, because the herbage intake estimations were less variable with this ratio than with the C_{32}:C_{31} alkane ratio. However, the estimations by the n-alkanes overestimated herbage intake compared to the energy requirements of the animals. The sward cutting technique gave highly variable results and is, therefore, not recommended for herbage intake estimations of individually grazing dairy cows.

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Chapter 4

Perennial ryegrass (*Lolium perenne* L.) cultivar effects on grass productivity, nutritive quality and herbage intake under grazing

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Abstract

Four perennial ryegrass (*Lolium perenne* L.) cultivars were compared for differences in grass production, nutritive quality and DM intake during the summers of 2002 and 2003. Two paddocks were sown with pure stands of four cultivars in a randomized block design with three replicates. Each plot was subdivided in 14 fields (22×6 m) which were grazed by one cow during 24 hours. All cows were assigned to one cultivar for a period of two weeks in a 4×4 Latin square experimental design; the experiment lasted 8 weeks. Sward structural (sward surface height, dry matter yield, green leaf mass, bulk density and tiller density) and morphological characteristics were measured. Samples were taken for chemical analyses (DM, ash, NDF, ADL, crude protein, water-soluble carbohydrates, and digestibility). The sward was also examined for infestation by crown rust (*Puccinia coronata* f. sp. *lolii)*. Herbage intake was estimated with the n-alkanes technique. Cultivar differences for all sward structural characteristics were found except for bulk density and tiller density in 2003. Cultivars differed for proportions of pseudostem, stem (in 2003 only) and dead material. The chemical composition was different among cultivars, especially the water-soluble carbohydrates concentration showed large variation (>25%). Cultivars were different for susceptibility to crown rust (P<0.001). Herbage intake differed (P<0.05) among cultivars in 2002, but not in 2003. Herbage intake was positively associated (P<0.05) with sward height, dry matter yield and green leaf mass. Canopy morphology did not affect herbage intake. ADL concentration was the only chemical component associated with herbage intake. Crown rust affected herbage intake negatively. It is concluded that breeders should aim for high yielding perennial ryegrass cultivars, low in lignin concentration and highly resistant against crown rust.

Keywords: Dairy cows, *Lolium perenne*, varieties, herbage intake.

Introduction

Breeding of new herbage varieties should be based on animal responses (Beerepoot and Agnew, 1997; Casler and Vogel, 1999; Wilkins and Humphreys, 2003). Perennial ryegrass (*Lolium perenne* L.) is one of the most important forages for dairy cows in temperate regions (van Wijk *et al.*, 1993), because it has a high dry matter yield (per hectare) and nutritive value and hence provides a cheap feed in comparison with silages or concentrate feed (Gibb *et al*., 1999). Traditionally, perennial ryegrass cultivars were mainly bred for high dry matter yields. Also crown rust (*Puccinia coronata* f.sp. *lolii*) resistance is one of the breeding goals in the Netherlands (Bonthuis *et al.*, 2004). Recently, also feeding value has become more of interest to breeders. Important traits for feeding value are *in vitro* digestibility and the proportions in DM of crude protein (CP), water-soluble carbohydrates (WSC) and fibre (NDF) (Wilkins and Humphreys, 2003).

One of the main constraints in grazing systems is the limited dry matter intake (DMI) by high productive dairy cows, resulting in the inability to meet their nutrient (energy and protein) requirements for milk production (Chilibroste, 1999). One of the ways to achieve this goal might be through breeding. There are several publications reporting consistent differences in traits important for DMI among perennial ryegrass cultivars (Miller *et al.*, 2001; Lee *et al*., 2002; Gilliland *et al*., 2002; chapter 2). In grazing systems, the interface between grass and animal is critical to optimise intake. Sward surface height (SSH), green leaf mass (GLM), bulk density (BD), dry matter yield (DMY), grass morphology, and tiller density (TD) are identified as important canopy structural components for this interface (Orr *et al*., 2004; Laca *et al*., 1992; Hazard *et al.*, 1998). Cultivar differences for these components have been found (chapter 2; Gilliland *et al.*, 2002), but their actual effects on animal production have not yet been quantified.

Cultivar effects on DMI have been found in sheep (Orr *et al.*, 2003; Hazard *et al.*, 1998) and beef cattle (Lee *et al.*, 2002; O'Riordan *et al.*, 1998). In dairy cows, cultivar effects have been found between diploid and tetraploid cultivars (Hageman *et al.*, 1992) showing that tetraploid cultivars have a high potential for grazing animals, such as higher WSC level, and a high GLM. However, until now the majority of selected cultivars on the Dutch Recommend List of Varieties are diploid (Bonthuis *et al.*, 2004). Also, differences have been found between late and intermediate heading cultivars (Gowen *et al.*, 2003), showing that late heading cultivars have a better potential for high DMI by grazing dairy cattle in spring and summer. In contrast, no effects of cultivar on DMI by dairy cows were found by Miller *et al*. (2001) and Tas (2005). However, this might be related to the fact that these experiments were stall-feeding experiments and there was no real interface between the animal and the standing crop.The aims of this chapter were to determine cultivar differences in DMI of four diploid perennial ryegrass cultivars during grazing by highly productive dairy cows, and to relate this to sward structural and chemical characteristics.

Material and Methods

Plots and soils

During the summers of 2002 and 2003, an experiment was conducted with four diploid perennial ryegrass cultivars. Two paddocks of Wageningen University, the Netherlands (51°58'N / 5°40'E, 7 m a.s.l.) were used, situated on a clay soil. The season prior to the experiment, both paddocks were cultivated with barley. Paddock A was established in autumn 2001 and top-dressed during spring 2002, whereas paddock B was established in spring 2002. Both paddocks had a randomized block design with four cultivars (cvs 1-4) in three replicates, resulting in twelve plots (22×84 m). Cultivars 1 and 2 were intermediate heading cultivars (May 27) and cultivars 3 and 4 were late heading cultivars (June 10). These cultivars were earlier analyzed for morphological traits (chapter 2) when used for a stall-feed experiment (Tas, 2005). Soil analyses of the layer 0-25 cm were taken in March 2002. The pH (KCl) at paddock A and paddock B was 7.2 and 6.7, the percentage organic matter 2.8 and 3.4, and the percentage soil particles < 2 µm 36 and 40%, respectively.

Table 1 Fertilization (kg ha^{-1}) during grazing trials in 2002 and 2003.

	2002						2003					
	Paddock A			Paddock B			Paddock A			Paddock B		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Spring application							*25 m^3 pig manure applied*					
Prior exp.	77	42	0	91	35	0	104	111	155	65	90	125
Prior P1	53	0	0	-	-	-	-	-	-	74	0	0
Prior P2	-	-	-	59	0	0	74	0	0	-	-	-
Prior P3	80	64	48	-	-	-	-	-	-	74	0	0
Prior P4	-	-	-	79	20	35	73	0	0	-	-	-
Total	210	106	48	229	55	35	251	111	155	213	90	125

Experimental set-up

A grazing trial was carried out to measure the effect of cultivar on DMI of dairy cows. Each of the twelve established plots of a paddock was subdivided into 14 small fields (22×6 m). A strip grazing system was applied and one field (22×6 m) was grazed by one cow during 24 h. A mobile fencing system was used. After two weeks, cows were moved to a new paddock were a similar set-up was applied. The grazing trial lasted for 8 weeks that were divided into four periods (P1-4) of two weeks. All cows were assigned for a period of two weeks to one cultivar in a 4×4 Latin square design. In 2002, the experiment started with P1 on July 5 (Paddock A), P2 on July 19 (Paddock B), P3 on August 2 (Paddock A), and P4 on August 16 (Paddock B). In 2003, the experiment started with P1 on July 11 (Paddock B), P2 on July 25 (Paddock A), P3 on August 8 (Paddock B) and P4 on August 29 (Paddock A).

Pasture management

Before the start of the experiment and after each grazing period, the grass was cut using a front mower with reciprocating blades set at a height of 6 cm. Cutting and fertilizing regimes were similar for all cultivars (Table 1). The fertilization rate, during for the experimental periods, originally was set on 55 kg N ha^{-1}. However during the first two periods (P1 and P2) of the experiment in 2002 grass growth was lower than foreseen, therefore the fertilization rate was raised to 75 kg N ha^{-1}. In 2002, grass was allowed approximately 23 days of regrowth, and 29 days in 2003. The target herbage mass was 2000 kg DM ha^{-1}, resulting in a herbage allowance of 25 kg DM cow^{-1} d^{-1} to ensure that cows grazed fresh grass *ad libitum*. When grass growth was retarded, the field size was enlarged to 1.5 or twice the size, in a similar way for all cultivars during each day.

Weather conditions

The weather conditions during the experiment are summarized in Table 2 and compared to a long-term average. The weather conditions in 2002 were rather similar compared to the long-term average, but August was warmer, July and August were wetter and September dryer than average. In 2003, it was warm and dry from June till August and irrigation was needed. Furthermore, the daily global radiation was higher than average. Paddock A was irrigated with 15 mm on July 18 and August 6, 12, 14, 18, 20 and 25. Paddock B was irrigated with 15 mm on July 3 and 23 and on August 1, 5, 8 and 15.

Table 2 Weather data during two grazing experiments in 2002 and 2003 compared with the long term average in Wageningen.

	Climate 1951-1999				2002				2003			
Month	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep
Mean temp. (^{o}C)	15.4	17.1	16.8	14.1	16.8	17.4	18.8	14.6	18.3	19.0	19.4	14.3
Max. temp. (^{o}C)	20.2	21.9	21.8	18.8	21.7	22.2	23.6	20.0	23.9	24.3	25.9	20.8
Min. temp. (^{o}C)	10.2	12.2	11.9	9.6	11.3	12.8	14.1	9.0	11.5	12.8	12.5	7.3
Precipitation (mm month^{-1})	69.2	73.9	70.3	66.7	67.3	107.0	113.3	11.0	44.9	45.4	29.7	45.4
Global radiation (MJ m^{-2} d^{-1})	17.2	16.2	14.2	9.9	18.9	16.8	13.6	11.3	21.6	18.5	16.8	12.2

Animals

In 2002, twelve Holstein-Friesian cows were used. Four of these cows were equipped with a rumen fistula. They were in their second to fourth lactation and 67 days (SEM = 4.2) in milk (DIM) and milk production was 33.0 kg FPCM d^{-1} (SEM = 0.38) at the start of the experiment. In 2003, again twelve Holstein-Friesian cows were used, none of them was equipped with a rumen fistula. They were in their second to fifth lactation and 79 (SEM = 3.7) DIM and milk production was 29.8 kg FPCM d^{-1} (SEM = 0.25) at the start of the experiment.

All cows were adjusted to a fresh grass diet three weeks prior to the start of the experiment. In 2002, during P1 and P3, cows grazed paddock A, whereas during P2 and P4, cows grazed paddock B. In 2003, the sequence was reversed and during P1 and P3, cows grazed paddock B, whereas during P2 and P4 cows grazed paddock A. Cows were grouped in three groups of four animals, based on either carrying a fistula or on their ranking of pre-experimental milk production. Cows were further supplemented with 3.0 kg concentrate feed per day in two equal portions at milking. Cows were milked twice a day at 6:00 and 16:00 h.

Sward measurements

A strip grazing system was applied and cows were moved daily to a new plot at 12:00 h. On days 10, 11, 12 and 13 of each experimental period, prior to grazing, the fields were measured for sward surface height, fresh herbage yield, dry matter percentage and dry matter yield, and the morphological composition of the sward was measured.

SSH was measured using a falling plate meter (‘t Mannetje and Jones, 2002). Ten recordings per field were made and the average was used in further calculations. Herbage dry matter yield was measured by cutting at least 5% of the total area with a mowing machine (Agria 3200) at a stubble height of 4 cm. The grass was collected with a rake and put in plastic bags. Fresh weight was recorded in the lab. Duplicate core samples of approximately 200 g of fresh material were taken and dried at 70 °C for 24 hours. Grass morphology was determined by hand-separating a plucked sample of approximately 30 g fresh material into stem (representing all generative organs), leaf blade, pseudo-stem (split at the ligule of each leaf) and dead material (all plant material showing no green colour). Green leaf mass was calculated with DMY and leaf blade proportion. Bulk density was calculated by dividing DMY by SSH minus 4 cm (cutting height). Herbage and leaf blade allowance were calculated by multiplying DMY and GLM, respectively, by plot size.

Tiller density, extended tiller height and sheath length

On day 5 of each experimental period, samples were taken to determine the tiller density. Per plot two strips of 216 cm^2 were cut with a knife at soil level and collected in plastic bags. In the lab, all living tillers were counted. Random sub samples of 40 intact tillers were taken to determine the extended tiller height (ETH). Also sheath length, the distance till the ligule of the youngest leaf, was determined. The ETH and sheath length were measured with a ruler.

Crown rust

In 2002 in P2, P3, and P4 and in 2003 in all periods, additional grass samples were taken from all plots on day 13 of each period as a sub sample of the herbage sampled for yield measurements. Severity of crown rust (*Puccinia coronata* f.sp. *lolii*) infestation was scored into three classes: no infestation, medium infestation (1-5 spots per leaf) or severe infestation (>5 spots per leaf) and expressed as percentage of total leaves in each class. At least 150 leaves per sample were counted.

Chemical composition

Daily, three pluck samples of the grass were taken at 13:00 h, 20:00 h and the next morning at 7:00 h at the same spot and a similar depth as where the cow had previously been grazing. Samples were taken from day 8 to day 13.

Table 3 Regression statistics of various chemical components (g kg^{-1} DM) and Digestibility (DOM) estimated by NIRS and the laboratorial measurements.

Component	Range	R^2	Bias	N
Ash	78-130	0.37	14.3	100
NDF	370-529	0.63	–40.5	100
ADL	11-33	0.29	–9.2	100
CP	138-225	0.96	–4.8	100
WSC	80-154	0.97	16.2	52
DOM	501-1052	0.73	5.3	98

NDF: neutral detergent fibre; ADL: acid detergent lignin; CP: crude protein; WSC: water soluble carbohydrates; DOM: digestible organic matter.

Samples were dried at 60 °C, and determined for DM, then ground to pass a 1 mm sieve, and analyzed with Near Infrared Reflectance Spectroscopy (NIRS) for ash, NDF, acid detergent lignin (ADL), CP and WSC, using equations developed by Centre de Recherch Agronomiques de Gembloux (CRAG) and Digestible Organic Matter (DOM), using an equation developed by Barenbrug Holland BV. A validation experiment was conducted to know the precision and the bias of these equation lines (Table 3). Ash was determined at 550 ºC; CP was analyzed using the Kjehdahl method; NDF and ADL were analyzed according a modified method of Van Soest as described by Goelema *et al.* (1998). For the determination of WSC, the method of van Vuuren *et al.* (1993) was used, with using millipore water instead of 40% ethanol. DOM was determined using method of DeBoever *et al.* (1996).

Dry matter intake (DMI)

To determine DMI, each cow was dosed with C_{32}-alkane concentrates twice daily during milking. The alkane dosing started one week prior to the experiment and continued throughout the experiment, to get a stable alkanes-flow through the gastrointestinal tract. Faeces were sampled daily, by sampling each dung patch in the field where the cow had been grazing the previous 24 hours. n-Alkanes were analyzed in the grass, faeces and concentrates as described in chapter 3.

The DMI was calculated with the following equation:

$$Herbage\ \ Intake\ \ (kg\,DM\,/\,d) = \frac{CI \times C_{32}}{\frac{F_{32}}{F_{33}} \times H_{33} - H_{32}} \qquad (1)$$

Where, F_{32} and F_{33} are faecal concentrations of alkanes C_{32} and C_{33} (mg kg^{-1} DM); H_{32} and H_{33} are the concentrations of the alkanes C_{32} and C_{33} in the grass (mg kg^{-1} DM); *CI* is concentrate intake (kg DM d^{-1}) and C_{32} are concentrations of alkane C_{32} in the concentrate (mg kg^{-1} DM). In this equation, it is assumed that the apparent digestibility of the alkanes is constant, independent of its source (natural occurrence in grass and concentrate or dosed) and chain length (C_{32} or C_{33}).

Statistical analyses

Data were analyzed averaging the daily data to a mean value for each period, each plot or animal. Sward characteristics and chemical parameters were analyzed with the General Linear Model procedure (SPSS for Windows, Rel. 11.0 Chicago: SPSS Inc.), according to the following model:

$$Y_{ij} = \mu + C_i + P_j + C_i \times P_j + e_{ijk} \qquad (2)$$

where, μ is general mean; C_i is cultivar effect (i = 1…4); P_j is period effect (j = 1..4); $C_i \times P_j$ is interaction between cultivar and period; e_{ijk} is residual term. LSD test was used for all pair wise comparisons.

DMI was statistically analyzed as a 4 × 4 Latin square design, with the mean effects of four cultivars measured over four periods with three cows as replicates, according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + A_k + e_{ijkl} \qquad (3)$$

where, μ is general mean; C_i is cultivar effect (i = 1…4); P_j is period effect (j = 1..4); A_k is animal effect (k = 1…12); e_{ijkl} is residual term. A LSD test was used for pair wise comparisons. The relations between DMI and sward and chemical parameters were analyzed using a two-tailed Pearson correlation procedure.

Results

Sward structure

Grass cultivars differed (P<0.001) consistently in SSH and DMY (Table 4). In both years cv 3 had the lowest SSH and DMY, and cv 4 the highest. Cv 1 was similar to cv 4 in 2002 and to cv 3 in 2003, whereas cv 2 was intermediate. In 2002, an interaction between cultivar and period was observed, this was related to cv 1 showing the highest SSH in P2 and P4 and intermediate in P1 and P3. SSH and DMY were positively correlated within each year. In 2003, the SSH was much lower, whereas the DMY was slightly higher. This resulted from a much denser sward in 2003 than in 2002, also reflected by the higher bulk density in 2003 (Table 4).

Leaf blade proportion did not differ among cultivars in 2002, but cv 3 was lowest in leaf proportion in P1, P3 and P4, but highest in P2, causing a significant interaction. The leaf proportion of cv 3 was complementary with the proportion of dead material. In 2003, cv 1 had a higher proportion of leaf blades than cvs 2 and 3. The stem proportion was generally low in the experiment, as the starting date of the experiment was chosen to enable comparison of leafy swards. In 2002, the sward of paddock B was vegetative as it was sown in spring. At the onset of the experiment in 2003, in the beginning of July, cv 2 had a slightly higher stem proportion in the sward than the other cultivars, but the proportion of stem went down to lower than 3% after P2, causing the interaction with cultivar. Cv 1 had the highest pseudo stem proportion in 2002 and cv 4 in 2003. Cv 3 had the least pseudo stem in both years. The proportion of dead material was highest in cv 3 in both years, and lowest in cvs 1 and 4, especially in periods with high crown rust infestation. In 2003, the proportion of dead material was much higher than in 2002, mainly due to the dry and hot summer (Table 2).

Although the total DMY in 2002 was lower than in 2003, the green leaf mass was higher, because of the higher concentration of dead material in 2003. Cv 4 had in both years the highest GLM, whereas cv 3 had the lowest GLM. In both years, no difference in bulk density among cultivars was found. There was a marked difference between the two experimental years, in 2002 bulk density was 1.58 kg DM/m^3 and in 2003 this was 2.06 kg DM/m^3. Tiller density was lowest in cv 2 in 2002. In 2003, tiller density of cvs 2, 3 and 4 had increased compared to 2002, but not in cv 1 due to winter damage. Nevertheless, in 2003 no significant differences for tiller density could be detected among cultivars. The extended tiller height was different in both years among cultivars. Cv 3 always had the lowest, whereas cvs 2 and 4 always had the highest ETH. The sheath length did not vary among cultivars in 2002, but in 2003 cvs. 2 and 4 had longer sheaths than cvs 1 and 3. The sheath length was in both years 21% of the extended tiller height.

Table 4 Sward structural and morphological characteristics of four perennial ryegrass cultivars during a two-year grazing experiment. The significances of effects of cultivar (C) and Period (P) and their interaction were tested

Parameter	Year	Cultivar				Mean	s.e.d.	Sign.		
		1	2	3	4			C	P	C×P
SSH	2002	17.8^{bc}	17.4^{b}	16.4^{a}	18.0^{c}	17.4	0.21	***	***	*
(cm)	2003	14.3^{a}	15.6^{b}	14.6^{a}	15.9^{b}	15.1	0.43	***	***	NS
DMY	2002	2204^{c}	2048^{b}	1933^{a}	2179^{c}	2091	43	***	***	NS
(kg DM ha^{-1})	2003	2102^{a}	2344^{ab}	2128^{a}	2429^{b}	2250	96	**	***	NS
Leaf blade	2002	77.3	77.7	76.1	77.6	77.2	0.7	NS	***	*
(% of DM)	2003	69.7	66.1	67.1	67.7	67.6	1.5	NS	***	NS
Stem	2002	1.3	2.1	1.6	1.3	1.5	0.5	NS	***	NS
(% of DM)	2003	3.2^{ab}	4.6^{b}	2.5^{a}	2.8^{a}	3.3	0.6	**	***	***
Pseudo stem	2002	16.6^{c}	14.6^{b}	11.8^{a}	16.2^{c}	14.8	0.5	***	***	NS
(% of DM)	2003	13.5^{b}	12.6^{ab}	12.2^{a}	15.1^{c}	13.4	0.6	***	***	NS
Dead	2002	4.8^{a}	6.1^{b}	10.7^{c}	5.2^{ab}	6.7	0.5	***	***	***
(% of DM)	2003	13.7^{a}	16.6^{ab}	18.3^{b}	14.4^{a}	15.8	1.5	*	***	NS
GLM	2002	1708^{c}	1599^{b}	1484^{a}	1695^{c}	1621	32	***	***	*
(kg DM ha^{-1})	2003	1448^{ab}	1539^{bc}	1409^{a}	1635^{c}	1508	60	**	***	NS
Bulk density	2002	1.62	1.54	1.56	1.58	1.58	0.04	NS	***	NS
(kg DM cm^{-3})	2003	2.10	2.02	2.07	2.05	2.06	0.05	NS	***	NS
Tiller density	2002	8385^{b}	7061^{a}	8314^{b}	8547^{b}	8077	418	**	***	NS
(# m^{-2})	2003	8489	9149	9157	9438	9058	654	NS	***	NS
ETH	2002	24.2^{b}	25.3^{b}	21.4^{a}	24.6^{b}	23.8	0.97	**	***	NS
(cm)	2003	18.9^{a}	20.5^{b}	18.2^{a}	21.2^{b}	19.7	0.54	***	***	NS
Sheath length	2002	5.30	5.26	4.71	5.16	5.11	0.26	NS	***	NS
(cm)	2003	3.70^{a}	4.34^{b}	3.70^{a}	4.63^{b}	4.10	0.18	***	***	NS

SSH: sward surface height; DMY: dry matter yield; GLM: green leaf mass; ETH: extended tiller height.

[a,b,c,d] means within the same row followed by the same subscript are not significantly different (P>0.05). ***: P<0.001; **: P<0.01; *: P<0.05; NS: Not significant.

Table 5 Total herbage and leaf allowance (kg DM day^{-1}) of four perennial ryegrass cultivars over a two-year grazing experiment. The significance of effects of cultivar (C) and Period (P) and their interaction were tested

	Cultivar				Mean	s.e.d	Sign.		
2002	1	2	3	4			C	P	C×P
HA	38.6^{c}	35.4^{b}	33.8^{a}	38.9^{c}	36.7	0.73	***	***	**
GLA	29.7^{c}	27.5^{b}	25.8^{a}	30.0^{c}	28.3	0.61	***	***	**
2003									
HA	30.2^{a}	33.9^{b}	30.9^{a}	35.1^{b}	32.5	1.46	**	***	NS
GLA	21.1^{ab}	22.6^{bc}	20.6^{a}	23.9^{c}	22.1	0.90	**	***	NS

HA: herbage allowance; GLA: green leaf allowance

^{a,b,c,d} means within the same row followed by the same subscript are not significantly different (P>0.05). ***: P<0.001; **: P<0.01; *: P<0.05; NS: Not significant.

Herbage allowance and green leaf allowance

The characteristics presented in Table 4 were crop-related, the allowance of herbage and green leaf (Table 5) were also dependent on the actual field sizes that were modified according to meet the cows' requirements. In 2002, herbage and green leaf allowance was lowest for cv 3 and highest for cvs 1 and 4. Cultivar 1 and 4 were alternately giving the highest allowance, causing a significant interaction with period. In 2003, herbage and green leaf allowance were again lowest for cv 3, but highest for cvs 2 and 4, thus cvs 3 and 4 had the largest contrast, in both years.

Chemical composition

Table 6 describes the chemical composition of all cultivars during the two-year grazing experiment. The variation in dry matter and ash concentration was rather small, 5% to 7%, respectively. In both years cv 3 has the highest DM and ash concentration, whereas cvs 2 and 4 had the lowest DM concentration and cv 1 the lowest ash concentration. Experimental years differed, 2003 had a very dry season, resulting in a high DM concentration and a lower ash concentration compared to 2002. Nevertheless, cultivars ranked almost similar in both years.

The neutral detergent fibre concentration and acid detergent lignin were slightly higher in 2003 than in 2002, which can be related to one year older sward in 2003. Cv 1 had in both years the lowest NDF concentration. In 2002, cvs 2, 3 and 4 had comparable NDF concentration (± 477 g/kg DM). In 2003,

Table 6 Chemical composition of four perennial ryegrass cultivars in a two-year grazing experiment. The significances of effects of cultivar (C) and Period (P) and their interaction were tested.

	Cultivar				Mean	Range	s.e.d	Sign.		
2002	1	2	3	4				C	P	C×P
DM	179^{b}	171^{a}	180^{b}	173^{a}	176	5%	1.8	***	***	**
Ash	94^{a}	96^{b}	102^{c}	97^{b}	98	7%	1.3	***	***	NS
NDF	458^{a}	481^{b}	477^{b}	475^{b}	473	5%	3.3	***	***	NS
ADL	26^{b}	26^{b}	28^{c}	24^{a}	26	15%	0.4	***	***	***
ADL/NDF	57^{c}	54^{b}	59^{c}	51^{a}	55	15%	1.02	***	***	***
CP	183^{b}	175^{a}	182^{b}	179^{a}	180	4%	1.9	**	***	**
WSC	149^{c}	132^{b}	110^{a}	144^{c}	134	29%	2.7	***	***	NS
DOM	911^{d}	886^{b}	879^{a}	897^{c}	893	4%	2.8	***	***	***
2003										
DM	219	216	222	216	218	3%	4.2	NS	***	NS
Ash	80^{a}	83^{b}	86^{c}	85^{bc}	84	6%	1.1	***	***	NS
NDF	470^{a}	490^{b}	484^{b}	474^{a}	479	4%	3.7	***	***	NS
ADL	32^{bc}	32^{b}	33^{c}	30^{a}	32	12%	0.6	***	***	NS
ADL/NDF	69^{c}	65^{b}	69^{c}	62^{a}	66	10%	1.1	***	***	*
CP	210^{b}	195^{a}	207^{b}	194^{a}	202	8%	3.2	***	***	NS
WSC	114^{b}	107^{b}	87^{a}	131^{c}	110	40%	3.9	***	***	NS
DOM	856^{c}	833^{a}	841^{ab}	854^{b}	846	3%	3.9	***	***	NS

DM: dry matter content (g kg^{-1}); NDF: neutral detergent fibre (g kg^{-1} DM); ADL: acid detergent lignin (g kg^{-1} DM); ADL/NDF: acid detergent lignin (g kg^{-1} NDF); CP: crude protein (g kg^{-1} DM); WSC: water soluble carbohydrates (g kg^{-1} DM); DOM: digestible organic matter (g kg^{-1} DM).

^{a,b,c,d} means within the same row followed by the same subscript are not significantly different (P>0.05). ***: P<0.001; **: P<0.01; *: P<0.05; NS: Not significant.

cvs 1 and 4 had a lower value (± 472 g/kg DM) than cvs 2 and 3 (487 g/kg DM). The ADL concentration was low in both years, but was 6 g/kg DM higher in 2003 than in 2002 ($P<0.001$). Cultivars ranked similar during years, cv 4 being lowest and cv 3 highest in lignin. In 2002, an interaction ($P<0.001$) between cultivar and period was observed, cvs 2 and 3 increased more in ADL concentration than cvs 1 and 4. In both years, the concentration of ADL in NDF was lowest in cultivar 4 and highest in cvs 1 and 3, where cultivar 1 was highest in the first two periods and cultivar 3 in last two periods.
The crude protein concentration was slightly lower in 2002 than in 2003. The variation among cultivars was small (4% in 2002 and 8% in 2003), however the consistency was rather strong. In both years, cvs 1 and 3 had the highest and cvs 2 and 4 the lowest CP concentration. However, cv 3 showed a strong decrease in CP concentration during the last period in 2002, causing an interaction ($P<0.01$) with period.
The variation in water-soluble carbohydrates concentration among cultivars was 29% and 40%, respectively, in 2002 and 2003. The cultivars showed a consisted pattern; cultivar 3 had in both years the lowest WSC concentration, cultivar 4 was in both years among the cultivars with the highest WSC concentration. Cultivar 1 had in 2002 the highest WSC concentration, but in 2003 an intermediate WSC concentration. In 2003, the WSC concentration was lower ($P<0.05$) than in 2002, this might be explained with the higher fertilization regime in 2002 than in 2003 and the trade-off with a higher CP in the cell content.
Cv 1 had in both years the highest digestibility, cvs 2 and 3 the lowest. In 2002, digestibility decreased over the season ($P<0.001$). This decrease was for cvs 2 and 3 stronger than for cvs 1 and 4. In 2003, the digestibility varied among the periods ($P<0.001$), but no clear trend was visible.

Crown rust

The cultivars differed ($P<0.001$) in their susceptibility to crown rust (Figure 1). Cultivar 3, which was the oldest cultivar, was most affected by crown rust, while the newer cultivars 1 and 4 were most resistant. This pattern was observed in both years. The sward was less infested by crown rust in 2003 than in 2002 (Figure 1). This might be related to the fact that in the first period of 2002 no leaf spot data were recorded. The visual impression in that period was that crown rust was not present in any of the cultivars and crown rust was not seen as a major factor in the cultivar comparison by that time

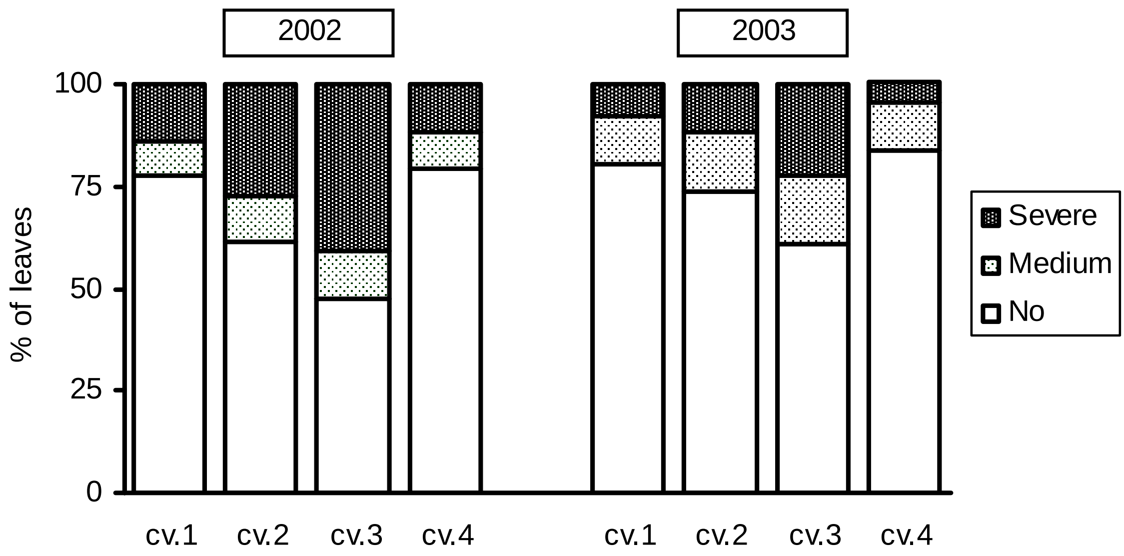

Figure 1 Severty of crown rust (*Puccinia coronata* f.sp. *lolii*) infection of the sward in two grazing experiments in 2002 and 2003, expressed as percentage of leaves infested: no infestation (0 spots per leaf), medium infestation (1-5 spots per leaf) or severe infestation (>5 spots per leaf).

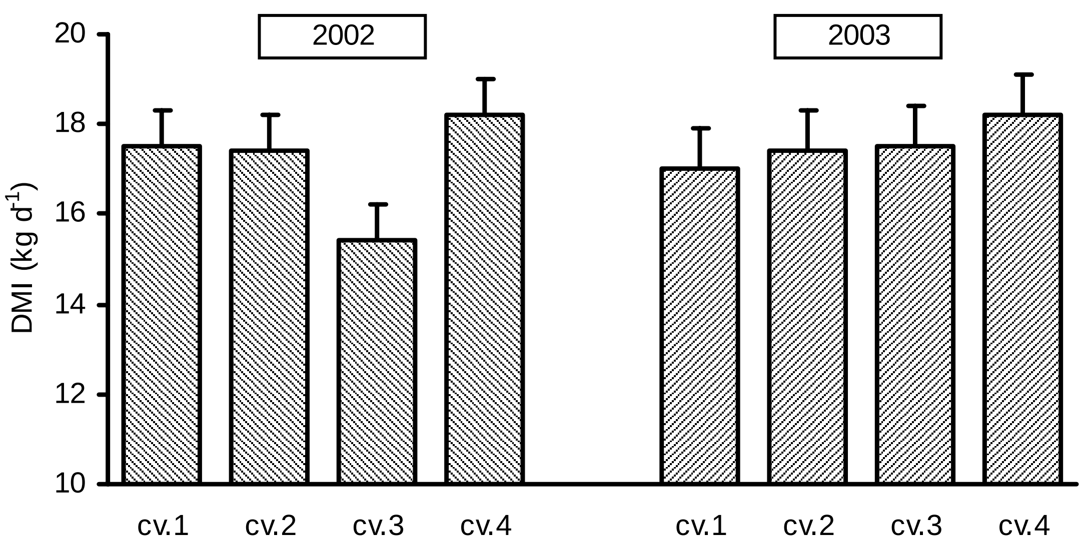

Figure 2 Herbage intake (kg DM day^{-1} animal^{-1}) of four cultivars of perennial ryegrass in 2002 and 2003. ^{a,b} columns with the same subscript are not significantly different (*P*>0.05).

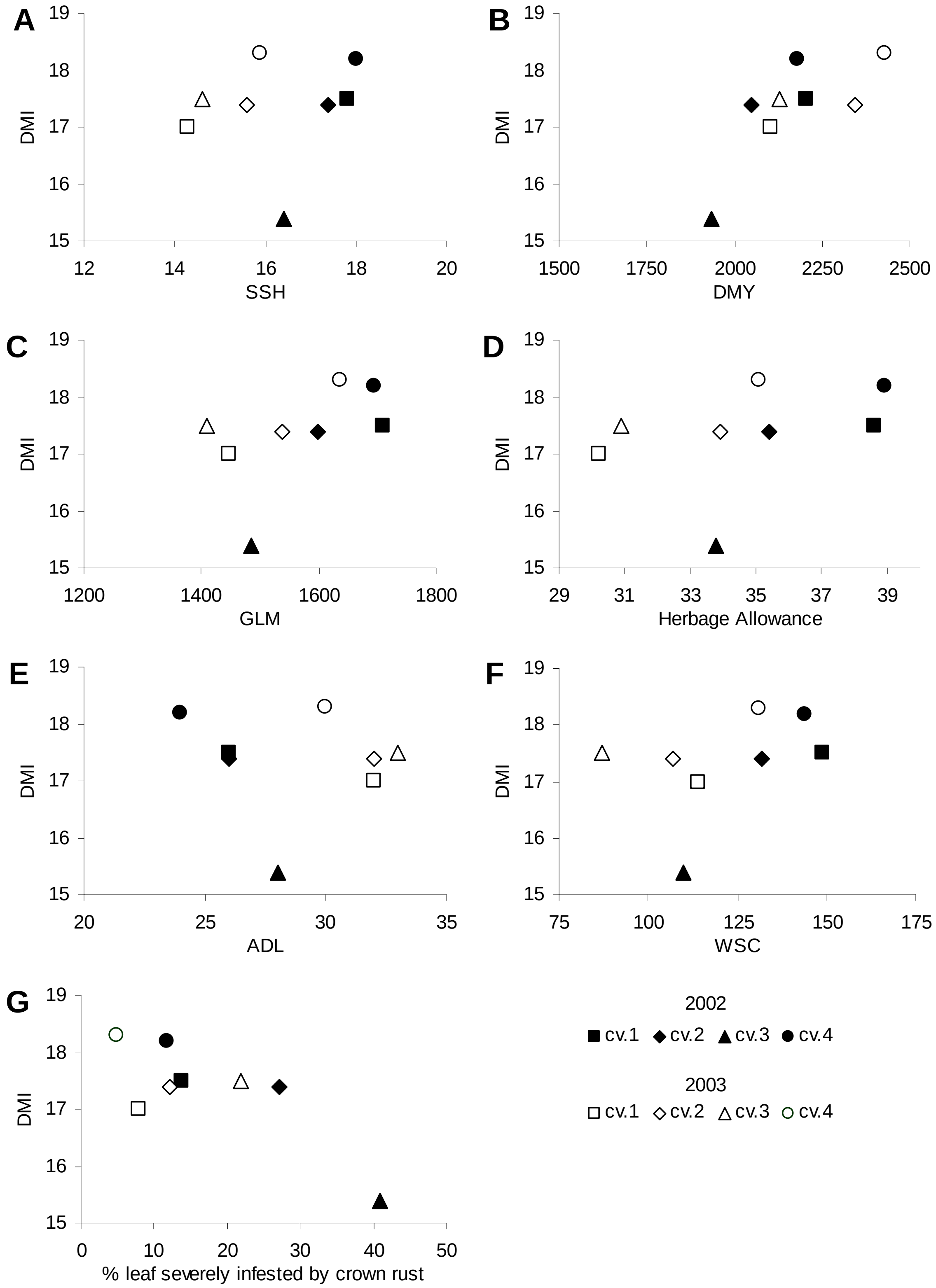

Figure 3 (A) sward surface height (SSH; cm); (B) dry matter yield (DMY; kg ha^{-1}); (C) green leaf mass (GLM; kg ha^{-1}); (D) herbage allowance (kg DM d^{-1}); (E) acid detergent lignin (ADL; g kg^{-1} DM); (F) water soluble carbohydrates (WSC; g kg^{-1} DM); (G) percentage of leaves severely infected by crown rust in relation to dry matter intake (DMI; kg d^{-1}) of four perennial ryegrass cultivars in 2002 (closed symbols) and 2003 (open symbols).

Dry matter intake of herbage

The results of the DMI analysis are presented in Figure 2. In 2002, a significant difference (P<0.05) among cultivars for DMI was found. Dairy cows consumed on average significantly less herbage of cv 3 throughout the experiment (15.4 kg DM day^{-1}). The DMI of herbage of the other cultivars was similar, while cv 4 tended to have the highest intake. In 2003, no significant differences could be found among cultivars (Figure 2), although again cv 4 was consumed most. The results for DMI expressed as g DM kg BW$^{-0.75}$ (data not shown) were almost identical.

Relations between sward structure and DMI

Variation was found in several sward structural characteristics important for intake; SSH, DMY and GLM differed among cultivars in both years. These three parameters were strongly intercorrelated. In this experiment, all three parameters were positively related with DMI. SSH was positively associated with DMI (Figure 3A). The two experimental years were clearly different (P<0.01), but within each year the trend was clear. A similar positive association with DMI was found for DMY (Figure 3B) and for GLM (Figure 3C). DMY was the only sward structural component which did not show a clear year effect (P=0.167) and showed a correlation (R=0.747, P=0.033) with DMI. There was also a positive association between herbage allowance and DMI (Figure 3D). Due to a similar field size for all cultivars during the same day, herbage allowance was highly correlated with DMY (R=0.981, P<0.001) within each year.

The relations between DMI and morphological parameters were limited. Only the proportion of pseudo stem had a positive relation with DMI. This might be related to the coinciding effect of a high percentage of pseudo stem with a higher SSH and DMY.

Relations between chemical composition and DMI

Effects of chemical composition on DMI were limited, the variation among cultivars in ash, NDF and CP concentration in both years did not result in differences in DMI in dairy cows. In both years, ADL concentration was different among cultivars (P<0.01), but levels were generally low, 26 and 32 g kg^{-1} DM in 2002 and 2003, respectively. However, DMI showed a significant negative correlation with ADL concentration (Figure 3E).

The largest difference in chemical composition among cultivars was found in the WSC-concentration. Variation of more then 25% was found, which was consistent over years. The consistent variation makes WSC concentration an attractive breeding goal. In 2002, a positive trend was observed between the WSC concentration and DMI (Figure 3F). In both years, cv 4 had a high WSC concentration, and was also consumed most by dairy cows. In contrast, cv 1 had the highest WSC concentration in 2002, but was intermediate in DMI. In 2003, cultivar 1 was intermediate in WSC concentration, but lowest in DMI (not significant). Furthermore, the variation among cultivars for WSC was much larger in 2003 than in 2002 (29% vs. 37%), whereas the variation for DMI diminished in 2003.

Relations between crown rust and DMI

The percentage of leaves infested with crown rust showed a negative correlation with DMI when controlled for year. The crown rust infestation, especially in 2002, may have played a large role in the effect on DMI during the grazing experiments (Figure 3G).

Discussion

The main question to be answered in this chapter was; "*Is there scope for breeding perennial ryegrass with a higher potential for dry matter intake of dairy cows during grazing?*" In this experiment, variation among cultivars in DMI was only found in the first experimental year. In the second year, no significant differences could be detected. The variation among the four diploid perennial ryegrass cultivars in the first year of this study could be due to several factors, that will be discussed hereafter: sward structural characteristics (Meijs, 1981; Hazard *et al*., 1998; Casey and Brereton, 1999), heading date (Gowen *et al*., 2003), chemical characteristics (Lee *et al*., 2002) and infestation by crown rust.

Sward structure and morphology

Among cultivars variation was found in several sward structural characteristics important for intake; SSH, DMY and GLM differed among cultivars in both years. The three parameters were strongly intercorrelated. Therefore, no strong conclusions can be drawn. DMY, GLM and SSH were pointed out previously (Meijs, 1981; Hazard *et al*., 1998; Casey and Brereton, 1999) as potential traits for breeding for high DMI. In this experiment, all three parameters were positively related with DMI.

A higher DMY implied a higher herbage allowance, because the field size on any day was similar for all four cultivars. A higher allowance meant increased possibilities for selection and this could be positively related with herbage intake (Meijs, 1981). The vertical gradient in herbage quality (Hazard *et al*., 1998; Delagarde *et al*., 2000; Smit and Elgersma, 2004) gave grazing cows the opportunity to select within a canopy for a higher quality.

Hazard *et al*. (1998) reported that herbage intake of grazing sheep was well correlated with GLM (above 10 cm). In this experiment, also a positive relation between GLM and DMI of grazing dairy cows was found. However, in this study GLM was determined deeper into the sward (above 4 cm). Cultivars did not differ in leaf proportion, therefore the variation among cultivars in GLM was mainly explained by the variation in DMY.

SSH is in short term intake studies often determined as the major constraint of intake during grazing (Wade *et al*., 1989; McGilloway *et al*., 1999; Casey and Bereton, 1999). In these short term studies, a low SSH limited intake by bite mass through limited grazing depth, biting rate remained similar at different SSH's (McGilloway *et al*., 1999). Casey and Bereton (1999) showed that cultivar differences can affect bite mass. In this study, herbage intake was stimulated by a higher SSH. A higher DMI was also positively associated with a higher proportion of pseudo stem. However, the ranking of cultivars for proportion of pseudo stem was positively related with SSH and DMY. Cultivars with a taller and heavier sward had more pseudo stem in the harvested herbage, this was due to the fixed cutting height of 4 cm. The average length of the leaf sheath was 21% of the extended tiller length. This value was also found in two earlier seasons (Chapter 2). The ratio between leaf sheath and ETH was independent of cultivar in this experiment. The position of the youngest ligule could become important when the grazing depth reaches below the sheath length, in situations where herbage allowance is limited and animals are forced to graze deep into the sward (Gowen *et al*., 2003).

Heading date

In this experiment, two intermediate (cvs 1 and 2) and two late heading cultivars (cvs 3 and 4) were tested, but, in contrast to Gowen *et al*. (2003), heading date did not have any effect on DMI. This was probably because this experiment started one month after heading date of the latest cultivar. The aim was to study inherent genetic differences for DMI in leafy swards, not biased by the reproductive stage of the sward.

Chemical composition

Chemical composition in this study was estimated using NIRS. The validation experiment showed, however, that ash and ADL were difficult to measure very precisely. The samples in this experiment were grass samples of three to four weeks of regrowth and had therefore a limited range in especially ash and ADL. The equations developed by CRAG were established with over 1000 samples, the range of measurements was larger, and their precision was better (R^2 > 0.8). The values were therefore used, but interpretation should be done with caution.

Variation among cultivars was found in several chemical characteristics important for intake; NDF, ADL, CP and WSC differed among cultivars in both years. Herbage intake of ruminants is related to the concentration of NDF in the diet (van Soest, 1982; Waldo, 1986). The slow digestion of the NDF fraction in the herbage may have a large influence on passage rate and therefore the fibre fraction is believed to have a direct effect on rumen fill. The physical constraint of rumen fill is thought to be one of the main constraints in ruminant feeding (Forbes, 1995). Voluntary intake is limited by fill to a greater extent for forages with low digestibility and high fibre concentrations (Allen, 1996). The digestibility of all grass cultivars used in this experiment was high (>80%). The variation in NDF concentration among perennial ryegrass cultivars observed in this experiment was significant, but small and there was no correlation between NDF and DMI.

In both years, ADL concentration was different among cultivars (P<0.01) and was associated with a negative effect on DMI. But lignification as indicator for DMI was also reported by van der Aar *et al.* (1981). They indicated that during grass maturation lignification takes place, which reduces digestibility. However, in this experiment a higher DMY reduced the ADL concentration and there was no relation between ADL concentration and DOM. There was a positive relation (P<0.05) between ADL and percentage of leaves severely infested by crown rust. This is in agreement with results obtained by Isawa *et al.*, 1974; Isawa and Nishihara, 1975), who also found a higher ADL concentration in severely infested leaves. The grass might have a defence mechanism against fungal attack, indirectly causing reduced intake by other herbivores as well.

The genetic variation among cultivars for CP concentration was small (4-8%), but consistent over years. However the differences in protein concentration among cultivars (P<0.01) did not have an effect on DMI in this experiment.

The cultivar with the lowest protein concentration (cv 2 in 2002; 175 g kg^{-1} DM) still exceeded the critical level of 140 g kg^{-1} DM needed to maintain herbage intake during grazing (Peyraud and Astigarraga, 1998; Valk *et al*., 2000).
Reported effects of an increased WSC concentration in perennial ryegrass on DMI by cattle were variable, some authors found a higher DMI (Mayland *et al*., 2000b; Lee *et al*.; 2002), others did not (Peyraud and Astigarraga, 1998; Valk *et al*., 2000). The reason of the increased DMI could be selection (Jones and Roberts, 1991; Mayland *et al*. 2000b) or by faster available energy (van Vuuren, 1993; Miller *et al*., 2001; Lee *et al*., 2002). The fact that Lee *et al*. (2002) found an increased DMI in grass with a higher WSC concentration might be due to the fact that their cultivars differed more in WSC concentration (>40%) compared with our experiments (29-40%). Furthermore, it might also be related with a much larger variation in NDF concentration (>15%) in their experiment than in this study (4%), and therefore larger variation for digestibility of the herbage. If cows can select, the animals may prefer high WSC cultivars (Mayland *et al*., 2000b; Chapter 5), but this experiment showed that when cows were allotted for two weeks to only one cultivar, they did not ingest more from a cultivar with increased WSC concentration than from one with a lower WSC concentration.

Crown rust

The direct effects of crown rust on grazing dairy cows are difficult to investigate and the effects are not well documented (Lewis and Hopkins, 2000). In this experiment, crown rust was associated with lower levels of DMY and green leaf mass, and more dead material in the herbage. This supported results obtained by Lancashire and Latch (1966). The decreasing effect of crown rust they found on tiller density, however, was not found in this experiment. Crown rust infestation was associated with an increased ADL concentration, a lower WSC concentration and lower digestibility. Therefore forage quality was reduced and forage palatability may have been lower. Isawa *et al.* (1974) and Potter (1987) reported that besides effects on ADL, WSC and DOM, crown rust also increased the CP concentration; this was not found in our experiment. Isawa *et al.* (1974) reported that crown rust infestation had also an effect on mineral concentration, by increasing calcium and decreasing sodium. In this experiment, sodium was not determined, but crown rust was positively associated with calcium concentration, so one could speculate on a decreased sodium level. This could have had a reducing effect on palatability (Mayne *et al.*, 2000).

Conclusion

The grazing experiments showed some scope for breeders to select for higher herbage intake. It was concluded that high yielding ability of cultivars were positively related with a high herbage intake. In this study, the variation among cultivars in nutritive value was too small to influence herbage intake. Lignin concentration showed some effect on herbage intake, and might be taken into account in breeding programmes, although the estimation of lignin by NIRS is still inaccurate. Finally, breeding for crown rust resistance is of evident importance to maintain a high yielding capacity, nutritive value and herbage intake.

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Chapter 5

Cattle grazing preference among six cultivars of perennial ryegrass

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Seerp Tamminga
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Abstract

Six diploid perennial ryegrass cultivars were tested for cattle grazing preference in July and September 2003 and May 2004. Three groups of dairy cows were allowed to select among the six cultivars that were sown in a randomized blocks with twelve plots (36.7 x 2.0 m). Each experiment lasted four days, and every day cows were offered a new field with twelve plots. Herbage intake was measured using the sward cutting method, measuring herbage yield before and after grazing. The Chesson-Manly index was used to quantify cattle grazing preference. Dairy cows did select among the cultivars in every period. The pattern of selection was consistent over three periods. In July 2003 and May 2004 cultivars differed in morphological characteristics, but this was not related with the selection among cultivars. In all three periods, distinct differences were found among cultivars in chemical composition and digestibility. Dairy cows preferred the cultivars with low ash and fibre (NDF), and a high dry matter and water-soluble carbohydrates concentration and high digestibility.

Keywords: *Lolium perenne*, diploid cultivars, preference, dairy cattle.

Introduction

Although large herbivores are often considered to be generalized grazers, still they are known to select if they have the opportunity (Newman *et al*., 1995). Preference among various plant species is a well-recognized concept, and has been frequently studied (Heady, 1964; Belovsky, 1978; Stephens and Krebs, 1986; Provenza *et al*., 1996). Cattle showed preference among cultivars of one species in tall fescue (*Festuca arundinacea*) (Shewmaker *et al*., 1997), annual ryegrass (*Lolium multiflorum)* (Aderibigbe *et al*., 1982) and perennial ryegrass (*Lolium perenne*) (O'Riordan *et al*., 1998). The mechanisms behind this preference are unclear. Simon and Daniel (1983) concluded for sheep and O'Riordan *et al*. (1998) for cattle, that tetraploids were preferred over diploid cultivars of perennial ryegrass. Jones and Roberts (1991) reported that sheep selected among four diploid perennial ryegrass cultivars, which form the largest part of the seed market in the Netherlands and are widely used in practice (Bonthuis *et al*., 2004) and concluded that cultivars with a high water soluble carbohydrates (WSC) concentration were preferred. Sward surface height (SSH) (Griffiths *et al*., 2003), bulk density (Laca *et al*., 1992) and sward morphology (Dumont *et al*., 1995) have been reported as key parameters in grazing behaviour studies.

Most of the preference experiments were done with sheep (Simon and Daniel, 1983; Hazard *et al*., 1998) or non-lactating beef cattle (O'Riordan *et al*., 1998) in short term experiments and experimental animals are often fasted before experiments (Jones and Roberts, 1991). Detailed information on the preference of grazing dairy cows in the long term (>1 day) is still lacking.

Aim of the experiment

In this experiment, the preference of Holstein Friesian cows was determined with respect to six diploid perennial ryegrass cultivars, throughout the grazing season to investigate whether there is scope for selecting cultivars for preference and to analyze plant and sward factors related to preference.

Material and Methods

Fields

Three four-day grazing experiments were conducted on the same paddock. Two experiments took place in 2003, in July and September, and the third experiment in May 2004. Experimental fields had been established in 1999, cut for three years during the grazing season and grazed by sheep during winter. Experimental details were reported earlier (chapter 2). All experiments had a similar set-up, as shown in Figure 1. Six diploid perennial ryegrass cultivars (Abergold, Agri, Barezane, Barnhem, Herbie and Respect) were sown in duplicate in a randomized block design with 4 replicate blocks, resulting in 48 plots (110 × 2 m), which were separated by 0.2 m bare soil. For this experiment, blocks were split in three parts with electric wires, resulting in 12 grazing areas of 36.7 × 26.4 m. Each grazing area consisted of 12 subplots (36.7 × 2 m) in which all six cultivars were present twice (Figure 1). Cows were moved to a new grazing area daily, as cultivars were randomized and in a different order every day, cows did not know where cultivars were located.

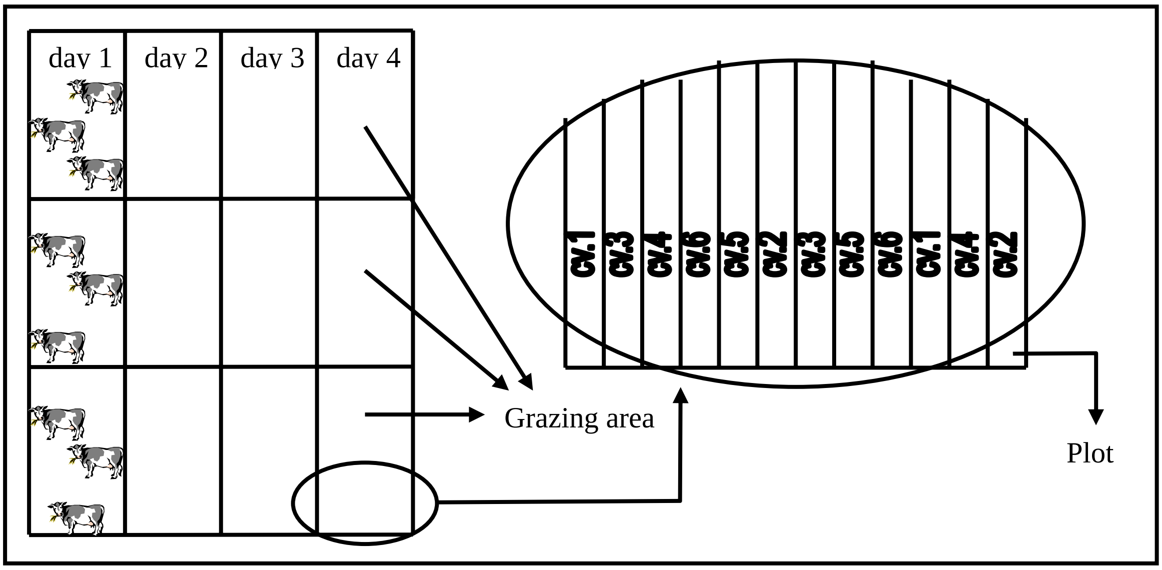

Figure 1 Set-up of grazing experiments, with an example of the grazing area with the 6 randomized cultivars offered during one day.

Management

Weather data during the three experiments are presented in Table 1. On March 21, 2003, the paddock was fertilized with 25 m^3 pig manure per ha and an additional 35 kg N ha^{-1} was applied. On May 15, a silage cut was made, and the fields were fertilized with 55 kg N ha^{-1}. On June 6 and 10, the fields for days 1-2 and days 3-4 were harvested, respectively, and 75 kg N ha^{-1} was applied directly after harvesting. The first grazing experiment took place from June 30 to July 4. Thereafter, the residual herbage was removed on July 10. On August 19, the sward was harvested and fertilized with 60 kg N ha^{-1}. The second grazing experiment took place from 15th to the 19th of September.

During the winter of 2003 to 2004 the sward was grazed by sheep, which removed all residual herbage. On April 13, 2004 the paddock was fertilized with 60 kg N ha^{-1}. The third grazing experiment took place from 10^{th} to the 14^{th} of May 2004.

Animals

Each experiment consisted of four consecutive days of grazing. The group size, some animal characteristics and the amount of concentrate fed during the three experiments are presented in Table 2. All cows had been grazing at least one week prior to the start of the experiments. Additional concentrate was supplied in portions of 1.5 kg during milking twice daily.

Table 1 Average meteorological data during the three grazing experiments.

Exp.	Date	Mean Temp (°C)	Max. Temp (°C)	Min. Temp (°C)	Precipitation (mm d^{-1})	Radiation (MJ m^{-2})
1	June 30 – July 4 2003	16.0	19.4	13.3	4.4	11.3
2	Sept. 15 – Sept. 19 2003	16.7	25.5	8.6	0.0	15.2
3	May 10 – May 14 2004	11.5	16.3	7.4	0.0	15.7

Table 2 Group size, body weight (BW), milk production (MP) and concentrate fed during three preference experiments.

Exp.	Date	No. of cows per group	BW (kg)	MP (kg d^{-1})	Concentrate (kg product)
1	June 30 – July 4 2003	5	533	30.8	3
2	Sept. 15 – Sept. 19 2003	3	553	26.2	3
3	May 10 – May 14 2004	3	680	Dry cows	0

Field Measurements

Each morning, the sward surface height (SSH) was measured with a falling plate (diameter 48 cm). Five measurements of SSH were taken in each subplot and averaged. Then a pre-grazing cut was taken with a mowing machine (Agria 3200) at a cutting height of 4 cm, to measure initial herbage mass. Per subplot an area of 3.5 m^2 (2.80 × 1.25 m) was cut. The grass was collected in plastic bags and fresh weight was recorded in the lab. Subsamples (200 g fresh weight) were taken with a grass core and dried at 70 ^oC for 24 hours to determine the DM content.

At noon, cows were moved to the next field. The SSH of the grazed plots was measured and post-grazing cuts were also taken with an Agria mower at a cutting height of 4 cm to measure residual herbage mass. Two strips of 3.5 m^2 were cut per plot. The two strips were situated immediately adjacent to the strip that had been cut prior to grazing. The cut grass was processed in the same way as the pre-grazing samples.

Morphological and chemical analysis

A plucked sample of the cut herbage of approximately 25 g fresh material was collected and hand-separated into leaf blade, pseudo stem and dead material.
The subsamples of the pre-grazing herbage used to determine DM content, were ground using a 1 mm sieve (Peppink, hammer mill) and analyzed for chemical composition (ash, Neutral Detergent Fibre (NDF), Acid Detergent Lignin (ADL), Water Soluble Carbohydrates (WSC), Crude Protein (CP)) and digestibility (DOM) with Near Infrared Reflectance Spectroscopy (NIRS). The NIRS predictions for chemical composition were validated with laboratory analyses (Chapter 3)

Methodology to express preference

To measure preference in a grazing situation, various methods have been reported. All authors define the food that is eaten most, as the most preferable. Many studies measured preference indoors, weighing the offered herbage and the residual and expressing preference as food consumed (offered-residual) (Aderibigbe *et al*., 1982; Provenza *et al*., 1996; Tolkamp *et al*., 1998). In a grazing situation, this is also possible by estimation of herbage yield before and after grazing, either visually (Shewmaker *et al*., 1997), or using the sward cutting techniques (Meijs, 1981; Macoon *et al*., 2003). Prior to grazing, a small area is cut to measure the pre-grazing yield, and after a period of grazing this is repeated to determine the post-grazing yield. By subtraction, the consumption is calculated (O'Riordan *et al*., 1998; Jones and Roberts, 1991; Chapter 3).
Manly *et al*. (1972) and Chesson (1978; 1983) developed a methodology to take food availability and depletion into account. They assumed that food depletion, and therefore the probability of encountering a preferred cultivar, decreases more rapid for a preferred than for a less preferred cultivar.

Chesson-Manly index (CM):

$$\alpha_i = \frac{\ln\left[1-(consumed_i / available_i)\right]}{\sum_{j=1}^{m}\ln\left[1-(consumed_j / available_j)\right]} \qquad (1)$$

Statistical analysis

The experimental unit in these experiments was the cultivar in each field, so the two subplots occurring in each field were averaged per cultivar. Pre- and post-grazing yield, consumption, SSH, chemical parameters and preference were analyzed as a randomized block design with six cultivars and three grazing areas and four days as replicates. Sward morphological parameters were analyzed as a randomized block design with six cultivars and four days in each experiment, in which the three grazing areas within one day were pooled. Data were analyzed for each experiment separately with a two-way ANOVA, with cultivar and day as independent variables. Correlation coefficients were calculated between preference and morphological and chemical parameters.

Results

Morphology

The morphological characterization of the swards is presented in Table 3. In July 2003 (experiment 1), over 30% of the canopy consisted of reproductive material. There were significant differences (P<0.001) among cultivars. Cultivars 3 and 4 had the highest proportion of leaf blade, cvs 2 and 6 had the highest proportion of stem and the lowest proportion of pseudo stem. In September 2003 (experiment 2), no inflorescences were present in the sward. The cultivars did not differ in DM allocation. The amount of dead material was very high (22.5 %), due to the hot and dry weather conditions during the summer of 2003. In May 2004 (experiment 3), the late heading cultivars, 3, 4 and 5, had the highest (P<0.001) proportion of leaf blade and pseudo stem, whereas the intermediate-heading cultivars, 1, 2 and 6 had the highest (P<0.001) proportion of stems.

Chemical composition

Table 4 shows the chemical composition of the six cultivars. The dry matter (DM) content of the herbage was high (>20%) in experiments 1 and 2, but did not differ among cultivars. In experiment 3 the level was lower and showed more variation.

The variation among cultivars in neutral detergent fibre (NDF) concentration was small with 8, 4 and 7 % in experiment 1, 2 and 3, and could be explained by the variation in stem proportion (Table 3). Cultivars with a high stem proportion also had a high NDF concentration and vice versa. Variation in lignin (ADL) concentration was most apparent in experiments 1 and 3 and could also be related to the stem proportion, cultivars with a high stem proportion had a lower ADL concentration. In experiments 1 and 3, the water soluble carbohydrates (WSC) concentration was much higher (165 and 166 g kg^{-1} DM) than in experiment 2 (104 g kg^{-1} DM). The WSC concentration had the largest variation of all examined traits among cultivars: 23, 40 and 16% in experiments 1, 2 and 3 respectively. Crude protein (CP) varied among cultivars in experiments 2 and 3 and had a stronger inverse relationship with the WSC (R^2=0.75) then with the NDF concentration (R^2=0.15). The digestibility of organic matter (DOM) was 81.7, 76.8 and 82.3 % in experiments 1, 2 and 3, respectively. Significant (P<0.001) variation of 5% for DOM among cultivars was found in each of the three preference experiments.

Table 3 Sward morphology (percentage of leaf blade, stem, pseudostem and dead material) of six perennial ryegrass cultivars during three grazing preference experiments.

		Cultivar								
Exp.	Morphological Component	1	2	3	4	5	6	Mean	s.e.d.	Sign.
1	Leaf blade	53.3^{bc}	44.0^{a}	58.4^{cd}	63.8^{d}	52.6^{bc}	46.3^{ab}	53.1	3.55	***
	Stem	33.9^{bc}	45.4^{d}	26.0^{ab}	19.9^{a}	30.7^{b}	41.2^{cd}	32.8	5.07	***
	Pseudostem	9.3^{ab}	6.2^{a}	11.5^{b}	10.3^{b}	10.1^{b}	6.2^{a}	8.9	1.56	**
	Dead	3.5	4.4	4.1	6.1	6.6	6.4	5.2	1.57	NS
2	Leaf blade	59.4	62.4	59.0	60.6	62.0	62.5	61.0	2.94	NS
	Pseudostem	14.8	17.1	16.4	18.7	15.5	16.4	16.5	1.28	NS
	Dead	25.8	20.5	24.6	20.8	22.5	21.1	22.5	2.68	NS
3	Leaf blade	42.8^{a}	48.2^{b}	64.7^{cd}	68.6^{d}	63.5^{c}	45.9^{ab}	55.6	2.35	***
	Stem	45.3^{b}	45.1^{b}	4.9^{a}	4.5^{a}	12.3^{a}	44.6^{b}	26.1	4.22	***
	Pseudostem	10.7^{a}	5.8^{a}	28.6^{b}	25.8^{b}	23.1^{b}	8.1^{a}	17	2.77	***
	Dead	1.1	0.9	1.8	1.2	1.1	1.4	1.2	0.35	NS

^{a,b,c,d} means within the same row followed by the same subscript are not significantly different (*P*>0.05). ***: *P*<0.001; **: *P*<0.01; *: *P*<0.05; NS: Not significant.

Table 4 Dry matter (DM), ash, neutral detergent fibre (NDF), lignin (ADL), water soluble carbohydrates (WSC), protein (CP) concentration (g kg^{-1} DM) and digestibility of organic matter (DOM, in %) of six perennial ryegrass cultivars in three preference experiments.

		Cultivar								
Item	Exp.	1	2	3	4	5	6	Mean	s.e.d.	Sign.
DM	1	222.2	198.6	215.0	225.0	213.8	213.5	214.7	12.5	NS
	2	233.9	222.5	228.4	228.6	224.0	223.6	225.2	6.4	NS
	3	181.2^{bc}	169.3^{a}	183.0^{c}	174.3^{abc}	180.0^{bc}	172.9^{ab}	176.8	4.5	*
Ash	1	69.1^{a}	71.8^{ab}	77.0^{c}	77.5^{c}	71.8^{ab}	74.1^{bc}	73.6	1.9	***
	2	76.4^{a}	81.4^{ab}	89.1^{c}	81.2^{ab}	83.9^{b}	83.2^{b}	82.5	2.6	***
	3	87.1^{a}	93.6^{bc}	94.9^{bcd}	98.2^{d}	95.8^{cd}	91.8^{b}	93.6	2.0	***
NDF	1	465.4^{b}	496.2^{c}	470.2^{b}	456.9^{a}	472.2^{b}	496.1^{c}	476.2	3.6	***
	2	467.3^{a}	480.8^{b}	487.6^{b}	472.2^{a}	481.5^{b}	486.1^{b}	479.2	3.4	***
	3	491.9^{b}	505.1^{bc}	477.4^{a}	476.4^{a}	481.3^{a}	509.6^{c}	490.2	4.8	***
ADL	1	29.4	29.1	29.9	28.2	29.2	28.0	29.0	0.7	NS
	2	24.5^{cd}	23.3^{bc}	25.7^{d}	21.9^{a}	24.9^{d}	23.0^{ab}	23.9	0.6	***
	3	28.7^{ab}	28.1^{a}	30.6^{d}	29.4^{bc}	30.1^{cd}	28.8^{ab}	29.3	0.5	***
WSC	1	181.5^{d}	153.6^{b}	161.8^{bc}	179.4^{d}	168.7^{c}	143.5^{a}	164.7	4.7	***
	2	118.8^{c}	104.6^{b}	81.7^{a}	122.7^{c}	99.1^{b}	99.1^{b}	104.3	4.3	***
	3	187.4^{b}	157.2^{a}	160.7^{a}	160.6^{a}	167.5^{a}	162.4^{a}	166.0	7.3	**
CP	1	171.3	168.2	176.4	174.0	173.3	172.1	172.6	2.8	NS
	2	208.4^{d}	200.2^{b}	207.0^{cd}	193.8^{a}	205.2^{cd}	203.5^{bc}	203.0	2.0	***
	3	146.4^{ab}	153.4^{bc}	164.4^{d}	163.6^{d}	155.3^{c}	145.8^{a}	154.8	3.7	***
DOM	1	83.0^{bc}	79.0^{a}	83.1^{bc}	83.6^{c}	82.3^{b}	79.4^{a}	81.7	0.45	***
	2	79.0^{c}	76.6^{b}	74.8^{a}	76.8^{b}	76.4^{ab}	76.9^{b}	76.8	0.82	***
	3	82.2^{c}	80.9^{b}	83.9^{d}	83.9^{d}	83.1^{cd}	79.9^{a}	82.3	0.44	***

^{a,b,c,d} means within the same row followed by the same subscript are not significantly different (P>0.05). ***: P<0.001; **: P<0.01; *: P<0.05; NS: Not significant.

Table 5 Pre- and post-grazing sward surface height (SSH) and the SSH difference (cm) of six perennial ryegrass cultivars grazed by dairy cows in three preference experiments.

		Cultivar								
Exp.	SSH (cm)	1	2	3	4	5	6	Mean	s.e.d.	Sign.
1	pre-grazing	18.5^{ab}	21.2^{b}	15.8^{a}	16.4^{a}	18.1^{a}	21.3^{b}	18.6	1.38	***
	post-grazing	10.3^{a}	13.4^{b}	9.5^{a}	10.1^{a}	10.8^{a}	13.8^{b}	11.3	1.09	***
	difference	8.3^{b}	7.8^{b}	6.4^{a}	6.3^{a}	7.3^{ab}	7.5^{ab}	7.3	0.63	**
2	pre-grazing	13.7	14.1	13.9	14.6	14.1	13.8	14.0	0.48	NS
	post-grazing	7.4^{a}	8.8^{bc}	9.2^{c}	8.4^{b}	8.5^{b}	8.5^{b}	8.5	0.26	***
	difference	6.3^{b}	5.2^{ab}	4.7^{a}	6.2^{b}	5.6^{b}	5.4^{ab}	5.6	0.35	***
3	pre-grazing	28.3^{c}	28.0^{c}	20.7^{a}	22.2^{b}	22.8^{b}	30.4^{d}	25.4	0.67	***
	post-grazing	21.8^{b}	23.0^{b}	17.5^{a}	16.9^{a}	18.1^{a}	26.7^{c}	20.7	0.81	***
	difference	6.5^{d}	5.0^{c}	3.2^{a}	5.3^{c}	4.7^{bc}	3.7^{ab}	4.7	0.57	***

^{a,b,c,d} means within the same row followed by the same subscript are not significantly different (*P*>0.05). ***: *P*<0.001; **: *P*<0.01; *: *P*<0.05; NS: Not significant

Sward surface height

The SSH of the pre- and post-grazing swards and their difference is shown in Table 5. The difference between pre- and post-grazing SSH was always highest for cv 1 and always lowest for cvs 3 and 6. The SSH measurements were strongly correlated (R^2=0.85 *P*<0.001) with corresponding yield measurements (Table 6).

Pre- and post-grazing yield, intake and preference

Table 6 describes the pre- and post-grazing yields of the six cultivars during the three experiments. In experiments 1 and 3, pre-grazing yield differed among cultivars. The intermediate-heading cultivars 2 and 6 were always among the highest yielding cultivars, whereas the late-heading cultivars 3, 4 and 5 had always the lowest yields. In experiment 2, however, the cultivar differences tended to be opposite. In all experiments, post-grazing yields differed among cultivars. In general, the ranking for the post-grazing yield followed that of the pre-grazing yield, especially in situations where there was large variation in pre-grazing yields as in experiments 1 and 3.

Table 6 Pre- and post-grazing yields, consumption of herbage (DMY in kg ha^{-1}), daily herbage intake (kg DM $animal^{-1}$) and preference index (%) of six perennial ryegrass cultivars grazed by dairy cows in three preference experiments.

		Cultivar								
Exp.	Item	1	2	3	4	5	6	Mean	s.e.d.	Sign.
1	pre-grazing	2697^{bc}	3082^{d}	2350^{a}	2585^{ab}	2640^{ab}	3026^{cd}	2730	172.3	***
	post-grazing	1303^{a}	1805^{b}	1286^{a}	1388^{ab}	1512^{ab}	1824^{b}	1520	218.5	*
	consumed	1394	1278	1088	1172	1129	1207	1211	126.5	NS
	intake	4.1	3.8	3.2	3.4	3.3	3.5	3.6	0.3	NS
	preference	20.7^{c}	15.1^{ab}	17.3^{b}	17.0^{ab}	15.6^{ab}	14.2^{a}	16.7	1.48	***
2	pre-grazing	2046	2083	2170	2287	2124	2042	2126	98.3	NS
	post-grazing	1155^{a}	1443^{b}	1585^{c}	1458^{bc}	1408^{b}	1362^{b}	1402	75.6	***
	consumed	892^{c}	640^{a}	586^{a}	829^{bc}	716^{ab}	681^{ab}	724	81.5	**
	intake	4.4^{c}	3.1^{a}	2.9^{a}	4.1^{bc}	3.5^{ab}	3.3^{ab}	3.5	0.4	**
	preference	23.0^{c}	14.9^{ab}	12.7^{a}	17.8^{b}	16.2^{b}	15.5^{ab}	16.7	1.51	***
3	pre-grazing	3523^{b}	3684^{b}	2926^{a}	3075^{a}	2993^{a}	3743^{b}	3324	193.0	***
	post-grazing	2600^{b}	2822^{b}	2331^{a}	2430^{a}	2237^{a}	3175^{c}	2599	141.0	***
	consumed	923	862	596	645	756	568	725	182.0	NS
	intake	4.5	4.2	2.9	3.2	3.7	2.8	3.5	0.9	NS
	preference	22.4^{b}	17.5^{ab}	14.7^{a}	14.6^{a}	20.6^{ab}	10.3^{a}	16.7	3.73	*

^{a,b,c,d} means within the same row followed by the same subscript are not significantly different (P>0.05). ***: P<0.001; **: P<0.01; *: P<0.05; NS: Not significant.

The level of consumption in experiment 1 was much higher than in experiments 2 and 3, because the group of dairy cows in one grazing area consisted in experiment 1 of five cows whereas in experiments 2 and 3 it consisted only of three cows. The intake of herbage per cow did not differ significantly in the three experiments. The consumed herbage only differed among cultivars in experiment 2. Cultivar 1 was consumed most in all experiments, although this was only significant in experiment 2. In each of the experiments differences (P>0.05) in preference among cultivars was found. Cultivar 1 was always among the best preferred cultivars, whereas cvs 2 and 6 were always among the least preferred cultivars.

Table 7 Correlation coefficients between preference and chemical components (dry matter (DM), ash, neutral detergent fiber (NDF), lignin (ADL), water soluble carbohydrates (WSC) and crude protein concentration), digestibility (DOM), morphological components (leaf, stem, pseudo stem and dead material) and sward structural components (dry matter yield (DMY), green leaf mass (GLM)) examined in the three preference experiments.

	DM	ASH	NDF	ADL	WSC	CP	DOM	Leaf	stem	Pseudo	dead	DMY	GLM	SSH
Preference	0.471*	-0.528*	-0.482*	-0.027	0.695**	-0.051	0.580*	0.005	0.015	0.030	-0.374	-0.230	-0.216	-0.216
DM		-0.033	-0.747***	0.192	0.481*	0.266	0.622**	0.435	-0.457	0.466	0.309	-0.534*	0.209	-0.006
ASH			-0.075	0.287	-0.619**	0.494*	-0.123	0.565*	-0.652*	0.412	0.161	-0.345	0.588*	-0.079
NDF				-0.186	-0.580*	-0.503*	-0.898***	-0.785***	0.792**	-0.767***	-0.119	0.783***	-0.534*	-0.029
ADL					-0.409	0.584*	0.089	0.263	-0.656*	0.366	0.208	-0.474	-0.065	0.048
WSC						-0.302	0.638**	0.064	0.001	0.092	-0.159	-0.118	0.008	0.095
CP							0.483*	0.742***	-0.890***	0.702**	0.361	-0.731***	0.489*	0.034
DOM								0.666**	-0.729*	0.576*	0.038	-0.741***	0.372	0.177
Leaf									-0.951***	0.888***	0.192	-0.849***	0.815***	-0.054
Stem										-0.969***	-0.344	0.866***	-0.777**	0.200
Pseudo											0.289	-0.854***	0.613**	-0.235
Dead												-0.158	0.147	0.218
DMY													-0.391	0.126
GLM														0.068

***: $P<0.001$; **: $P<0.01$; *: $P<0.05$.

Relations with preference

Preference is a relative principle (van Dyne and Heady, 1965; Westoby, 1974; Chesson, 1983), it is always determined in relation to the other options the animal has. Preference is therefore expressed as a relative figure. To enable comparison of this relative figure to morphological, sward structural and chemical components, every component was expressed as an index value (100 = mean), relative to the mean during each experimental period. The relationship between morphological, sward structural and chemical components and preference is shown in Table 7, the significant effects are presented in Figure 2.

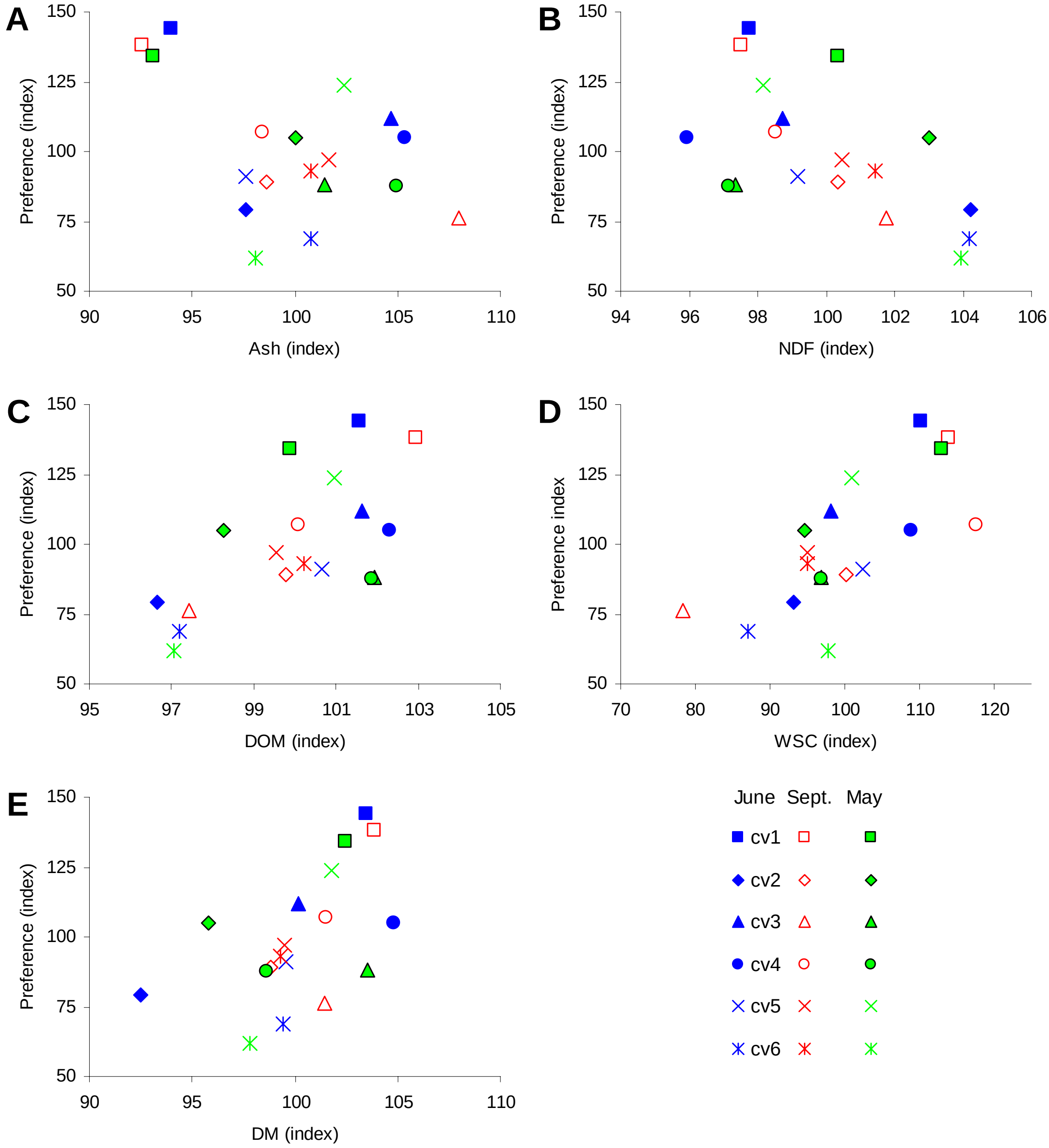

Figure 2 The relation between ash (A), NDF (B), digestibility (C), water soluble carbohydrates (D) and DM concentration (E) expressed as index figure to preference of six cultivars (cv) in July (black), September (open symbols) and May (grey symbols).

Preference was significantly related (*P*<0.05) to five parameters; negatively with ash and NDF and positively with DM, WSC and DOM. WSC showed the highest correlation with preference (Figure 2D). The positive relation between water soluble carbohydrates and preference was found by many others (Heady, 1964; Mayland *et al*., 2000b; Ciavarella *et al*., 2000), but to our knowledge, never in diploid perennial ryegrass consumed by dairy cows. WSC was related to the other parameters that had an effect on preference: ash (R=-0.619, *P*<0.01), NDF (R=-0.580, *P*<0.01), DM (R=0.481, *P*<0.05) and DOM (R=0.638, *P*<0.01). The relation between WSC and preference is, therefore, probably a combined effect of a high WSC concentration and digestibility and a low ash and NDF concentration. These interactions are inevitable and it is therefore wise to focus not on one parameter, but take all into account.Morphology did not have a direct effect on preference in this experiment, but leaf, stem and pseudo stem proportions did have large effects on the NDF concentration, one of the determinants of preference. So, indirectly, they had an effect. DMY and SSH did not have an effect, which is a consequence of the preference index chosen, as its calculation already takes availability (DMY) into account.

Discussion

Selection, preference, palatability

In the strict definition (given by Parsons *et al*., 1994) in this experiment selection by dairy cattle among perennial ryegrass cultivars was observed and no preference. Preference is a decision process in the head of the animal and selection is a function of preference (Stephens and Krebs, 1986). Nevertheless the definition by Hodgson (1979) states: "*Preference is a general term describing the discrimination exerted by animals between areas of the sward or the components of a sward canopy. Preference ranking is the ranking of a series of swards, herbage samples or morphological units, based if possible on the relative intakes determined in free-choice trials*". In the experiments conducted, animals had free choice to eat a certain cultivar. Therefore, we consider the cultivar which was consumed most as the most preferred cultivar. In this experiment, we tried to find factors (chemical and morphological) that were positively or negatively related to preference by dairy cows. This will give breeders for perennial ryegrass the opportunity to select for those traits for preferable cultivars. We avoided the term palatability, because it is too much related to taste and it is unknown whether taste is the driving key behind preference.

Preference needs a signal

Preference behavior is based on the ability of animals to recognize different characteristics. For an animal it is not possible to recognize such things as “cultivars” or “species” and even terms as “ash”, “fibres”, “digestibility” or “soluble sugars” do not exist in the animal perspective and need to be related to the animal’s reality (Arnold, 1981). The process of selection is multidimensional, and certain characteristics can be interrelated. The researcher can just give associations with preference, and show in specific experiments which choices animals make. Factors important for preference could be morphology, taste, color, texture or odor, etc.

Sward surface height and morphology

In vegetative swards cattle tend to select for taller swards (Bailey, 1995; Dumont *et al.*, 1995; Griffiths *et al.*, 2003). In this experiment, however, only in experiment 2 the sward was completely vegetative, and then no differences among cultivars were found for SSH. In experiments 1 and 3, the sward was partly in a reproductive stage. No selection against cultivars with a high percentage of stems was found. Cattle can, however, graze selectively between stems and leaves (van Dyne and Heady, 1965) and generally tend to select for the vegetative patches, when the sward height is 18 cm (Dumont *et al.*, 1995). In this experiment, cultivars were in different stages of their reproductive phase. The intermediate-heading cultivars (1, 2 and 6) had more inflorescences, whereas the late-heading cultivars had more pseudo stem. This, however, did not have an effect on preference by dairy cows.

Chemical composition

Chemical composition of forage is one of the major important and best studied factors in preference behavior (Heady, 1964). In this experiment, negative effects on preference behavior were found for ash and NDF and positive effects were found for DM, DOM and WSC among cultivars of perennial ryegrass. The ash concentration or minerals usually shows a positive relation with preference (Westoby, 1974; Belovsky, 1978). In contrast, in this study a negative relation is found, this might be explained by the fact that cultivar 1 had always the highest WSC concentration and had always a low ash concentration. The ash fraction does not exist on a molecular level in the plant, but can be related to sodium or potassium salts as trigger for preference (Belovsky, 1978; Arnold, 1981). No specific data were available for these fractions.

Digestibility was positively associated with preference, whereas NDF concentration was negatively associated with preference. The two parameters were strongly (P<0.01) interrelated, and there was negative relation between the NDF concentration and digestibility in each of the experiments. Heady (1964) reported a negative relation between cell wall constituents (lignin and crude fibre) and preference behavior of large herbivores, mainly in association with positive relations with sugars, proteins and fats. Wright and Illius (1995) linked the aversion of herbivores to species with a higher NDF concentration to higher energy expenditure for grazing and chewing. To minimize costs and maximize benefits (Illius *et al*., 1995) animals should avoid species or cultivars with a high NDF concentration. The same principle is valid for DM concentration; animals minimize their energy expenditure per bite, when selecting cultivars with a higher DM concentration.

Ciavarella *et al*. (2000) directly related preference to WSC concentration. A pasture that mainly consisted of *Phalaris aquatica* was partly shaded, causing a distinct decrease in WSC concentration. When sheep were given the choice between the shaded and the unshaded part, sheep consumed 2.6 times more of the unshaded (high WSC) part of the pasture. Researchers concluded that WSC played a large role in the diet selection of large herbivores and stated that it might be useful to select for high WSC cultivars. This was supported by Mayland *et al*. (2000b), who described an experiment in which cows could select between eight tall fescue cultivars. They found that cattle chose for an energy-dense diet and selected cultivars with high non-structural carbohydrate levels. In a large examination of 24 perennial ryegrass cultivars in Ireland, clear differences were found among cultivars (O'Riordan *et al*., 1998). No chemical analyses were carried out, but tetraploids were more preferred than diploids. Tetraploids have larger a cell content than diploids and therefore a higher digestibility, crude protein and WSC concentration (Smith *et al*., 2001b). In this experiment with diploid cultivars, in all experiments (July, September and May) a significant (P<0.001) positive effect of WSC on preference was found. This might be related to nutritional wisdom (Provenza, 1996), i.e., ruminants generally tend to select a constant ratio of protein to energy (Wang and Provenza, 1996). In this experiment, all perennial ryegrass cultivars had a high nitrogen concentration (172-203 g kg^{-1} DM). This would then result in the preference for energy dense grasses (read: high WSC grasses). However, under lower nitrogen fertilization levels, the preference might change. Several studies (Heady, 1964; Tolkamp *et al*., 1998) have also found a positive effect of protein on preference of forages.

Cows can taste the four primary flavors sweet, salt, sour and bitter (Chiy and Phillips, 1999) and seem to prefer sweet diets (Nombekela *et al*., 1994). However, WSC in itself cannot be tasted (Arnold, 1981), but is a collective noun of all mono-, di-, tri- or polysaccharides, each of which will give a different sweetness response. Detailed information on saccharides in forages as a key to palatability is still lacking. Sucrose is found to be preferred in concentrate feeds (Chiy and Phillips, 1999; Nombekela *et al*., 1995). In perennial ryegrass, however, sucrose is only a small amount of the total WSC fraction (15% of WSC), fructans form the largest part (60% of WSC) (McGrath, 1988). Mayland *et al*. (2000b) found a relationship between total WSC and preference, but could not find significant relation with a single saccharide (Mayland *et al*., 2000a). Arnold (1981) comments that despite reservations about considering a gross indicator such as WSC, it may still be a useful trait for breeding for preference in absence of better guidelines, and we agree fully.

Implications

Dairy cows selected among six diploid perennial ryegrass cultivars. Preference was not related to morphological parameters, but was positively related to the water soluble carbohydrates and dry matter concentration and the level of digestibility and negatively related to ash and fibre (NDF) concentration. There are options for grass breeders and farmers to select for cultivars preferred by dairy cows.

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Chapter 6

General discussion

Limitations of this study

Choice of cultivars

The perennial ryegrass cultivar was the most important factor in this study, and the conclusions of this study are determined by the choice of cultivars. The ultimate aim of this study was to see whether there is scope to improve herbage intake of dairy cows by the choice of cultivar. Trials were conducted in the Netherlands and to ensure the use of adapted germplasm, only cultivars were chosen that were listed at the Dutch National List of Recommended varieties (Ebskamp and Bonthuis, 1998). At the start of the experiment in 1999, approximately 35 cultivars were available at the National List of varieties. Three criteria were used to select the cultivars used in the experiments; heading type, digestibility and water soluble carbohydrates concentration.

Data were collected from 17 intermediate- and late-heading cultivars, from experiments by Barenburg B.V. and the official Dutch grass cultivar testing body. A larger collection of varieties for screening would have permitted a more accurate choice of cultivars. However, these data were not available and the 17 screened varieties form a proper reflection of the cultivars used in the Netherlands (Jan Visscher, personal communication)

Six cultivars, Abergold, Agri, Respect, Barnhem, Barezane and Herbie, were chosen, because they represent contrasting cultivars according to the schedule presented in Table 1. The data from the 17 cultivars were not analyzed in one experiment, but in separated experiments. The selection could have been more powerful if the data had been collected in one complete experiment. Nevertheless the six cultivars did differ in all four years for the selection criteria.

In the stall feeding experiment in 2000 and 2001 (Tas, 2005) the six cultivars were examined in three 2-week periods (Chapter 3), during surrounding periods (four 2-week periods) cultivars Barlet and Magella were fed (Table 1). The four cultivars used in the grazing experiment in 2002 and 2003 were chosen as the divergent within the eight cultivars used in the stall-feeding experiments.

Table 1 The cultivars selection schedule.

Cultivar	Heading Type	DOM[1]	WSC[2]
Abergold	Intermediate	+	++
Agri	Intermediate	+/-	+
Respect	Intermediate	-	--
Barnhem	Late	+	++
Barezane	Late	+/-	--
Herbie	Late	-	--
Barlet	Intermediate	++	+/-
Magella	Intermediate	--	+/-

[1] DOM: Digestible Organic Matter

[2] WSC: Water Soluble Carbohydrates

Near infrared reflectance spectroscopy (NIRS)

The samples in all grazing experiments (Chapters 3, 4 and 5) were analyzed for their chemical composition using Near Infrared Reflectance Spectroscopy. About 100 samples were selected during the 4 years period in which also wet chemistry was performed. This set was used to validate the equation lines used by NIRS to estimate the chemical composition. The results of this validation experiment are presented in Figure 1.

The validation showed that the prediction of crude protein and water soluble carbohydrates was quite good (R^2>0.95), with only a small bias from the x = y line. The prediction of ash, NDF and Digestibility by NIRS was moderate (R^2 = 0.66-0.82). The prediction could improve within a larger range of samples. The samples used in this study were 100 samples from pure perennial ryegrass taken at the same location and in the same stage of growth. However, the equation lines for these components need improvement when breeding programmes want to use them in the future.

Lignin concentration was estimated poorly in 2000 and 2001 (R^2<0.2), but in 2002 a high and significant correlation was found (R^2 = 0.84, P>0.001). The poor correlation coefficient in 2000 was probably due to a very small range in ADL concentrations within the 26 samples (6.6 g kg^{-1} DM), whereas in 2001 the poor predictions were due to first 14 days in this experiment, in which samples with a high proportion of stems were examined (Chapter 2). The NIRS was not able to accurately predict ADL concentration in these samples, whereas it preformed better in the samples with vegetative material only.

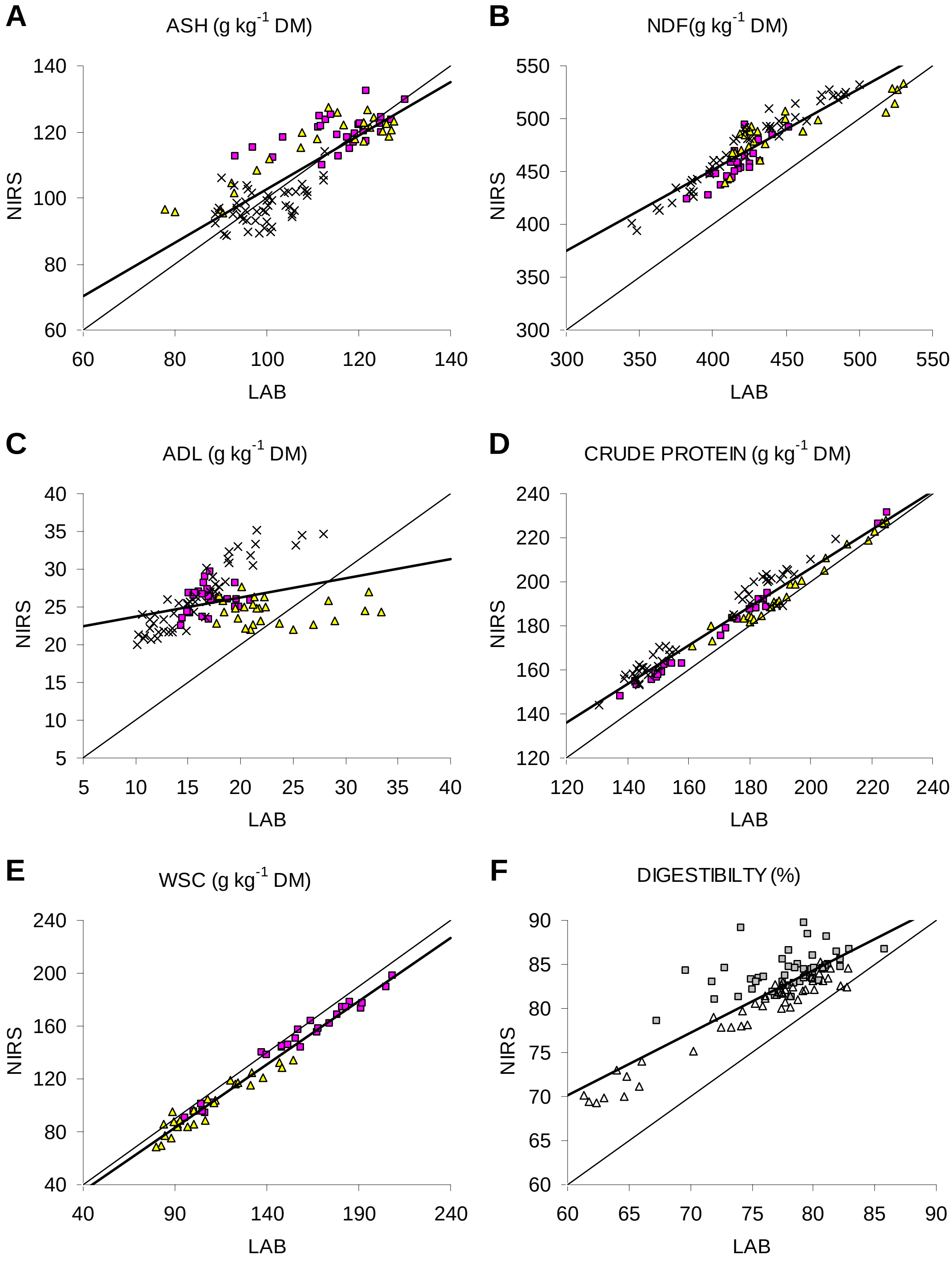

Figure 1 Chemical composition and digestibility estimated by NIRS and the wet chemical laboratory analysis. (A) ash, 500°C (ISO 5984), 3 h.; (B) NDF van Soest (Goelema *et al.*, 1998); (C) ADL van Soest (Goelema *et al.*, 1998); (D) crude protein, Kjeldahl (ISO 5983); (E) Water Soluble Carbohydrates (van Vuuren, 1993); (F) Digestibility (DeBoever *et al.*, 1996). NIRS-equations were obtained from Centre de Recherches Agronomiques de Gembloux in Belgium (Biston *et al.*, 1998).

Grazing season: July, August, September

The grazing season in the Netherlands starts approximately mid April and continues to the end of September. Experiments in this study were mainly conducted in July, August and the beginning of September. The period of booting and heading, normally from mid-May to mid-June was intentionally avoided, because the cultivars were to be tested under vegetative conditions. However, this was not always the case, see Chapter 2.

Soil type

All experiments in this study were conducted on clay soils, and can therefore not directly represent all dairy systems. However, improvements in forage nutritional value are generally consistent over environments, management and harvesting systems (Wilman *et al*., 1992; Casler, 1998).

Measuring individual herbage intake during grazing

The total herbage intake of an animal determines the intake of every nutritional component during grazing, therefore measuring herbage intake of individual animals during grazing is essential for the understanding of the nutritional processes.

In Chapter 3 the use of n-alkanes was promoted to be used in experiments in which the individual herbage intake of animals is to be estimated. This method was less variable and closer related to the expected intake based on net energy requirements of the animals. The sward cutting method was found to be too variable in experiments with individually kept animals.

In Chapter 5 the sward cutting method was used again. However, this time the method was applied in a selection experiment with small groups of cattle. In selection experiments among different species, the n-alkanes method can also be used (Dove *et al*., 2000; Martins *et al*., 2002). The intake of different plant species in the diet is then estimated from the pattern of n-alkanes in the faeces and in the herbage, using least square procedures (Dove and Moore, 1995; Martins *et al*., 2002). To obtain accurate results, there is a need for a distinct difference in n-alkane pattern among components of the diet offered. This could be done artificially by spraying the pasture with an even-chain alkane (Ciavarella *et al*., 2000) or by differences in natural occurring n-alkanes. Some differences were found among the cultivars in the grazing experiment in Chapter 4 (Table 2), but these differences were too small to be used in a selection experiment.

Table 2 Composition of n-alkanes (mg kg^{-1} DM) and standard errors (s.e.) in four cultivars of perennial ryegrass in 2002 and 2003. (2002 n=48; 2003 n=44).

Year	Cultivar	n-alkanes							
		C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{35}
2000	Abergold	16.9	7.4	60.8	7.9	135.4	14.7	145.6	44.7
	s.e.	0.9	1.3	0.8	0.5	4.1	1.5	4.9	2.2
	Agri	17.6	9.6	71.2	8.5	163.0	13.6	133.2	39.0
	s.e.	1.3	0.9	2.5	0.7	5.0	1.3	4.4	1.9
	Barezane	20.6	9.4	61.8	7.4	132.3	11.9	135.1	39.7
	s.e.	1.8	0.8	1.7	0.5	1.8	1.2	4.0	1.7
	Barnhem	16.6	8.1	63.0	7.8	144.8	11.4	120.6	33.8
	s.e.	0.9	0.9	1.4	0.4	4.5	1.0	3.2	1.9
2001	Abergold	25.4	3.5	102.8	12.4	211.5	12.4	178.9	27.3
	s.e.	1.2	0.7	3.0	0.9	6.3	0.7	4.2	0.6
	Agri	25.8	3.5	118.0	12.6	270.2	12.2	166.9	21.0
	s.e.	1.6	0.7	4.0	0.7	10.6	0.5	6.4	1.0
	Barezane	29.0	4.7	111.5	11.1	224.2	12.6	171.9	25.7
	s.e.	1.8	1.0	4.8	1.1	10.1	0.7	4.3	1.4
	Barnhem	26.6	4.7	100.3	11.7	212.0	11.9	139.1	17.8
	s.e.	3.0	0.9	6.1	0.6	8.4	0.8	4.2	1.2
Sign.	Year	0.000	0.000	0.000	0.000	0.000	0.392	0.000	0.000
	Cv	0.118	0.359	0.000	0.283	0.000	0.281	0.000	0.000
	Y. x Cv	0.951	0.506	0.275	0.954	0.022	0.411	0.181	0.607

The sward cutting method remained as the only option in the selection experiments. When used in a small group of dairy cows, the method gave comparable results over three experimental periods, however, there were daily fluctuations. The estimated intake of 21.3±1.0 kg DM animal^{-1} d^{-1} seemed rather high. It might be that cows ingest more herbage in a grazing situation where they do have more choice or that cows in a situation with a higher herbage allowance, ingest more herbage (Peyraud *et al.*, 2004) or that the method gives a slight overestimation of the herbage intake (Meijs, 1981).

Breeding perennial ryegrass for improved nutritional value

Herbage for high producing dairy cows has a limited nutritional value, e.g. a dairy cow cannot sustain milk production higher than 28 kg d^{-1} (van Vuuren, 1993). One of the options to improve herbage quality is by breeding. Grass breeding has focused until now mainly on agronomical traits. In Europe, most of the breeding programs are primarily yield-oriented and mainly based on cutting regimes (Gilliland *et al.*, 2002; Bonthuis *et al.*, 2004). From a dairy farmer's point of view, however, not only the quantitative herbage yield, but also the conversion from grass into milk is very important. There is a need for cultivars with a high nutritional value, which can improve milk yields from pasture and more effort could have been put in the breeding of improved herbage nutritional value, especially under grazing. Breeding efforts to improve herbage nutritional value fall apart into two categories (Casler, 1998):

1. Breeding against anti nutritional factors, e.g. chemical toxicities, mineral imbalances and physical characteristics.

2. Breeding in favour of increased nutrient (energy and/or protein) intake and availability to the ruminant.

In this study, several characteristics of perennial ryegrass cultivars were examined, among others morphology and sward structural parameters, chemical and physical parameters and disease resistance. The first category contains lower stem fraction, lower fibre and lignin concentrations, and lower tensile strength and the second category contains higher leaf fraction, higher digestibility (DOM), higher dry matter content, water soluble carbohydrates (WSC) and protein concentration (CP).

Variation among Perennial ryegrass cultivars

Yield

As expected from the National List, cultivars differed for yield expressed as dry matter yield. The variation between the cultivars was in every year over 10% (Chapters 2 and 3) as shown in Figure 2. However, the ranking of the cultivars was not consistent, especially cultivar Abergold had very variable yields. In the first season after sowing (2000 and 2002) it yielded highest, but in the second season after sowing (2001 and 2003), both years with drier weather conditions,

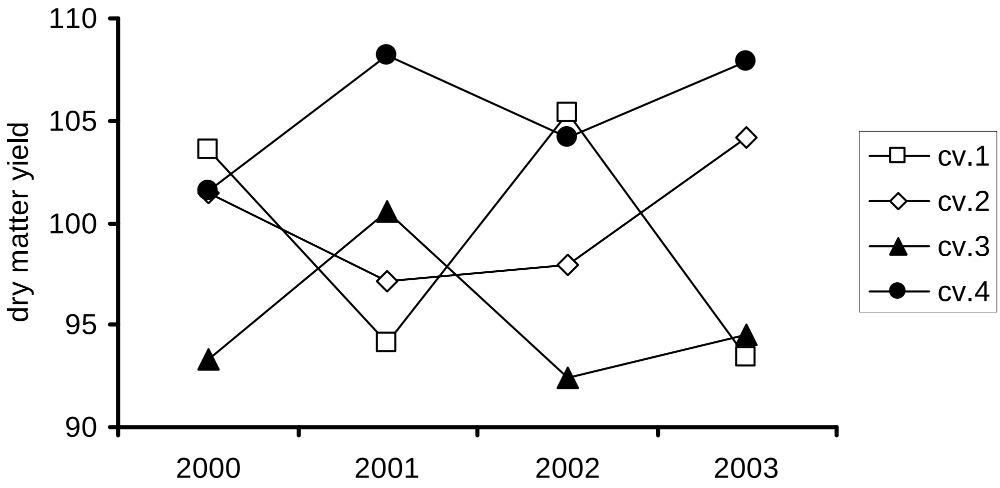

Figure 2 The variation in dry matter yield estimated expressed as index figure to the annual mean (100) of four perennial ryegrass cultivars (1. Abergold, 2. Agri, 3. Barezane and 4. Barnhem) during the four years of this study.

it yielded lower (P<0.01) than average. Cultivar Barezane behaved in an opposite way, yielding higher in the second year after sowing. However, it was always among the lowest yielding cultivars and was removed from the Dutch National List in 2000 (Ebskamp and Bonthuis, 2000). Cultivar Barnhem had a rather consistent yield that was always among the highest yielding cultivars. The relatively large differences between the cultivars gives breeders still options to select for higher dry matter yields in the future.

Yield expressed as total digestible yield (kg DOM ha^{-1}) or total yield of Nitrogen (kg N ha^{-1}), showed a similar pattern as total DM yield, because the variation in DM yield exceeded the cultivar variation in DOM (g kg^{-1} DM) and crude protein concentration (see below).

Sward structure and morphology

All experiments in this study showed clear differences among cultivars for several sward structural and morphological characteristics. This study showed that they also differed in sward surface height and green leaf mass (Chapters 2 and 3), two parameters also mentioned as potential characteristics to improve intake rate during grazing (Laca *et al*., 1992; Hazard *et al*., 1998).

Differences among cultivars were also found in the morphological parameters in that leaf blade proportion and (pseudo)-stem proportion differed among cultivars under cutting (Chapter 2) in both years. Under grazing (Chapter 4) especially the pseudostem fraction differed among cultivars, but no cultivar effect was found in the leaf blade proportion. The proportion of dead material also differed among cultivars, but this was associated with the severity of crown rust infestation. The absolute level of dead material was, however, more associated with the water availability.

Chemical properties

This study showed clearly that diploid perennial ryegrass cultivars did differ in several chemical properties. The variation among cultivars was in all years approximately of the same magnitude. The largest difference in chemical composition among the examined cultivars in this study was found in WSC concentration (20-40%) (Figure 3A).

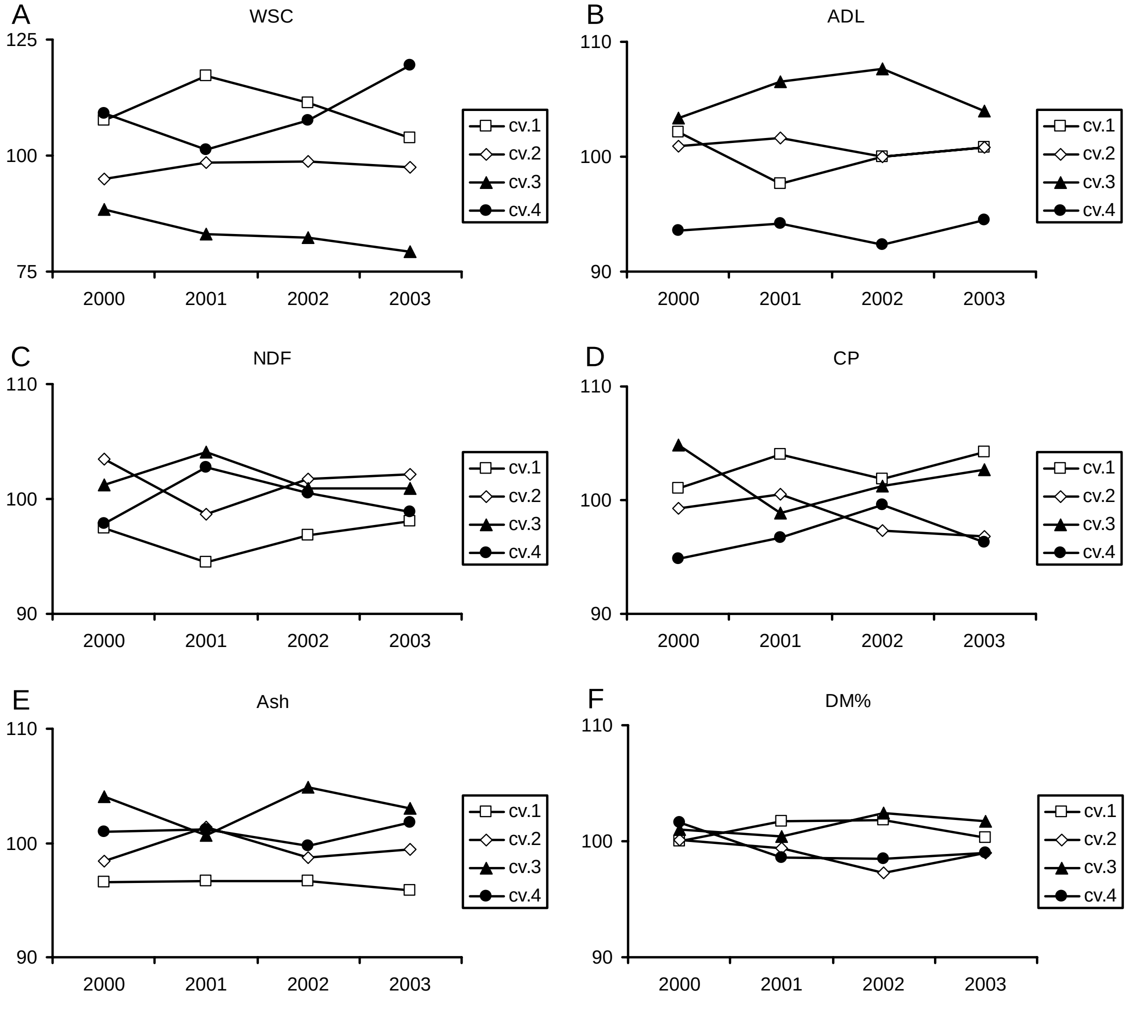

Figure 3 The variation in (A) water soluble carbohydrates (WSC); (B) acid detergent lignin (ADL); (C) neutral detergent fibre (NDF); (D) crude protein (CP); (E) ash and (F) dry matter concentration (DM%) expressed as index figure to the annual mean (100) of four perennial ryegrass cultivars (1. Abergold, 2. Agri, 3. Barezane and 4. Barnhem) during the four years of this study.

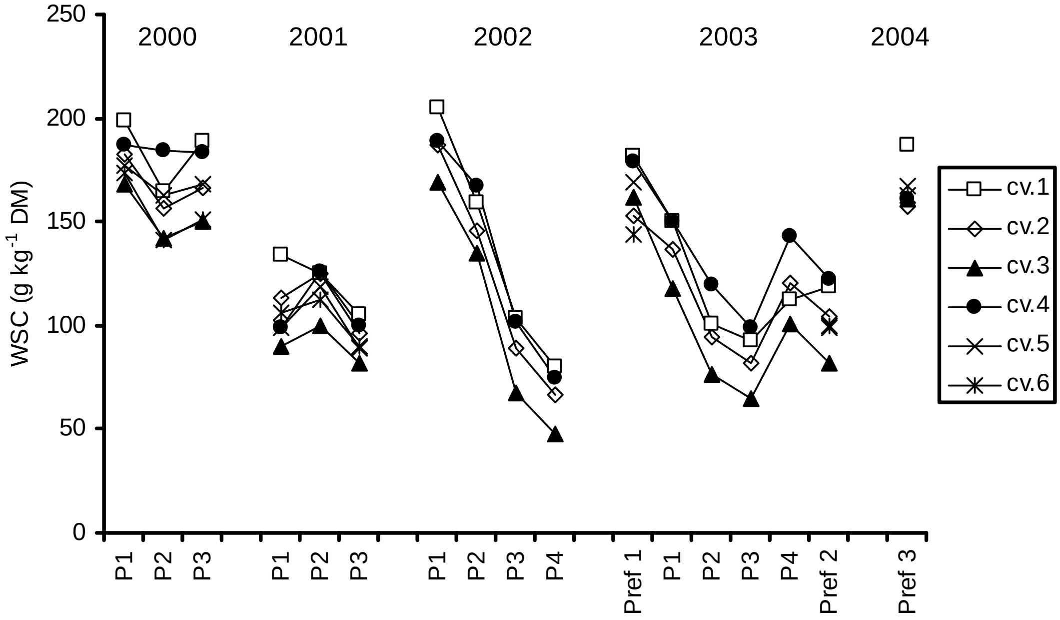

Figure 4 WSC concentration (g kg^{-1} DM) of six perennial ryegrass cultivars (1. Abergold, 2. Agri, 3. Barezane, 4. Barnhem, 5. Herbie and 6. Respect) during the experimental years of this study.

Gilliland *et al.* (2002), who examined six diploid perennial ryegrass cultivars, also reported a variation among cultivars of 20% for WSC concentration averaged over the year. They found the largest variation in July and August, the same months in which this experiment was performed. There is a general decreasing trend in WSC trough the season, as shown in Figure 4. The reduction of WSC concentration in autumn has several reasons. At first, the reduced solar radiation will decrease photosynthetic activity, which results in a reduced primary production of non-structural carbohydrates. It has been shown (Fulkerson and Donaghy, 2001) that WSC concentrations are closely related with hours of sunlight. Furthermore, pasture plants respire and use the produced WSC and if this is inadequate (e.g. during nighttime or cloudy weather) also the non-structural carbohydrates reserves are used (Fulkerson and Donaghy, 2001). The higher night temperatures in autumn will induce respiration during nighttime (McGrath, 1988). The altering source and sink relations deplete WSC reserves and hence the WSC concentration in the consumed herbage.

The acid detergent lignin (ADL) concentration also showed a significant variation among cultivars, but lower (7-15%). The variation in WSC and ADL among cultivars was rather consistent throughout the four experimental years of this study (Figures 3A and B), which gives good perspectives for future breeding (Casler, 1998; Wilkins and Humphreys, 2003). The variation in other chemical traits: NDF, CP, ash and DM concentration, was lower than 10% and did not show a very consistent picture (Figure 3C-F) during the four experimental years. Therefore, it will be more difficult for breeders to use these as traits in future breeding programs.

Physical characteristics

Toughness of leaves and their component tissues is of biological significance, playing a major role in the resistance of leaves to herbivores, pathogens and other physical damage (Choong *et al.*, 1992). Tough leaves could be avoided by herbivores, because of their energetic expenses, but also because of a lower palatability (Wright and Vincent, 1996). Naturally this information is of use to the plant breeder who can select for the important characteristics (Vincent, 1982). Of more fundamental importance is the fact that from a mechanical point of view, grass is probably the simplest of all plants and is therefore a good starting point to understand the mechanical properties of plants (Vincent, 1982).

In this study, the cultivars were examined for their tensile breaking force (N), as described by Henry *et al.* (1997), but using the conventional clamps as described by Theron and de Booysen (1968) and the Zwick apparatus (Z2.5). The method is graphically described in Figure 5.

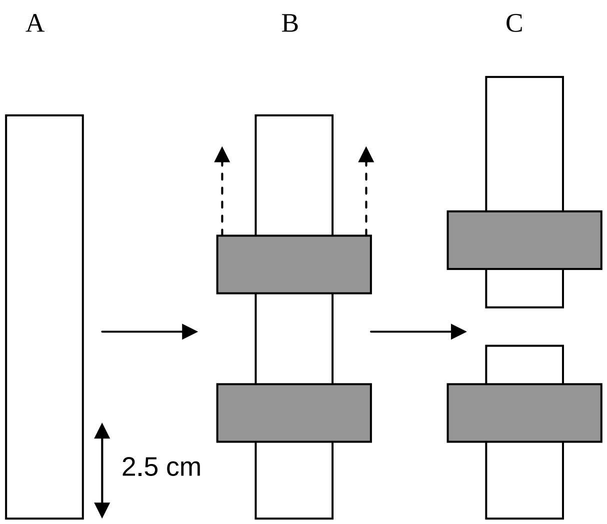

Figure 5 Method of measuring tensile breaking force.

A. An undamaged leaf of 15 to 20 cm is selected.
B. The leaf is tightened in clamps, at 2.5 cm from the ligule (LE distance: 10 mm) and the upper clamp is moving upwards (10 mm min^{-1}).
C. The computer measures force deformation, energy and the maximal breaking force.

In three years the maximal breaking force was measured and the results are shown in Table 2. Abergold had in all years the lowest and Agri had always the highest breaking force. Tensile strength parameters might be better expressed as N mm^{-2} (Vincent, 1982, 1991; MacAdam and Mayland, 2003), but this study, the leaf thickness could not be measured and leaf tensile strength was related to the width of the leaf (P<0.001, R^2 =0.72). Further research should investigate whether the leaf tensile properties also differ among cultivars when expressed in relation to their transverse leaf blade area.

Table 2 The maximum breaking force (N), tested among six cultivars in 2000, six cultivars in 2001 and four cultivars in 2002.

Year	*Cultivar*						*s.e.d.*
	Abergold	Agri	Barezane	Barnhem	Herbie	Respect	
2000	5.6	7.1	6.0	6.0	6.3	6.6	0.45
2001	6.5^{a}	7.8^{d}	6.9^{abc}	6.7^{ab}	7.1^{bc}	7.4^{cd}	0.27
2002	7.8	8.5	8.3	8.6			0.36

[a,b,c] means within the same row followed by the same subscript are not significantly different (P>0.05).

Table 3 Proportion of leaves severely infested by crown rust (*Puccinia coronata* sp. *lolii*) of eight cultivars used in this study, in 2000 in September only, in 2002 and in 2003 July and August.

Year	*Cultivar*						*s.e.d*
	Abergold	Agri	Barezane	Barnhem	Herbie	Respect	
2000	9.5^{a}	14.0^{b}	20.1^{c}	5.3^{a}	17.2^{bc}	15.4^{b}	6.6
2001	No data recorded*						
2002	13.8^{a}	27.1^{b}	40.9^{c}	11.8^{a}			3.7
2003	7.8^{a}	12.1^{a}	21.9^{b}	4.9^{a}			4.4

*In 2001, no data were recorded. The visual impression was that no crown rust infestation occurred.

[a,b,c] means within the same row followed by the same subscript are not significantly different (P>0.05).

Disease resistance

Infestation of crown rust mainly takes places during humid, warm weather and in situations of reduced growth. In autumn, the growing conditions for the fungi are favourable. The effects of crown rust on palatability are not well described in the literature, but it is generally accepted that animals do not like to eat infested herbage, because of a bitter taste (Potter, 1987; Tas, 2005). As shown in Table 3, there was large variation in resistance among diploid cultivars of perennial ryegrass. In all experimental years, the two newest cultivars (Abergold and Barnhem) were least infested by the fungi, whereas the oldest cultivar Barezane was most infested (Table 3). Clearly there was an improvement of crown rust resistance in the past, but resistance might further be improved by using molecular marker techniques (Reheul and Ghesquière, 1996; Smith *et al.*, 1998; Kimbeng, 1999). In this study, no complete resistance was found, even in the most resistant cultivar spots of crown rust were found, especially in periods of reduced growth rates (in autumn or dry conditions 2002 and 2003). In the past, cultivars with a high WSC concentration were reported to be more susceptible to crown rust infestations (Smith *et al.*, 1998). However, this was not found in this study, where the newer cultivars (Abergold and Barnhem) both had a higher WSC concentration and were less susceptible to crown rust.

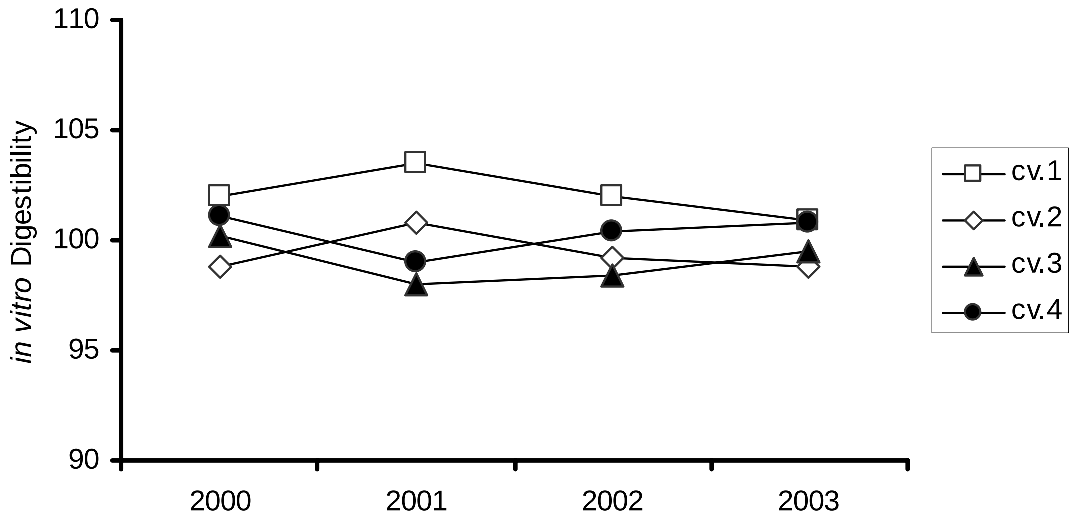

Figure 6 The variation in *in vitro* digestibility estimated by NIRS expressed as index figure to the annual mean (100) of four perennial ryegrass cultivars (1. Abergold, 2. Agri, 3. Barezane and 4. Barnhem) during the four years of this study.

Digestibility

The cultivars used in the experiments described before did differ significantly and consistently for *in vitro* digestibility estimated by NIRS as shown in Figure 6. The digestibility had a variation of between 2 to 6% among cultivars. A small variation among cultivars in 2000 in *in vitro* digestibility could not be confirmed *in vivo* in a stall feeding situation (Tas, 2005). The small number of animals used in the *in vivo* experiments might be an explanation for this. In 2001, when the variation *in vitro* was 6%, a variation of 4% among cultivars was demonstrated *in vivo*. Cultivars Abergold and Barnhem had the highest *in vivo* digestibility, whereas cultivars Agri and Barezane were lowest in *in vivo* digestibility (Tas, 2005). The *in vivo* measurements could only be performed during stall feeding and no figures of the grazing experiments (2002 and 2003) can be presented. Beerepoot and Agnew (1997) also found consistent differences in *in vitro* digestibility between two diploid perennial ryegrass cultivars (Barlet and Magella). *In vitro* digestibility is considered by some breeders to be not important in perennial ryegrass (Boller *et al.*, 1994), whereas others (Ingram and Beerepoot, 1994) consider the digestibility value of great value. The potential financial impact of small improvements could be large, e.g. a 5% increase in herbage digestibility could lead for an average farmer up to 100 euro ha^{-1}. This premium is achieved by substitution of concentrate feed for grass (Vellinga and van Loo, 1994). The effects, however, on animal performance remained limited.

Cultivar effects on the animal

Herbage intake and palatability

Cultivars of perennial ryegrass varied in different traits considered important for intake. Cultivar effects in sward morphology did not have effects on the herbage intake of the dairy cows during stall feeding (Tas, 2005), restricted grazing (Chapter 4) or free choice grazing (Chapter 5). However, DMY did have a significant effect (Tas, 2005; Chapter 4). Yield can have positive and negative effects on herbage intake. In the stall feeding experiment, the high yielding cultivars were consumed less (Figure 7A), but during the grazing experiment in 2002 (chapter 4), the high yielding cultivars were consumed more (Figure 7B). This difference can be explained by the different feeding systems. In a stall feeding situation, the dairy cows could consume a

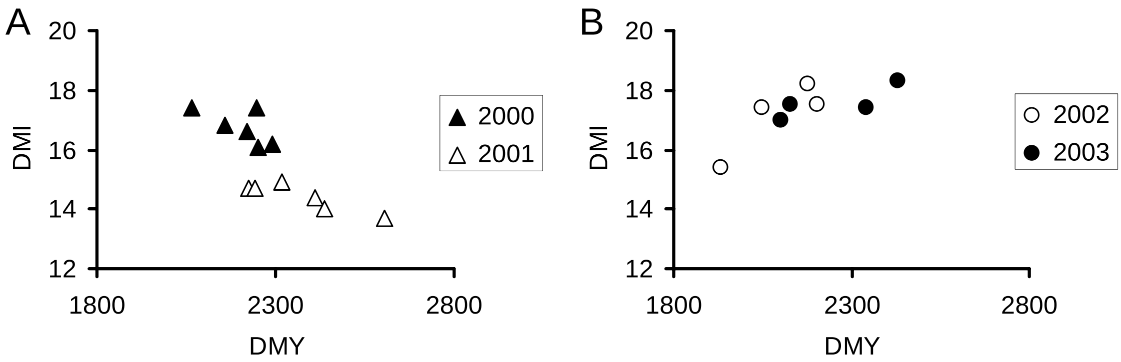

Figure 7 The relation between dry matter yield (DMY) (kg ha^{-1}) and herbage intake (DMI) (kg day^{-1}) during stall feeding (A) and grazing (B).

homogeneously cut herbage, which was *ad libitum* available and the quality of the sward was the most important factor (Minson, 1987). Quality, e.g. digestibility, decreases with a higher DMY and therefore the consumption of herbage will decrease. In a grazing situation, dry matter yield affected also herbage allowance and played therefore a different role. The animals were offered more herbage to select from, and intake was higher in cultivars with a higher DMY. This mechanism will continue until the animal is saturated, and it seemed that especially above 2000 kg ha^{-1} the herbage intake does not increase anymore (Figure 7B) (Lantinga, 1985; Minson, 1987).

The diet of a dairy cow can be very general and the variation among cultivars of perennial ryegrass found in several characteristics was clearly insufficient to have a large effect on dairy cows' performance. The variation in chemical composition did not lead to a higher herbage intake by dairy cows when fed a single cultivar, neither in a stall feeding (Tas, 2005) nor in a grazing situation (Chapter 4). Only in 2002, a significant cultivar effect on herbage intake was observed, but this was probably due to a severe crown rust infestation in the most susceptible cultivar (Barezane). The reduction of herbage intake could, however, not clearly be pinpointed to crown rust alone. Cultivar Barezane had in the same year also a lower SSH, DMY, GLM and WSC concentration and a higher ADL concentration than the other cultivars. In 2003, however, the same cultivar had again a lower SSH, DMY, GLM and WSC concentration and a higher ADL concentration, but in this year the crown rust infestation was less severe and no cultivar effects on herbage intake were found. The main cause of the reduced herbage intake is therefore thought to be the crown rust infestation.

It is concluded that the possibilities to enhance dry matter intake in a grazing situation through breeding can mainly be achieved by a better crown rust resistance and a higher dry matter production. A higher dry matter production would give more opportunities to select and might, therefore, enhance herbage intake (Meijs, 1981).

In the experiment described in Chapter 5, the dairy cows were offered a choice between the six cultivars described earlier. In the choice situation, a clear distinction was observed among the offered cultivars and it was concluded that cows can distinguish among diploid perennial ryegrass cultivars. Dairy cows showed a clear preference for the cultivars with an increased WSC concentration and digestibility and a decreased NDF and ash concentration.

So, if the herbage of a dairy cow consisted only of one single cultivar, voluntary intake was not influenced by quality parameters, such as digestibility, WSC and NDF concentration, whereas in a free choice situation quality parameters did play a decisive role in the selection process. The trade-offs in the feeding behavior of animals may be different when they are given access to one food rather than more foods as a choice (Kyriazakis and Oldham, 1997). The decision process in the head of the cow remains unknown. Literature suggests that WSC has an effect of increased palatability (Heady, 1964; Jones and Roberts, 1991; Mayland *et al.*, 2000b). Others (van Vuuren, 1993; Taweel, 2004) point to the effect that feeding cultivars with elevated WSC concentration lowers the NH_3-N concentration in the rumen. The NH_3-N concentration in the rumen could play a role in long term diet selection (Kyriazakis and Oldham, 1997). Still, the question remains, why dairy cows do select for high sugar cultivars. This needs further examination.

Rumen kinetics

A few options to increase herbage intake and animal performance were investigated. A faster rumen degradability of the cell walls in the herbage might lead to a faster passage and a higher intake. However, the variation in NDF degradability among the investigated cultivars remained limited, only 1% (Taweel, 2004).

A higher energy concentration in the rumen can lead to more efficient nitrogen utilization (van Vuuren, 1993; Lee *et al.*, 2002). This can be achieved by offering cultivars of perennial ryegrass with an enhanced WSC concentration (Miller *et al.*, 2001; Taweel, 2004; Tas, 2005; Chapter 4). The NH_3 concentration in the rumen was significantly lower when feeding the high sugar varieties, and the rumen fermentation shifted slightly towards propionate at the expense of acetate (Taweel, 2004; Tas, 2005).

Feeding ruminants a high level of WSC in the herbage might lead to a lower pH in the rumen (Lantinga, 1985; Obara *et al*., 1991). However, in this study, feeding of cultivars with an enhanced WSC concentration (difference of 40 g kg^{-1} DM) did not lead to a lower pH in the rumen (Taweel, 2004). At higher differential magnitude, there might be an effect, although Lee *et al*. (2002) did not observe any reduction in pH, because of feeding high WSC grass (with 82 g kg^{-1} DM differential magnitude from the low sugar grass).

Milk production

The ultimate goal of the project was to breed perennial ryegrass cultivars, that would enhance milk production when fed to dairy cows. In this study, the variation in herbage quality among commercial diploid cultivars on the National List did exist, but differences were too small to induce effects on milk production.

The effects on milk composition were limited. The urea concentration in the milk was lower in the group fed with cultivars with increased WSC concentration (Taweel, 2004; Tas, 2005), due to a lower NH_3 concentration in the rumen and consequently a lower urea concentration in the blood.

General conclusions

- There are differences among commercial diploid cultivars of perennial ryegrass for yield, sward parameters and morphological characteristics.

- There are clear differences among commercial diploid cultivars of perennial ryegrass for the concentration of water soluble carbohydrates (WSC) and lignin (ADL). Differences in other chemical components, crude protein, neutral detergent fibre (NDF) and ash were minor.

- The effect of cultivar on herbage intake of dairy cows is limited when animals are restricted to grazing one single cultivar.

- When dairy cows can select between cultivars, there is an effect of cultivar on herbage intake. Dairy cows prefer cultivars with a high WSC concentration and high digestibility, and select against cultivars with high NDF and ash concentration.

- For estimating herbage intake of grazing dairy cows on perennial ryegrass pastures, the n-alkanes method is the best option. However, in selection experiments with several cultivars of perennial ryegrass, the sward cutting method will be the only option due to the lack of variation in the n-alkanes pattern between cultivars.

Implications of this study

Measuring individual herbage intake during grazing

The total herbage intake of an animal determines the intake of every nutritional component during grazing, therefore measuring herbage intake of individual animals during grazing is essential for the scientific understanding of the nutritional processes.

In Chapter 3 of this study, the use of n-alkanes was promoted for use in experiments in which the individual herbage intake of animals is to be estimated. This method was less variable and values were closer related to the expected intake based on net energy requirements. The sward cutting method was found to be too variable in experiments with individually kept animals.

More efficient grazing in autumn

The experience of farmers is that grazing in late summer and autumn is less efficient, due to lower palatability and decreased herbage intake (Holshof, personal communication, ASG-WUR). The main problems among others could be due to the infestation of crown rust (*Puccinia coronata* f.sp. *lolii*), which reduces the palatability (Potter, 1987), and the reduced concentration of water soluble carbohydrates in the herbage, which also reduces palatability (Chapter 5). This decreased palatability in autumn could partly be overcome by using highly crown rust resistant cultivars and high sugar cultivars. In this study, the two least-infested cultivars (Abergold and Barnhem) were also the high sugar cultivars and should, therefore, be extra palatable. This is supported by the observation that the variation among cultivars in crown rust infestation and in WSC concentration was highest is autumn, and that the variation in preference was also most clear in the autumn experiment (Chapter 5).

Improved nitrogen utilization

The efficiency of nitrogen in dairy systems in the Netherlands is poor (Ketelaars and van der Ven, 1992; Aarts *et al*., 1992; van der Hoek, 2002). This could be explained by the high protein levels in fresh grass, which cause a high concentration of ammonia in the rumen that cannot be utilized by the animal. The ammonia will be converted to urea that will disappear from the body via urine. A better regulation of the protein to energy ratio in the rumen could improve N utilization (van Vuuren, 1993), which could be realized by using cultivars of perennial ryegrass that have an enhanced WSC concentration. An increase in WSC concentration can have a reducing effect on the NH_3 level in the rumen (Taweel, 2004) and milk urea N in milk (Tas, 2005). A lower protein content in the herbage, through lower fertilization or feeding additional fodder with a lower N content, will be necessary to increase N efficiency (van Vuuren, 1993).

Breeding for improved herbage quality

The results from this study show that there was variation among the current perennial ryegrass cultivars for sward morphological and nutritional important parameters. Although effects of improved cultivars on herbage intake and animal performance remained rather limited in this study, the potential financial impact of small improvements could be large. For grass breeders, breeding for improved yield will still be the first category to breed for, but nutritional quality should not be forgotten.

Options for future research

Four years experience in the field of grazing ecology gives an avalanche of new ideas and future research plans. The most promising ideas are listed below.

Proteases inhibitors

Although large efforts have been made in the last two decades, losses of nitrogen to the environment is still a major problem in Dutch dairy production (van der Hoek, 2002). The efficiency of nitrogen utilization by ruminants is low (16%) (Ketelaars and van der Ven, 1992). The rate and extent of protein breakdown in the rumen frequently exceeds the level at which the released amino acids can be incorporated by the microorganisms. This imbalance results in deamination and loss of ammonia across the rumen wall, one of the main causes of inefficient N retention by ruminants (Falconer and Wallace, 1998; van Vuuren, 1993). This is especially a problem with animals fed on young and fresh grass, because young grass contains a large amount of rapidly degradable protein.

The literature describes three options to solve this problem

- An energy dense diet, more WSC or starch in the diet.
- An easily digestible fibre fraction, which can provide a more rapidly available source of energy.
- A slower degradation of the protein fraction.

In the last option, protease inhibitors can be used. Protein degradation or proteolysis has been reported to be normally present in the cells of plants, animals, bacteria and fungi (Storey, 1986). In the cell, enzymes (proteinases) split the proteins into amino acids that are then available to be used by cell organs. An organism uses this process inside its own cells for removal of abnormal or damaged proteins, for reallocation of nitrogen in the cell, for its supply of amino acids, its control of enzymatic pathways and programmed cell death (Vierstra, 1996).
The proteolysis of protein can be stopped or slowed down by inhibitors. In perennial ryegrass both cysteine and aspartic endopeptidase are present and can be inhibited by specific inhibitors of cysteine (antipain, cystatin and E-64) and aspartic (pepstatin A) endoproteinases in *in vitro* assays (Wetherall *et al.*, 1995). An addition of extra cystatin might lead to a reduced degradation of protein in perennial ryegrass.

Measuring spatial intake behavior

Advances have been made in the estimation of herbage intake behavior of individual cows (Chapter 3). This is crucial in the understanding of utilization processes of herbage under grazing condition. Further progress could be made if other technical tools can be used during grazing trials for measuring spatial patterns of grazing and grazing behavior activity. The use of differential Global Position System (dGPS) in combination with grazing recorders (Rutter *et al.*, 1997) could be very helpful in grazing trials. In Chapter 5, the position of the grazing cows was measured for three periods of two hours, but with these new technical tools it must be possible to have twenty-four hour (also during the night period) measurements of position (with an 0.2 to 1 meters accuracy) and grazing activity. This will lead to new insights in grazing behavior and in decisions, the animals make during grazing.

Flavor and odor

Chapter 5 showed clear differences in selective grazing behavior, and showed that this was related to an increased WSC concentration. The way animals differentiate among cultivars (and species) remained however unclear. Research of a more fundamental nature in the field of flavors and odors in relation to palatability of herbage is needed.
Odor plays a large role in the diet choice by animals (Chiy and Phillips, 1999). Also ruminants use volatile compounds for selection. The anatomy of the cow is such that the olfactory system is very close to the mouth. During harvest,

grass species are known to vaporize a distinct aromatic scent. During grazing, cows breathe deeply using the olfactory system (personal observation).

Scehovic *et al.* (1985) described an experiment in which unpalatable tall fescue was sprayed by a juice of very palatable Italian ryegrass and *vice versa*. This resulted in a significant modification of behavior of sheep towards the two species. Since this effect of these treatments was limited in time, the authors concluded that this was due to volatile substances. They concluded that volatile esters, aldehydes, ketones and volatile phenols are characterized as attractive. Sulphur-containing compounds, which are abundant in tall fescue, could be determining the low palatability of this species.

Van Tien *et al.* (1999) described an experiment in which the time taken by young sheep to get familiar with a new feed, in this case a rice bran concentrate, was determined. The sheep were significantly faster in familiarizing when the concentrate feed had a flavor or odor of grass.

Differences in volatile components have been found among cultivars of tall fescue (Mayland *et al*., 1997), however, in this experiment no relation could be established with preference (Shewmaker *et al.*, 1997).

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Summary

Grass is for the Dutch dairy cow the most important component of the diet, over 70% of the diet exists of fresh or ensiled grass. In the Netherlands, grasslands cover an area of almost one million hectares and are mainly located in the Friesland (North), Overijssel and parts of Gelderland (East) and the Groene Hart region (West). The majority of the farmers let their dairy cows graze at least partly during summer. Nevertheless, in the last decade there is tendency to summer stall feeding. An important reason for this tendency is that farmers cannot control the quality of fresh grass and have, therefore, less opportunity to balance dairy diets.

The most important grass species in the Netherlands is perennial ryegrass (*Lolium perenne* L.). The Dutch climate and soil conditions are ideal for this species, which is known for its high yields and excellent quality. An agronomically good pasture consists of over 50% perennial ryegrass. When less of this species occurs, the farmer will often decide to resow his grassland. Next to botanical composition, also the rotation with maize is an important reason for resowing pastures. On average, pasture is resown once in eight years.

At the moment of resowing, the farmer has the opportunity to optimize the botanical composition of the pasture. Perennial ryegrass is the largest component in the seed mixtures sold, but within the species there are options to choose between different perennial ryegrass cultivars. These cultivars are officially tested for several agronomical characteristics and are described in the National List of cultivars. The most important characteristics, for which the cultivars are tested, are the yield of the first cut and of the total year, resistance against crown rust (a fungal disease), winter hardiness and persistence. All these characteristics are related to the production of pastures, but none of these characteristics are related to the ultimate goal: herbage for dairy cows.

The aim of this study was to investigate the opportunities for grass breeders to influence the quality of perennial ryegrass cultivars in order to improve the herbage intake of dairy cows. This study focused on herbage intake, because this parameter largely controls the milk production of dairy cows.

The literature suggested that herbage intake of grazing animals is affected by sward and morphological characteristics. In Chapter 2 the differences in these characteristics among perennial ryegrass cultivars were examined. An experiment with six different perennial ryegrass cultivars (Abergold, Respect,

Agri, Herbie, Barezane, Barnhem) was set up. During two years (2000 and 2001) between June and September, these six cultivars were intensively studied during three two-week periods. There appeared to be differences between the cultivars for important characteristics influencing herbage intake, e.g. herbage yield, sward surface height and density. Furthermore, differences between cultivars were found for the proportions and yield of leaf, stem and pseudostem in the sward.

To examine the effects of cultivar on the herbage intake by grazing dairy cows, an accurate measurement tool had to be development. In Chapter 3, two methods were examined.

The first method was a sward cutting technique. The cows were allotted to a field with a specific area (in this case 132 m^2). Before the cows were turned in, a measured proportion of the field was harvested at a cutting height below the defoliation level of the cow, the yield was determined and the herbage allowance was calculated. Subsequently the cow was allowed to graze for 24 hours and after that a yield estimation of the herbage residual was made by cutting another measured proportion of the field. The difference between these two herbage masses and a correction for the daily regrowth gave an estimate of the herbage intake.

The second method was a marker method. The outer layer of grass leaves contain a wax layer, which plays a role in the defense mechanisms against water losses of the grass plant. This wax layer consists partly of substances, so-called alkanes. These alkanes are carbon-chains with a large number of C-atoms. In perennial ryegrass especially odd chain alkanes (C_{31} and C_{33}-alkanes) are present. Even chain alkanes (C_{32}-alkanes) are nearly absent. However, all these alkanes are practically similarly indigestible (± 85% is recovered in the manure). By dosing the cow daily with an exact amount C_{32}-alkane (in this case 1 gram) we could, with the alkanes content in herbage and the ratio between odd and even alkanes in the faeces, calculate the herbage intake.

These two methods were compared with an average that was calculated from the energy required for milk production and maintenance that is related to the live weight of the dairy cows. The sward cutting technique gave very variable results that fluctuated largely between cows and years. Furthermore, the average of the sward cutting technique did not match with the expected calculated intake. The n-alkanes method gave less variable results that were more related to the expected calculated intake values. Within the n-alkanes method, the ratio C_{32}:C_{33} gave the best results. Therefore it was concluded that herbage intake of grazing dairy cows on perennial ryegrass could best be estimated with the C_{32}:C_{33}-alkanes method.

The effects of perennial ryegrass cultivars on the intake of grazing dairy cows was examined in Chapter 4, by choosing the four most extreme cultivars out of the six described in Chapter 2. An experiment was set up with 12 dairy cows. During two weeks, three of the cows grazed each day a new field of the same cultivar. After this period the cows were allotted for two weeks to fields of another cultivar, until they had grazed all grass cultivars. During the experiment the cultivars were examined for agronomical traits and chemical composition. Herbage intake was estimated using the C_{32}:C_{33}-alkanes method. The experiment was conducted in 2002 and 2003. In the first year a clear difference was found between the cultivars, but in the second year no differences were found. An improved herbage intake was related with a higher mass of herbage and green leaf, a higher sward surface height, a lower infestation rate of the crown rust fungus and a lower lignin content (an indigestible substance) in the herbage. The cultivars differed too little in the other quality parameters to induce effects on the production of the cow. It was, therefore, concluded that grass breeders should aim for high producing cultivars, and the resistance against crown rust should have high priority. Furthermore, new cultivars should not contain a high level of lignin that might have negative effects on herbage intake.

In the experiment described above, we saw that quality parameters did not influence herbage intake by dairy cows, however, this was in a situation that animals were allotted for two weeks to the same cultivar. In Chapter 5, aspects of selection and preference were examined. An experiment was set up in which three groups of dairy cows could select among the six, earlier described, cultivars (Abergold, Respect, Agri, Herbie, Barezane and Barnhem). Herbage intake was measured using the sward cutting method; this was the only method which could be used, because the cultivars did not differ largely in alkane content. The experiment was conducted in spring, summer and autumn. The dairy cows very consistently consumed more of the cultivars with a high water soluble carbohydrates concentration (Abergold and Barnhem), a high digestibility, a low cell wall concentration and a low ash concentration. It was concluded that in the selection process the chemical quality of the herbage plays an important role.

Main conclusions of this study

- There are differences among diploid cultivars of perennial ryegrass for yield, sward and morphological characteristics.
- There are clear differences among diploid cultivars of perennial ryegrass for the concentration of water soluble carbohydrates and lignin.
- The effect of cultivar on herbage intake of dairy cows is limited when animals are restricted to grazing one single cultivar.
- When dairy cows can select between cultivars, there is an effect of cultivar on herbage intake. Dairy cows prefer cultivars with a high concentration of water soluble carbohydrates and a high digestibility, and select against cultivars with a high concentration of cell wall and ash.
- For estimating herbage intake of grazing dairy cows in perennial ryegrass pastures, the n-alkanes method is the best option. However, in selection experiments with cultivars of perennial ryegrass, the sward cutting method will be the only option, because of the lack of variation in the n-alkanes pattern between cultivars.

Samenvatting

Gras is voor de Nederlandse melkkoe het belangrijkste bestanddeel van het rantsoen, gemiddeld meer dan 70% van het rantsoen bestaat uit vers of ingekuild gras. Het areaal aan grasland in Nederland is bijna een miljoen hectare groot en ligt vooral in Friesland, Overijssel, delen van Gelderland en Zuid-Holland (het Groene Hart). Het overgrote deel van de boeren laat hun koeien tenminste een deel van het zomerseizoen buiten grazen, al is er in de afgelopen tien jaar een trend om melkkoeien meer op stal te houden. Een belangrijke reden voor deze trend is, dat boeren geen vat hebben op de kwaliteit van het verse gras en daardoor minder goed het rantsoen van de melkkoe kunnen sturen.

De belangrijkste grassoort die we in Nederland kennen is Engels raaigras (*Lolium perenne* L.). De Nederlandse klimaatsomstandigheden en bodem zijn ideaal voor deze soort, die bekend staat om zijn hoge opbrengst, smakelijkheid en kwaliteit. Landbouwkundig gezien bestaat een goed grasland voor meer dan 50% uit Engels raaigras en als er veel minder van deze soort voorkomt, zal een boer kunnen besluiten om zijn grasland opnieuw in te zaaien. Naast een slechte botanische samenstelling is, vooral op zandgrond, de rotatie met maïs een belangrijke reden voor herinzaai. In Nederland worden graslanden gemiddeld eenmaal in de 8 jaar opnieuw ingezaaid.

Op het moment van herinzaai heeft de boer de mogelijkheid om de botanische samenstelling van het grasland weer optimaal te maken. Engels raaigras vormt dan ook het grootste gedeelte van de verkochte graszaadmengels, maar binnen deze soort is er keuze tussen verschillende rassen of variëteiten. Deze rassen zijn officieel getoetst op een aantal agronomische kenmerken en staan beschreven in de nationale rassenlijst. De belangrijkste kenmerken waarop men de rassen onderzoekt, zijn de opbrengst van de eerste snede en van het hele jaar, de resistentie tegen kroonroest (*Puccinia coronata*, een schimmelziekte), wintervastheid en standvastigheid. Al deze kenmerken zijn gerelateerd aan de productie van het grasland, maar geen van de kenmerken is gerelateerd aan het uiteindelijke doel: voer voor de melkproducerende koe.

Het doel van dit onderzoeksproject was om te kijken of er mogelijkheden zijn om via veredeling de kwaliteit van rassen van Engels raaigras te beïnvloeden en na te gaan of de voeropname van melkvee door verschillen in graskwaliteit

verhoogd kon worden. Er werd in deze studie vooral gekeken naar voeropname, omdat deze parameter voor het overgrote gedeelte de melkproductie van koeien stuurt.

Uit de literatuur bleek dat de voeropname van grazende dieren beïnvloed wordt door gewasstructuur en morfologie. In Hoofdstuk 2 werd gekeken naar de verschillen in deze kenmerken tussen rassen van Engels raaigras. Daarvoor werd een maaiproef opgezet met zes verschillende rassen (Abergold, Respect, Agri, Herbie, Barezane en Barnhem), die gedurende twee jaar (2000 en 2001) tussen juni en september drie keer een periode van twee weken intensief onderzocht werden. Er bleken verschillen te zijn tussen de rassen voor belangrijke kenmerken die van invloed zijn op de opname, zoals grasopbrengst, grashoogte en dichtheid. Verder waren er verschillen tussen de rassen in hoeveelheden en percentage blad, stengel en schijnstengel in het gewas.

Om de effecten van grasras op de voeropname van grazende melkkoeien in de wei te onderzoeken, moest een accurate meetmethode worden ontwikkeld. In Hoofdstuk 3 worden twee methoden beschreven.
De eerste methode was een maaitechniek. De koeien kregen een veldje aangeboden van een bepaalde afmeting (in dit geval 132 m^2). Voordat de koeien het veld in gingen, werd er een kleine afgemeten oppervlakte gemaaid tot onder het niveau waar het dier kan grazen, hiervan werd de opbrengst bepaald. Zo was bekend hoeveel gras er aan het dier werd aangeboden. De koe kreeg vervolgens 24 uur de tijd om te grazen, daarna werd er opnieuw een opbrengstmeting gedaan, door weer een precies afgemeten oppervlakte te maaien. Zo was bekend hoeveel gras de koe had achtergelaten. Door de twee momentopnames van elkaar af te trekken en een correctie toe te passen voor de dagelijkse groei van het gras, kon de grasopname berekend worden.
De tweede methode was een merker-methode. De buitenste laag van een grasblad bevat een soort waslaag, die een rol speelt bij de waterhuishouding van de plant. Deze waslaag bestaat voor een deel uit stoffen, die we alkanen noemen. Deze alkanen zijn koolstofverbindingen met een groot aantal koolstof-atomen (C). In gras zijn vooral oneven alkanen (C_{31}- en C_{33}-alkanen) aanwezig. Even alkanen (C_{32}-alkanen) zijn nauwelijks aanwezig. Al deze alkanen zijn voor de koe vrijwel even moeilijk verteerbaar (± 85% vindt men terug in de mest). Door de koe een exacte hoeveelheid C_{32}-alkaan (1 gram) toe te dienen kon, met het gehalte aan alkanen in het gras en de verhouding van even en oneven alkanen in de mest, de grasopname berekend worden.

Deze twee methoden werden vergeleken met een gemiddelde dat berekend werd vanuit de energiebehoefte voor melkproductie en onderhoud dat gerelateerd is aan het gewicht van de koeien. De maaimethode gaf erg variabele uitslagen, die per koe en per jaar erg uit elkaar lagen. Verder lag het gemiddelde van de maaimethode erg ver van de verwachte berekende opname. De alkanen-methode gaf een minder variabel beeld en was beter gerelateerd aan de verwachte berekende opname. Binnen de alkanen-methode gaf de verhouding tussen C_{32}:C_{33} de beste resultaten. De C_{32}:C_{33}-alkanen-methode werd dan ook beschouwd als de beste methode om opname van Engels raaigras door grazend melkvee te meten.

De effecten van Engels raaigrasrassen op de opname van grazende dieren werden in Hoofdstuk 4 onderzocht. Hiervoor werden de vier meest extreme grasrassen uitgekozen uit de proef die eerder in Hoofdstuk 2 werd beschreven. Er werd een proef opgezet met 12 melkkoeien. Gedurende twee weken begraasden drie koeien iedere dag een veldje met hetzelfde grasras. Na deze periode kregen de koeien weer twee weken lang veldjes met een ander grasras, totdat ze alle vier de grasrassen begraasd hadden. De grasrassen werden gedurende de proef onderzocht op de agronomische kenmerken en chemische samenstelling. De voeropname werd bepaald door middel van de C_{32}:C_{33}-alkanen-methode. De proef werd uitgevoerd in 2002 en 2003. In het eerste jaar werden er duidelijk verschillen gevonden tussen de rassen, maar in het tweede jaar niet meer. Een hogere grasopname kon worden toegeschreven aan een hoge productie van totale biomassa en bladeren, een hogere grashoogte, lagere infectie door de kroonroest schimmel en een lager gehalte aan lignine (een stof die onverteerbaar is) in het gewas. De grasrassen verschilden in de overige kwaliteitsparameters te weinig om effecten op de productie van de koe te geven. Er werd daarom geconcludeerd, dat grasveredelaars door moeten gaan met het produceren van rassen die hoogproductief zijn en de resistentie tegen kroonroest moet een hoge prioriteit krijgen. Verder moeten nieuwe rassen niet een te hoog gehalte aan lignine hebben, omdat dit een negatieve invloed op de opname kan hebben.

In de hierboven beschreven proef zagen we dat graskwaliteit nauwelijks van invloed was op de opname door melkkoeien, dit was in de situatie dat dieren twee weken lang één ras konden begrazen. In Hoofdstuk 5 werden de aspecten van selectie en voorkeur tussen de verschillende Engels raaigrasrassen bekeken. Hiervoor werd een proef opgezet, waarbij drie groepen van melkkoeien vier dagen lang konden kiezen tussen de zes, eerder beschreven, grasrassen (Abergold, Respect, Agri, Herbie, Barezane, Barnhem). De grasopname werd bepaald door de maaimethode (beschreven in Hoofdstuk 2), dit was de enige bruikbare methode omdat de rassen niet duidelijk verschillen in alkaan gehaltes. De proef werd uitgevoerd in de lente, zomer en herfst. De melkkoeien aten telkens meer van de grasrassen met een hoog gehalte aan water-oplosbare koolhydraten (suikers), een hoge *in vitro* verteerbaarheid, een laag celwandgehalte en een laag asgehalte. Er werd geconcludeerd dat in het selectie proces de chemische kwaliteit van gras een grote rol speelt.

Belangrijkste conclusies van dit onderzoek

- Er zijn verschillen tussen diploïde rassen van Engels raaigras voor opbrengst, gewas en morfologische kenmerken.
- Er zijn duidelijke verschillen tussen diploïde rassen van Engels raaigras voor het gehalte aan water-oplosbare koolhydraten en lignine.
- Het effect van grasras op de grasopname van melkkoeien is beperkt als de koe gelimiteerd wordt tot het eten van één rasgras.
- Wanneer melkkoeien kunnen kiezen tussen grasrassen, is er een effect van grasras. Melkkoeien hebben een voorkeur voor grasrassen met een hoog gehalte aan water oplosbaar koolhydraten en een hoge verteerbaarheid en vermijden grasrassen met een hoog celwand- en asgehalte.
- Voor de schatting van grasopname van grazende melkkoeien in weides met Engels raaigras is de alkanen methode de beste optie. In selectie experimenten met rassen van Engels raaigras blijft de maaimethode de enige optie, omdat er geen grote variatie in alkaan-patroon tussen de rassen is.

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PE&RC PhD Education Statement Form

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (ECTS 3)

- Cultivar effects of perennial ryegrass on herbage intake by grazing dairy cows (2001)

Post-Graduate Courses (ECTS 4)

- Experimental Animal Course. Catholic University Nijmegen (2001)
- Summer School: The analysis of natural variation crops and model plants. EPS (2003)

Deficiency, Refresh, Brush-up and General Courses (ECTS 5)

- Basic Statistics. PE&RC (2000)
- Advanced Statistics. PE&RC (2001)
- Crop Ecology. WUR (2001)
- Techniques for scientific writing and presentations. PE&RC (2001)

PhD discussion groups (ECTS 4)

- Plant and Crop Ecology (2000-2004)

PE&RC annual meetings, seminars and introduction days (ECTS 1.5)

- PE&RC Science Day ‘Food Insecurity’ (2001)
- Studiedag Nederlandse Vereniging voor Weide and Voederbouw in Oosterbeek (2001)
- PE&RC Science Day ‘Ethics in Science’ (2002)
- Studiedag Nederlandstalige Voedingsdag in Leuven (2003)
- PE&RC Science Day ‘Biological Disasters’ (2004)
- Studiedag Nederlandse Vereniging voor Weide en Voederbouw in Lelystad (2004)
- Masterclasses for young researchers (2004)

International symposia, workshops and conferences (ECTS 5)

- Occasional European Grassland Symposium. Witzenhausen. Germany (2001)
- General European Grassland Symposium. La Rochelle, France (2002)
- International Symposium on Herbivore Nutrition. Merida, Mexico (2003)
- General European Grassland Symposium. Luzern, Switzerland (2004)
- International Grassland Symposium. Dublin, Ireland (2005)

Laboratory training and working visits (ECTS 1)

- DARNI (Northern Ireland), TEAGASC (Ireland) and IGER (Wales). Studytrip to visit several Grassland institutes in Ireland and the United Kingdom (2001)

Publications of the author

Full accepted papers

Smit, H.J., B.M. Tas, H.Z. Taweel and A. Elgersma (2005). Sward characteristics important for intake in six *Lolium perenne* varieties. *Grass and Forage Science, 60* (in press).

Smit, H.J., H.Z. Taweel, B.M. Tas, S. Tamminga and A. Elgersma (2005). Comparison of techniques for estimating herbage intake of grazing dairy cows. *Journal of Dairy Science, 88* (in press).

Taweel, H.Z., B.M. Tas, H.J. Smit, A. Elgersma, J. Dijkstra and S. Tamminga (2005). The effect of feeding perennial ryegrass with elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. Accepted in *Animal Feed Science and Technology*.

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Tas, B.M. H.Z. Taweel, H.J.Smit, A. Elgersma, J. Dijkstra and S. Tamminga (2005). Effects of perennial ryegrass cultivars on intake, digestibility, and milk production by dairy cows. Accepted in *Journal of Dairy Science*.

Conference proceedings papers (first author)

Smit, H.J., B.M. Tas, H.Z. Taweel, J. Dijkstra, A. Elgersma and S. Tamminga (2005). Effects of perennial ryegrass cultivars on traits for improved animal performance. Accepted for International Grassland Congress 2005.

Smit, H.J. and A. Elgersma (2004). Diurnal fluctuations in vertical distribution of chemical composition in a perennial ryegrass *(Lolium perenne* L.) sward during the season. *Grassland Science in Europe* 9: 951-953.

Smit, H.J., C. van den Anker, J. Galle, B. Tas, H. Taweel and A. Elgersma (2003). Cattle grazing preference between two cultivars of perennial ryegrass. *Tropical and Subtropical Agroecosystems* 3: 449-452.

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Smit, H.J., H. Schlepers, A. Brok, L. Westra, B. Tas, H. Taweel and A. Elgersma (2001). Influence of grass variety on herbage intake and milk production. *Grassland Science in Europe* 6: 304-306

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Smit, H.J., I.J.M de Boer (2000). Energy analysis of agriculture in two Dutch regions; a comparison of methods. In: *51st annual meeting of the European Association for Animal Production, Den Haag, the Netherlands, 21-24 August 2000.* pp. 200

<u>Conference proceedings papers (co-author)</u>

Elgersma A, H.J. Smit, G. Ellen and S. Tamminga (2005). Effect of perennial ryegrass cultivars with contrasting water-soluble carbohydrates concentrations on the fatty acid composition in milk of stall-fed cows. International Grassland Congress 2005.

Tas, B.M., H.Z. Taweel, H.J. Smit, A. Elgersma, J. Dijkstra, S. Tamminga (2003). Intake and milk production of cows grazing perennial ryegrass cultivars with different crown rust resistance. *Grassland Science in Europe* 9: 1080-1082.

Tas, B.M., H.Z. Taweel, H.J. Smit, A. Elgersma, J. Dijkstra and S. Tamminga (2003). Intake and milk production of cows grazing perennial ryegrass cultivars with contrasting WSC contents. *Tropical and Subtropical Agroecosystems* 3: 501-504

Tas, B.M., J. Lopes, H.Z. Taweel, H.J. Smit, A. Elgersma, J. Dijkstra and S. Tamminga (2003). Kalium als interne markeerstof voor bepaling van mest- en urineproducten bij koeien. In: *28ste Studiedag Nederlandstalige Voedingsonderzoekers, Leuven, 11 april 2003*. Leuven: KU-Leuven 2003. pp. 52 - 53.

Elgersma, A., H.J. Smit, B.M. Tas, H.Z. Taweel, J. Dijkstra, H. Boer and S. Tamminga (2002). Effect of perennial ryegrass cultivar on intake of fresh grass and milk production. In: *Xth European Intake Workshop Techniques for investigating intake and ingestive behaviour* *by farmed animals, Reykjavik, 2002*. Reykjavik: The Agricultural Research Institute, 2002. pp. 4.

Tas, B., H.Z. Taweel, H. Smit, J. Dijkstra, A. Elgersma and S. Tamminga (2002). Seasonal variation in degradation rate of eight different perennial ryegrass cultivars In: *53rd annual meeting European Association of Animal Production, Cairo, Egypt 1-4 September 2002.* Wageningen: Wageningen Press, 2002. pp. 79.

Tas, B., H. Taweel, H. Smit, S. Tamminga, J. Dijkstra and A. Elgersma (2002). Intake, digestibility and milk production of dairy cows fed perennial ryegrass cultivars during the season. *Grassland Science in Europe* 7: 262-263.

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De Boer, I.J.M. and H.J. Smit (2000). Embodied Energy and EMERGY analysis in a Dutch Agricultural region. In: *2nd International Workshop Advances in energy: exploring supplies, constraints, and strategies. Porto Venere, Italy 2000*. pp. 189-198.

Reports

Smit, H.J., I.J.M. de Boer (2000). *A comparison of Embodied Energy Analysis and EMERGY analysis for two Dutch agricultural regions.* A report explorative research on energy analysis, Animal Production Systems Group, Wageningen University.

Popular publications of the project

- Omroep Fryslân, 31 mei 2004.
- NOS 6-uur Nieuws, 10 september 2003.
- Volkskrant, 4 september 2003. Koe in de wei.
- Agrarisch journaal, 2 september 2003.
- Oogst, 20 september 2002. Grasras bepaalt hoeveel koe vreet en hoeveel melk ze geeft.
- Oogst, 22 juni 2001. Grasras beïnvloedt melkgift.
- Telegraaf, 30 november 2000. Hoofdrol koeien in culinair experiment.
- Veeteelt, 2 augustus 2000. Koeien veredelen gras.

Curriculum Vitae

Harm Jakob Smit was born on the 26th of September 1975 in Zeist. He followed high school at the Christelijke Lyceum in Zeist and graduated from the VWO in 1994. In the same year he started an MSc Animal Sciences at Wageningen University. During this study he completed two MSc-theses. His first thesis was at the Agronomy Group of Wageningen University about estimating digestibility of fresh grass with Near Infrared Reflectance Spectroscopy. Then he went for four months to New Zealand, where he joined the Institute of Natural Resources of Massey University, Palmerston North. He performed an experiment to clarify the effect of drought on the ammonia volatilization of fertilizers. When he returned he started a second thesis at the Animal Production System Group of Wageningen University that dealt with Nitrogen flows through agricultural systems in a peat soil region. When he graduated in January 2000, he was immediately appointed at the same group for a nine-month project on energy flows through agricultural systems. After that he started a PhD-project at the Crop and Weed Ecology Group at Wageningen University dealing with the possibilities of grass breeders to improve perennial ryegrass cultivars to enhance herbage intake during grazing. This PhD-project was preformed in close cooperation with the Animal Nutrition Group of Wageningen University and the grass breeding company Barenbrug Holland BV. He graduated in April 2005. Currently he is appointed as Post-doc researcher at the Crop and Weed Ecology Group.

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