Chemical composition of lamina and sheath of *Lolium perenne* as affected by herbage management

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Abstract

The quality of grass in terms of form and relative amounts of energy and protein affects both animal production per unit of intake and nitrogen (N) utilization. Quality can be manipulated by herbage management and choice of cultivar. The effects of N application rate (0, 90 or 390 kg N ha^{-1} $year^{-1}$), duration of regrowth period (2–3, 4–5, or 6–7 weeks), and cutting height (8 or 12 cm) on the mass fractions of nitrogen (N), water-soluble carbohydrates (WSC), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin and ash in lamina and sheath material of a high-sugar (Aberdart) and a low-sugar (Respect) perennial ryegrass (*Lolium perenne*) cultivar, were studied in a factorial field experiment during four seasons in 2002 and 2003. Expressing NDF and ADF mass fractions in g per kg WSC-free dry matter (DM) increased the consistency of treatment effects. The high-sugar cultivar had generally higher WSC mass fractions than the low-sugar cultivar, especially during the late season. Moreover, the relative difference in WSC mass fraction between the two cultivars tended to be higher for the lamina material than for the sheath material, which suggests that the high-sugar trait may be more important under grazing conditions, when lamina forms the bulk of the intake, than under mowing regimes. Longer regrowth periods and lower N application rates increased WSC mass fractions and decreased N mass fractions; interactions between regrowth period and N application rate were highly significant. The mass fractions of NDF and ADF were much less influenced. The NDF mass fraction in terms of g per kg WSC-free DM tended to be higher at lower N application rates and at longer regrowth periods. The effect of cutting height on herbage chemical composition was unclear. In conclusion, high-sugar cultivars, N application rate and length of the regrowth period are important tools for manipulating herbage quality.

Additional keywords: cutting height, feeding value, herbage quality, high-sugar cultivar, nitrogen application rate, perennial ryegrass, regrowth period, rotation length

Introduction

In many parts of Europe, a large proportion of the bovine's diet consists of grazed grass (Lantinga *et al.*, 1996). The quality of the grass intake affects both animal production and nitrogen (N) utilization (Rearte, 2005). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) mass fractions are important parameters of herbage quality as they affect dry matter intake and digestibility. Protein is an essential nutrient, but the N mass fraction of temperate pasture grazed at an immature stage is usually in excess of animal requirements. The water-soluble carbohydrates (WSC) are relevant as the main source of energy. However, the WSC mass fraction is generally too low to balance the high mass fraction of highly degradable protein. This imbalance results in large losses of N from the rumen and in a low N utilization by the grazing cow (Rearte, 2005).

The main tools to manipulate grass quality are regrowth duration, N application rate and cutting height, which have been the subject of many studies. Additionally, in the UK perennial ryegrass cultivars have been developed that have higher WSC mass fractions (Humphreys, 1994). The use of these cultivars has led to increased animal production (Lee *et al.*, 2001; Miller *et al.*, 2001) and reduced urinary N output (Miller *et al.*, 2001).

Designing management systems aimed at optimizing grass quality requires the prediction of grass chemical composition as affected by management tools and their interactions. Some interactions have been studied in great detail [e.g., those between N application rate and regrowth period, by Wilman *et al.* (1976)], other ones not. For example, little is known about the performance of the high-sugar cultivars under different herbage management regimes. Moreover, the studies tended to focus on the whole crop, rather than on individual plant parts. This makes it hard to apply the results obtained from cutting trials to grazing situations, as the lamina material forms the bulk of the intake under grazing (Brereton *et al.*, 2005).

The objective of this study was to quantify in detail the effect of herbage management tools and their interactions on the chemical composition of lamina as well as sheath fractions of a low or normal and a high-sugar perennial ryegrass cultivar throughout the growing season. The selection of management tools was based on a review paper by Hoekstra *et al.* (2007), who identified the most relevant management tools for manipulating the carbon and nitrogen mass fractions in grass herbage.

Materials and methods

Experimental design

The 36 factorial combinations of 2 cultivars of perennial ryegrass (*Lolium perenne* L.), 2 cutting heights, 3 regrowth periods and 3 fertilizer-N application rates were compared in a field experiment of the split-split-plot design, replicated 3 times, with cultivars as main factor, the factorial combinations cutting height × regrowth period as split factor, and the fertilizer-N application rates as split-split factor. Per cultivar and per replication, the six combinations cutting height × regrowth period were arranged in

Table 1. Sampling dates and weather data for the four measurement periods during the four seasons.

Season		Date start period	Regrowth period T1	T2	T3	Average max. temp.	Average min. temp.	Average rainfall
			---- (days) ----			-------- (°C) --------		(mm day^{-1})
S1	Late season	05.09.2002	15	29	49	16.0	9.6	4.1
S2	Early season	14.03.2003	19	33	47	12.4	5.4	2.0
S3	Mid season	08.05.2003	20	34	48	16.0	9.4	2.4
S4	Late season	24.07.2003	22	34	48	20.3	12.6	1.6

strips, so as to be able to cut the grass mechanically at the same height and on the same day with a plot harvester (Haldrup, Logstor, Denmark). The smallest experimental unit measured 1.5 m × 2 m.

A high-sugar (Aberdart) and a low-sugar cultivar (Respect) were used (coded HS and LS), both diploids with similar heading dates [27 and 23 May, respectively (Anon., 2001)]. The cutting heights were about 8 or 12 cm (coded LD and HD, respectively), the regrowth periods 2–3, 4–5, or 6–7 weeks (coded T1, T2 and T3, respectively) and the N application rates 0, 90 or 390 kg N ha^{-1} year^{-1} (coded N) split over seven equal applications.

Measurements were taken during four seasons: September/October 2002 (late season; S1), April 2003 (early season; S2), May/June 2003 (mid season; S3) and August/September 2003 (late season; S4) (Table 1). To enable the grass to recover from the sampling (see below), each replication (block) consisted of two similar sub-blocks, each comprising the 36 treatment combinations. One sub-block was harvested only during the seasons S1 and S3, the other one only during the seasons S2 and S4. This layout resulted in a total of 216 experimental units (2 cultivars × 2 cutting heights × 3 regrowth periods × 3 N application rates × 3 replications × 2 sub-blocks to allow harvesting in the four seasons).

The two cultivars were sown on 12 June 2002 at a seeding rate of 4 g m^{-2}, using a plot fertilizer spreader (Probe, Fiona, Denmark). The seed was worked in by hand, using a rake. At the 3-leaf stage a basal NPK (10:10:20) fertilizer dressing was applied equivalent to 50, 50 and 100 kg N, P and K per ha. After the first cut (8 weeks after sowing), the plots were cut every four weeks, using the plot harvester. Within four days after each cut the plots were fertilized with the assigned N rates (0, 13 or 56 kg N ha^{-1} per cut), using the plot fertilizer spreader. At the start of the four seasons the plots were cut at the assigned cutting height and sampling took place after 2–3, 4–5, or 6–7 weeks, according to the regrowth period.

The experiment was located on a clay soil at Johnstown Castle Research Centre, Wexford, Ireland. Minimum and maximum daily temperatures and daily rainfall were recorded at the centre's weather station (Table 1). The seasons S1 and S3 were very similar in temperature conditions, but S2 was much cooler and S4 much warmer and dryer than the other seasons.

Sampling

At each harvest, fresh grass samples of approximately 250 g were taken by cutting the plants at 1 cm above ground level from the middle of the plots, using electric shears (Wolf-Garten, Accu 75 Professional). Each sampling event started at the same time of the day (09:00 h) to avoid effects of diurnal changes in WSC mass fraction (Donaghy & Fulkerson, 1998). The samples were stored immediately at 4 °C. They were manually divided into sheath (sheath, stem and new leaves within the sheath tube), lamina, inflorescence and dead material (defined as leaves and sheaths of which more than 50% of the surface was dead). After all samples had been dissected, i.e., within three days after storing at 4 °C, the fractions were stored in a freezer. Earlier research to test the methodology had shown that our procedure had little absolute effect on the chemical composition and was very unlikely to influence treatment effects (N.J. Hoekstra, unpublished results).

During the last week of S1 not all the treatments could be harvested due to calves that had entered some of the plots.

Chemical analyses

The separated fractions of sheaths, laminae and inflorescences were freeze-dried and subsequently ground over a 1-mm sieve. To obtain sufficient material for chemical analysis the lamina and sheath material from the three replications was bulked per treatment. Bulking of the inflorescence material was done over the two cutting height treatments and the three replications. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin mass fractions were determined according to Van Soest *et al.* (1991). The samples were analysed for total N using a Kjeldahl-N analyser. Water-soluble carbohydrates (WSC) were determined colorimetrically with an automatic analysing device (Technicon autoanalyzer 2, Technicon Instruments Corporation, Tarrytown, NY, USA), using ferricyanide (Struik, 1983).

N, ADF and NDF mass fractions were expressed in g per kg dry matter (DM) and converted into g per kg WSC-free DM, using the following equation:

$$\frac{\text{Fraction (g per kg DM)}}{\text{1000 (g per kg DM)} - \text{WSC (g per kg DM)}} \times \text{1000 (g per kg DM)}$$

Detailed chemical analysis of the different fractions of total nitrogen was also carried out but will be reported in another paper (Hoekstra *et al.*, accepted).

Statistical analyses

Analysis of variance was carried out using the SAS GLM procedure (SAS Enterprise Guide version 8.2). Analysis of variance was done separately for the four seasons and for lamina and sheath material. The bulking of the material for chemical analyses resulted in single values for all factorial treatment combinations. All main effects and two-factor interactions were included in the model, resulting in 36 treatment combinations (d.f. error = 16).

Results

Lignin and ash

The acid detergent lignin mass fraction was 11.1 g per kg DM on average, and fluctuated strongly, which appeared to be due to analytical errors (which were large in comparison to the total variation) rather than treatment effects (data not shown).

The ash mass fraction averaged 82.9 and 73.1 g kg^{-1} for lamina and sheath material, respectively (data not shown). Variation appeared to be influenced by artefacts (soiling) rather than treatment *per se*.

Water-soluble carbohydrates

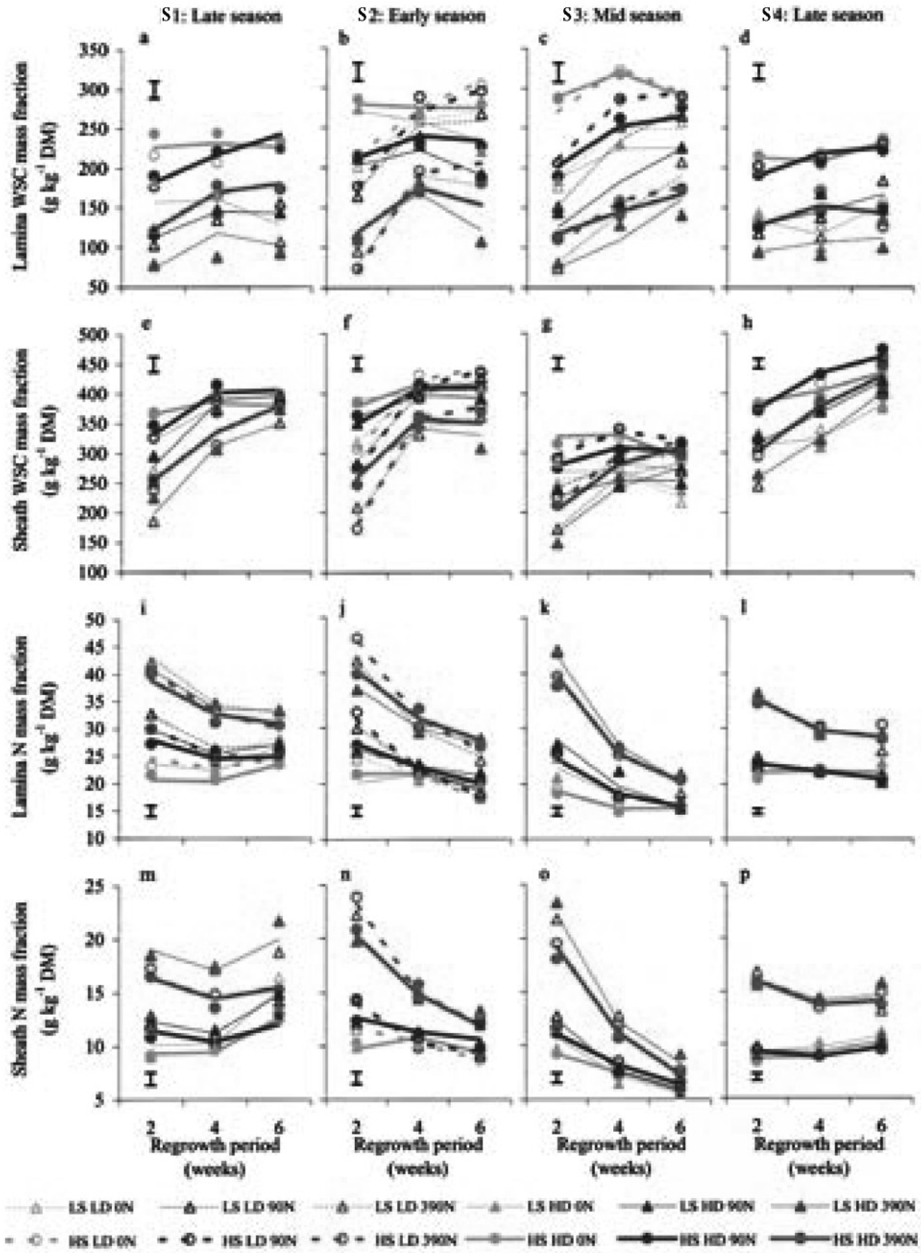
The water-soluble carbohydrates (WSC) mass fraction varied strongly, ranging from 74 to 323 g per kg DM in the lamina and from 149 to 473 g per kg DM in the sheath material (Figure 1). The WSC mass fraction of the lamina material tended to be higher during S2 and S3 (212 and 207 g per kg DM, respectively) than during S1 and S4 (160 and 159 g per kg DM, respectively), whereas the WSC mass fraction of the sheath material was lower during S3.

There was a statistically significant ($P < 0.01$) positive effect of the HS cultivar on the WSC mass fraction in all treatment combinations, except for sheath material during S2 (Table 2). For lamina material during S2, the difference was statistically significant only at longer regrowth periods (a significant ($P < 0.05$) T × C interaction). For laminae during S3 and S4 there was a statistically significant N × C interaction ($P < 0.05$) indicating that the difference between HS and LS cultivars was smaller at 390 than at 0 and 90 kg N ha^{-1} $year^{-1}$.

In all treatment combinations, WSC mass fraction was significantly ($P < 0.01$) reduced by an increase in N application rate, although in some cases the difference between the 0 and 90 kg N ha^{-1} $year^{-1}$ application rate was not statistically significant. For all seasons there was a statistically significant ($P < 0.01$) positive effect of the the length of the regrowth period (T) on WSC mass fraction. In the sheath material this average increase was stronger at the high N application rate [N × T interaction statistically significant ($P < 0.05$)].

The effects of cutting height on WSC mass fraction were inconsistent: they were statistically significant in a few cases only. During S3, the low cutting height significantly ($P < 0.01$) increased the WSC mass fraction of the laminae. In contrast, the WSC mass fraction was higher for the high cutting height for T1 and lower for T3 due to a statistically significant ($P < 0.001$) T × D interaction for S2 lamina and sheath material.

The relative increase in WSC mass fraction for the HS cultivar compared with the LS cultivar [(HS – LS)/LS × 100%] tended to be larger during S1 and S4 than during S2 and S3 and larger for lamina than for sheath material (Table 3).



and sheath (m–p) material as affected by cultivar (HS = high sugar; LS = low sugar), N application rate (0, 90 and 390 kg ha^{-1} year^{-1}), regrowth period (about 2, 4 and 6 weeks) and cutting height (LD = low height; HD = high height), for late season 2002 (a, e, i, m), early season 2003 (b, f, j, n), mid season 2003 (c, g, k, o) and late season 2003 (d, h, l, p). Lines represent the ANOVA model, averaged over non-significant effects. Error bars are 2× standard error for the comparison of individual model points.

Table 2. Statistical significance[1] of the main effects (one capital) and the two-factor interactions[2] (two capitals) of grassland management tools[3] on WSC, N and NDF mass fraction and WSC-free NDF and WSC-free ADF mass fractions of lamina and sheath material during four seasons (S1–S4). R^2_{adj} is the percentage variance accounted for by the ANOVA model.

	Lamina										Sheath									
	R^2_{adj}	N	T	C	D	NT	NC	ND	TC	TD	R^2_{adj}	N	T	C	D	NT	NC	ND	TC	TD
WSC (g per kg DM)																				
S1	0.89	***	**	***		*					0.89	***	***	**		*				
S2	0.94	***	***	**		*			*	***	0.96	***	***		**	**			*	***
S3	0.95	***	***	***	**	**	**		*		0.91	***	***	***		***		*		
S4	0.89	***	**	***			**				0.95	***	***	***		***				
N (g per kg DM)																				
S1	0.96	***	***	***	**	***					0.97	***	***	***		*				
S2	0.97	***	***	*		***				***	0.97	***	***			***				**
S3	0.98	***	***	***		***			**		0.99	***	***	*		***	*		**	
S4	0.95	***	***			***					0.98	***	**	*		***				
NDF (g per kg DM)																				
S1	0.76			***							0.93	***	***	***	*	**			*	**
S2	0.73	***	*	*						*	0.90	***	***			*			**	***
S3	0.91	***	***	***	***	***	***				0.87	**	***	***	***	***	***			*
S4	0.75		**	***							0.97	***	***	***		***		*	*	*
NDF (g per kg WSC-free DM)																				
S1	0.67	***		*		*					0.78	***	***							
S2	0.61	**	***					**			0.91	***	***		***					
S3	0.91	***	***			***					0.96	***	***	**	***	***	**			**
S4	0.66	***	***								0.72	***	***	*						
ADF (g per kg WSC-free DM)																				
S1	0.24	*									0.78	*	***	*						
S2	0.88	***	***	*		**		*			0.78		***							
S3	0.56		***								0.66	*	***		*					
S4	ns[4]										0.94	***	*	***	***	*			***	

[1] Levels of statistical significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

[2] The interaction C × D was never statistically significant and is therefore not included in the table.

[3] N = nitrogen application rate; T = length of regrowth period; C = cultivar; D = cutting height.

[4] ns = not statistically significant.

Table 3. Average relative increase[1] (%) in WSC mass fraction of the high sugar cultivar relative to that of the low sugar cultivar for lamina and sheath material during four seasons (S1–S4). Averages over all herbage management treatments. Standard error in brackets (S1, n = 16; S2–S4, n = 18).

Material	S1	S2	S3	S4	Mean
Lamina	60.8 (7.5)	9.2 (4.1)	29.8 (4.9)	49.5 (5.4)	36.7 (3.6)
Sheath	11.3 (3.1)	1.4 (1.8)	21.0 (2.1)	16.6 (1.7)	12.6 (1.4)

[1] Calculated as [(HS–LS)/LS] × 100%.

Dry matter versus WSC-free dry matter

All mass fractions on the basis of DM are by definition mutually correlated because they are expressed in relative terms. The range in WSC mass fraction as affected by the treatments was large, resulting in a strong effect on the other fractions. In order to eliminate this effect, the N, NDF and ADF mass fractions were also expressed on the basis of WSC-free DM. If expressed in this way the treatment effects on NDF and ADF became much more consistent. As to N mass fraction there was not much difference between the two methods (data not shown). So in the remainder of this paper only NDF and ADF mass fractions will be expressed in g per kg WSC-free DM, unless stated otherwise.

Nitrogen

The average N mass fraction did not vary much between the seasons, but was much lower for sheath than for lamina material: 11.8 and 25.6 g per kg DM, respectively (Figure 1).

There was a statistically significant effect of N application rate ($P < 0.001$) on the N mass fraction for all periods and for both lamina and sheath material (Figure 1 and Table 2). However, the difference in N mass fraction among N application rates tended to be smaller with longer regrowth periods (T), resulting in a statistically significant ($P < 0.001$; S1 lamina $P < 0.05$) N × T interaction.

During S2, but especially during S3, N mass fraction of lamina and sheath material decreased strongly and significantly ($P < 0.001$) with T. During S1 and S4 the effect of T was still statistically significant, but less pronounced at low N application rates.

During S3 and S1, the low-sugar cultivar generally had a significantly ($P < 0.05$) higher N mass fraction. For S1 this was mainly during the longer regrowth period, whereas during S3 the difference was only statistically significant for the short regrowth period.

In most cases, N mass fraction was not significantly affected by cutting height, except during S2 when a statistically significant ($P < 0.01$) T × D interaction was found. During S1 the N mass fraction of the lamina was slightly but significantly ($P < 0.01$) higher for the lower cutting height.

Neutral detergent fibre

The neutral detergent fibre (NDF) mass fraction tended to be lower in lamina than in sheath material (on average 558 and 687 g per kg WSC-free DM, respectively) and higher for S3 than for the other seasons (Figure 2).

N application rate had a statistically significant ($P < 0.01$) negative effect on NDF mass fraction (g per kg WSC-free DM) in all cases (Table 2). During S3 and for lamina material during S1 there was a statistically significant N × T interaction, the increase in NDF with T becoming larger as N application rate increased. There was a positive statistically significant ($P < 0.001$) effect of T on the NDF mass fraction in all cases, except for lamina material during S1. However, for sheath material during S1 and S4 the NDF mass fraction tended to decrease rather than increase with a longer regrowth period.

There was no statistically significant cultivar effect except for sheath material during S3 and S4 and for lamina material during S1, where the HS cultivar tended to have slightly higher NDF mass fractions with higher N application rates.

During S2 and S3, cutting height had a statistically significant ($P < 0.001$) effect on the NDF mass fraction of the sheath material. During S3 the difference in the NDF mass fraction of the sheath material between HD and LD decreased with T, resulting in a statistically significant ($P < 0.01$) T × D interaction.

Acid detergent fibre

The acid detergent fibre (ADF) mass fraction ranged from 252 to 451 g per kg WSC-free DM and tended to be higher during S3, especially for the sheath material (Figure 3).

The effect of N application rate was small and the ADF mass fractions either significantly ($P < 0.05$) decreased (lamina material during S1 and S2, and sheath material during S1 and S3) or significantly increased (sheath material during S4) with increasing N rate (Table 2).

During S2 and S3, ADF mass fractions (g per kg WSC-free DM) significantly ($P < 0.001$) increased with T. However, during S1 and S4 no effect of T was found on NDF mass fraction of the lamina material, whereas it was slightly but significantly ($P < 0.001$) negative for the sheath material.

Cultivar had a small negative effect on NDF mass fraction in sheath material during S1 and S4 due to a lower ADF mass fraction of the HS cultivar. There was a statistically significant effect ($P < 0.05$) of cutting height for sheath material during S3 and S4, as ADF mass fraction was slightly lower at the lower cutting height.

Inflorescences

Due to a shortage of material, the inflorescences could not be analysed for all treatment combinations and the number of missing values was too large for analysis of variance. Therefore, only average mass fractions of NDF, ash, N, and WSC in inflorescence material are presented in Table 4. These values are included to complete the picture of the chemical analysis of the herbage material.

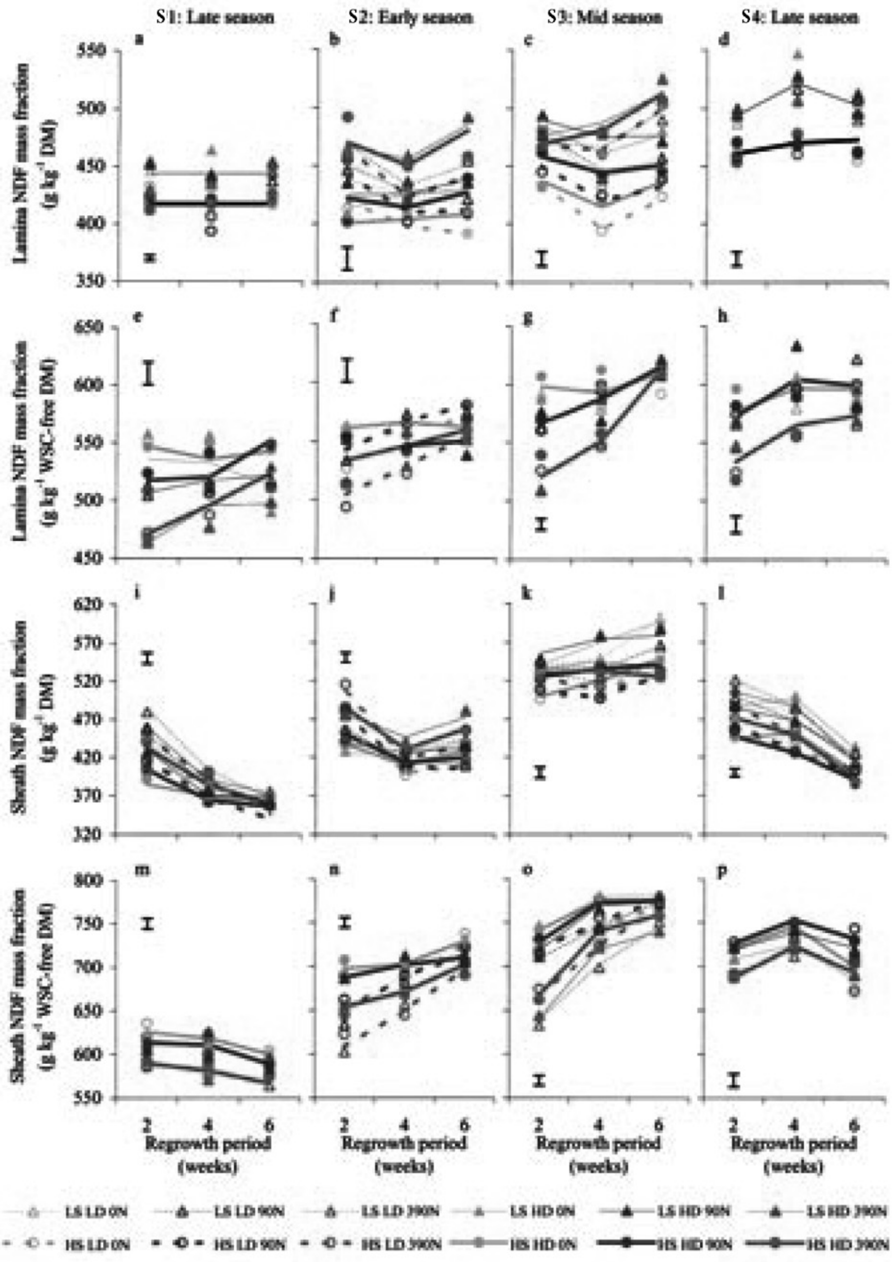


Figure 2. NDF mass fraction of lamina (a–h) and sheath (i–p) material expressed as g per kg DM (a–d; i–l) or g per kg WSC-free DM (e–h; m–p) as affected by cultivar (HS = high sugar; LS = low sugar), N application rate (0, 90 and 390 kg ha^{-1} year^{-1}), regrowth period (about 2, 4 and 6 weeks) and cutting height (LD = low height; HD = high height), for late season 2002 (a, e, i, m), early season 2003 (b, f, j, n), mid season 2003 (c, g, k, o) and late season 2003 (d, h, l, p). Lines represent the ANOVA model, averaged over non-significant effects. Error bars are 2× standard error for the comparison of individual model points.

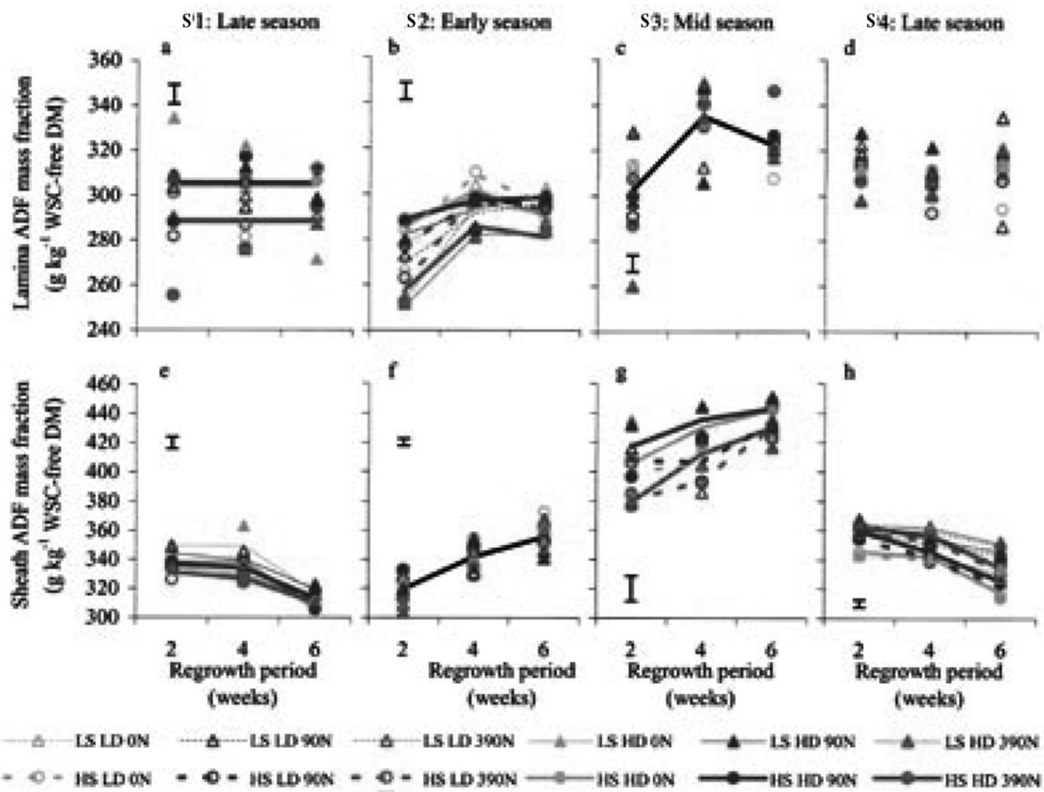


Figure 3. ADF mass fraction lamina (a–d) and sheath (e–h) material as affected by cultivar (HS = high sugar; LS = low sugar), N application rate (0, 90 or 390 kg ha^{-1} yr^{-1}), regrowth period (about 2, 4 or 6 weeks) and cutting height (LD = low height; HD = high height), for late season 2002 (a, e), early season 2003 (b, f), mid season 2003 (c, g) and late season 2003 (d, h). Lines represent the ANOVA model, averaged over non-significant effects. Error bars are 2x standard error for the comparison of individual model points.

Table 4. Chemical composition of inflorescence material (g per kg dry matter).

Component	Average[1]	SD[2]	n
N total	17.3	2.5	22
WSC	137.0	21.5	25
NDF	589.4	22.8	22
Lignin	30.3	4.0	17
Ash	45.8	2.7	29

[1] Average over all treatments.

[2] SD = standard deviation of the mean.

Discussion

Analysis of the gap

The mass fractions of the different chemical constituents did not always add up to 100%. We therefore asked other laboratories to re-run our analyses. These laboratories confirmed our findings. The gap cannot be explained by the fatty acids, which were not included in the analyses: fatty acids usually have low mass fractions. However, the gap was correlated with the mass fractions of the water-soluble carbohydrates (data not shown), suggesting that especially long-chain carbohydrates could have contributed. Also other studies have reported gaps in the analyses, and have suggested that this unexplained component is likely to have consisted of a mixture of lipids, organic acids, pectins, and other carbohydrate compounds (Van Soest, 1982; Smith *et al.*, 2002).

Attempts to analyse these non-structural poly-carbohydrates were not successful (data not shown).

Lignin and ash

The mass fractions of acid detergent lignin could not be assessed with high accuracy. But as the mass fractions were low the analytical error in assessing the lignin mass fraction did not interfere with the other results.

Average levels and variations in ash mass fractions were considerably larger than those in the mass fraction of lignin, but were more related to soiling than to herbage management treatments. Given the fact that the ash mass fractions were fluctuating, sometimes even considerably, it was an option to express mass fractions of chemical constituents on the basis of the organic matter instead of the dry matter. However, this was not considered to provide more insight than our current approaches of expressing mass fractions on either DM basis or WSC-free DM basis.

Dry matter versus water-soluble carbohydrates-free dry matter

When NDF and ADF were expressed as a proportion of WSC-free DM, treatment effects became much more consistent, which yielded some interesting insights. For example, when NDF was expressed in g per kg DM, no consistent effect of N application rate on NDF mass fraction was found, which is in agreement with the studies of Wilman *et al.* (1977), Valk *et al.* (1996) and Peyraud & Astigarraga (1998). However, when expressed in terms of WSC-free DM, higher N application rates significantly ($P < 0.001$) reduced the NDF mass fraction. In that case the higher N application rate resulted in a higher mass fraction of N, present mainly in the cell contents. As a result the amount of cell wall is relatively lower. Apparently, in terms of g per kg DM, this decrease in cell wall mass fraction is negated by the accompanied decrease in WSC. This phenomenon can also be derived from the data by Valk *et al.* (1996).

Generally the NDF and ADF mass fractions of grass are reported to be lower for high-sugar than for low-sugar cultivars (Smith *et al.*, 1998; 2001; 2002; Lee *et al.*, 2001;

Miller *et al.*, 2001). However, when NDF and ADF were expressed on a WSC-free DM basis, the cultivar effect largely disappeared, indicating that the increase in WSC mass fraction of the HS cultivar was partly obtained at the expense of NDF and ADF (less WSC converted into cell wall material).

In our study the expected increase in NDF and ADF mass fractions with longer regrowth periods and in all seasons was not observed when the mass fractions were expressed in g per kg DM. However, when expressed in g per kg WSC-free DM, it became apparent that this lack of effect was partly due to the relatively strong increase in WSC with the length of the regrowth period. This is in agreement with Wilson (1994), who stated that although the NDF mass fraction usually increases with the length of the regrowth period, this effect may be negated by an increase in storage of WSC components.

Expressing ADF and ADL on the basis of NDF instead of DM or WSC-free DM can provide detailed insight into the effects of herbage management on cell-wall digestibility, but such an approach did not serve our general objective of obtaining insight into the chemical composition of the dry matter.

Effects of the high-sugar cultivar

The higher WSC mass fraction of the HS cultivar compared with the LS cultivar was 43 g per kg DM, which is comparable to or lower than the mass fractions found in other studies (Humphreys, 1994; Miller *et al.*, 2001). The difference between HS and LS was most apparent during the mid and late seasons (S1, S3 and S4, Table 3). This would be an important feature as N losses from grazing tend to be larger during the late season, due to the higher N mass fractions of the grass (Beever *et al.*, 1978) and the increased risk of leaching. However, other studies have reported varying trends in the difference in WSC mass fraction between high- and low-sugar cultivars over the seasons (Jones & Roberts, 1991; Radojevic *et al.*, 1994).

On average, the WSC mass fraction of the HS cultivar compared with the LS cultivar tended to be higher in the lamina material than in the sheath material, especially during the late seasons (Table 3). This would imply that the high-sugar trait of the plant material taken in by the ruminant may be more pronounced under grazing, when lamina material forms the bulk of the intake (Brereton *et al.*, 2005). It is questionable, however, whether the ruminant may benefit from this trait as nutritional quality and intake by grazing dairy cows did not differ significantly among high-sugar and low-sugar cultivars (Smit *et al.*, 2005; Moorby *et al.*, 2006b). We confirmed this in our model studies (N.J. Hoekstra, unpublished results).

Conversely, in three other studies that have measured the WSC mass fraction of the different crop fractions, the relative WSC mass fraction tended to be higher in the 'stem' material (Smith *et al.*, 2001; 2002; Turner *et al.*, 2001). However, the methods these authors used were different from our methods as their experiments were performed under controlled environmental conditions, using different cultivars and seedlings (only up to 42 days of age) rather than mature plants.

For other recent information on the effects of grazing on or feeding of grass with an elevated mass fraction of water-soluble carbohydrates we refer to Moorby *et al.*

(2006a), Shewmaker *et al.* (2006), Smit *et al.* (2006) and Taweel *et al.* (2005; 2006). In general, also these papers report surprisingly little effect of the high-sugar trait on chemical composition, intake and nutritional quality, although dairy cattle obviously prefer the high-sugar grass when offered a choice.

In some cases N mass fraction tended to be slightly higher in the low-sugar cultivar, but this tendency was not consistent. This trend is confirmed in the literature, where higher (Smith *et al.*, 1998; 2001), equal (Lee *et al.*, 2001; Smith *et al.*, 2002) and lower (Miller *et al.*, 2001) mass fractions are reported for high-sugar cultivars compared with control cultivars. This would confirm that the sink–source related negative correlation between WSC and N does not apply across different genetic lines (Humphreys, 1994).

Effects of length of the regrowth period

The WSC mass fraction usually increases with the length of the regrowth period, as was also the case in our experiment. Immediately after cutting, the plant partly relies on WSC reserves for its regrowth and these can be replenished only if the photosynthetic capacity is restored (Fulkerson & Donaghy, 2001). The increase in WSC with time tended to be strongest in sheath material, whereas in the lamina, especially during the late season, hardly any increase took place. This can be explained by the fact that the sheath is the main storage site for WSC. The increase in WSC mass fraction of the sheath increased with N application rate to such an extent that at the last harvest date there was no difference in WSC level among the different N application rates. Apparently, with longer regrowth periods the increased photosynthetic capacity as a result of the increased growth rates at higher N application rates compensates for the amount of WSC that is used as a source of energy for the extra growth.

Generally, the NDF mass fraction increases with the regrowth period due to the maturation of the plant (Wilman *et al.*, 1977; Wilson, 1994; Groot, 1999). In our experiment the changes in NDF mass fraction (g per kg WSC-free DM) with regrowth period were relatively small, but the mass fraction tended to increase with time in all cases except for sheath material during the late season. The negative effect during the late season of 2002 may be explained by the fact that the young sward was still developing, resulting in an increased tillering rate. So while individual sheaths were ageing, the formation of new sheaths decreased the relative age, which resulted in a decrease of NDF material with time. This may also have been the case during the late season of 2003, as tillering rates during this part of the year are likely to be higher in order to compensate for tiller losses after generative regrowth. The effect of the length of the regrowth period on ADF mass fraction was very similar to the effect on NDF.

The observed decrease in N mass fraction with increasing length of the regrowth period is widely documented (Nowakowski, 1962; Wilman *et al.*, 1976). The initial uptake of N is driven by N supply and not by growth, resulting in a high level of N shortly after fertilizer application, which is diluted as the herbage mass increases with time. The N × T interaction that was found can be explained by the higher dilution rate at high N application rates, as both the initial boost in N uptake and growth rate are increased. So the difference in N mass fraction among the different N application rates becomes smaller as the periods of regrowth become longer. During the late season, the decrease in N mass fraction with regrowth period was less pronounced, and the N mass fraction of the sheath even increased. The lower dilution rates can be explained by the reduced growth rates during this season. As explained above, the increase in N mass fraction of sheath material during the late season may be the result of increased tillering rates.

The effect of N application rate

The strong negative effect of a higher N application rate on WSC mass fraction found in this study has been widely documented in earlier studies (Reid & Strachan, 1974; McGrath, 1992; Valk *et al.*, 1996). It can be attributed to an increased crop growth rate in combination with an increase in utilization of carbon for protein synthesis and for the production of energy required for the nitrate reduction preceding protein synthesis (Reid & Strachan, 1974).

N fertilization strongly increases the herbage N mass fraction (Nowakowski, 1962; Reid & Strachan, 1974; Wilman *et al.*, 1976). However, as discussed above, because of the statistically significant N × T interactions, the difference in both WSC and N mass fraction as affected by N application rate becomes smaller with longer regrowth periods.

The effect of cutting height

The effect of cutting height on herbage chemical composition was unclear, with effects being inconsistent and often relatively small. Also the literature on the effect of cutting height is inconclusive. There is some evidence that the digestibility of lamina in cocksfoot (*Dactylis glomerata*) is lower at high initial sward heights due to a decrease in leaf appearance rate (Duru & Ducrocq, 2002), reflecting higher NDF mass fractions. However, studies on the effect of initial sward height on leaf appearance rate of perennial ryegrass have yielded conflicting results (Grant *et al.*, 1981; Hernández Garay *et al.*, 2000). Moreover, the effect on leaf appearance rate is based on the difference in initial sheath length as affected by cutting height. The cutting height in our experiment may not have been low enough to cut off sheaths, which would explain the lack of a direct effect.

Lamina versus sheath material

Distinct differences were observed in chemical composition between lamina and sheath material so that their relative proportions in the intake have a pronounced effect on diet composition. Consistent with literature, the N mass fraction was higher (Grindlay, 1997) and the WSC mass fraction was lower (Smith *et al.*, 2002) for lamina than for sheath material. Generally, the NDF and ADF mass fractions are higher for sheath than for lamina due to the more structural role in lifting the lamina up towards the light (Lantinga *et al.*, 2002; Smith *et al.*, 2002). When expressed in g per kg WSC-free DM this was also found in the current experiment. However, when expressed in g per kg DM, NDF and ADF mass fractions of the sheath were equal to or lower than those of the lamina during all seasons except mid season. This can be partly explained by the sheath material during these seasons, which comprises the sheath tube including the young leaves developing within the tube. During mid season the sheath fraction consisted mainly of real stem material, which has a higher NDF mass fraction compared with sheath material (Minson, 1990).

Seasonal effects

In our experiment the results for the late seasons of 2002 and 2003 were very similar, suggesting that the observed effects were not just typical for newly sown swards or a particular year.

The sheath material had lower N and WSC and higher ADF and NDF mass fractions during mid season than during the other seasons, which could be attributed to the formation of real stems. In contrast to studies of Beever *et al.* (1978), the average N mass fraction in the current experiment was not higher during the late season.

Conclusions

- When NDF and ADF mass fractions were expressed in g per kg WSC-free DM, treatment effects became much more consistent than when expressed in g per kg DM, which yielded interesting insights. We therefore recommend this method for interpreting NDF and ADF mass fractions.
- As expected, the high-sugar cultivar generally had a higher WSC mass fraction than the low-sugar cultivar (on average 25% higher). However, the difference between the two cultivars was especially pronounced during the late season (35%), which would be an important feature to reduce the relatively high N losses from grazing during this period. Moreover, the higher WSC mass fraction of the high-sugar cultivar tended to be higher in lamina material (37% increase) than in sheath material (13% increase). This implies that the high-sugar trait may be utilized more effectively under grazing conditions, when lamina forms the bulk of the intake.
- Longer regrowth periods and lower N fertilizer rates increased WSC mass fractions and decreased N mass fractions; interactions between regrowth period and N application rate were highly significant. NDF and ADF mass fractions were less affected. The NDF mass fraction in terms of g per kg WSC-free DM tended to be higher at low N application rates and at longer regrowth periods.
- The prevalence of statistically significant interactions stresses the importance of evaluating the effects of N application, regrowth period and grass cultivar in factorial experiments, rather than in isolation.
- The effect of cutting height on herbage chemical composition was unclear. The effect probably depends more on the proportion of stem, lamina and dead material in the sward than on the changes in chemical composition of the individual components.

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