

Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows

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Abstract

Eight multiparous Holstein–Friesian dairy cows in late lactation were used to investigate the potential of using perennial ryegrass with a high concentration of water-soluble carbohydrate (WSC) to increase the efficiency of milk production. After a pretreatment period on a common pasture, the cows were each given *ad libitum* access to one of two varieties of zero-grazed grass continuously for 3 weeks. Treatments were: high sugar (HS), an experimental perennial ryegrass variety bred to contain high concentrations of WSC; or control, a standard variety of perennial ryegrass (cv. AberElan) containing typical concentrations of WSC. The two grass varieties were matched in terms of heading date. All animals also received 4 kg day⁻¹ standard dairy concentrate. Grass dry matter (DM) intake was not significantly different between treatments (11.6 vs. 10.7 kg DM day⁻¹; s.e.d. 0.95 for HS and control diets respectively), although DM digestibility was higher on the HS diet (0.71 vs. 0.64 g g⁻¹ DM; s.e.d. 0.23; $P < 0.01$) leading to higher digestible DM intakes for that diet. Milk yield from animals offered the HS diet was higher (15.3 vs. 12.6 kg day⁻¹; s.e.d. 0.87; $P < 0.05$) and, although milk constituent concentrations were unaffected by treatment, milk protein yields were significantly increased on the HS diet. The partitioning of feed N was significantly affected by diet, with more N from the HS diet being used for milk production (0.30 vs. 0.23 g milk N g⁻¹ feed N; s.e.d. 0.012; $P < 0.01$) and less being excreted in urine (0.25 vs. 0.35; s.e.d. 0.020;

$P < 0.01$). In a separate experiment, using the same grasses harvested earlier in the season, the fractional rate of DM degradation, measured by *in situ* and gas production techniques, was higher for the HS grass than for the control. It is concluded that increased digestible DM intakes of the HS grass led to increased milk yields, whereas increased efficiency of utilization of the HS grass in the rumen resulted in the more efficient use of feed N for milk production and reduced N excretion.

Keywords: nitrogen balance, nitrogen use efficiency, gas production, *in situ* degradation

Introduction

In the move towards more sustainable production systems, levels of milk production are limited by a low efficiency of utilization of grass nitrogen (N) for milk production, largely as a result of poor conversion efficiency of forage N to microbial N and consequential losses of N from the rumen (Beever *et al.*, 1986; Ulyatt *et al.*, 1988). A significant proportion of dietary N can be lost from the rumen as ammonia because of the rumen microbial population's inability to capture the non-protein nitrogen (NPN) released during the proteolysis of plant proteins (Huntington, 1984; Beever *et al.*, 1986; Kingston-Smith and Theodorou, 2000). This is partly because leaf proteins in grazed grass are highly soluble and rapidly degraded by plant and microbial proteases after ingestion (Wallace, 1995; Zhu *et al.*, 1999). When supplies of fermentable carbohydrates are readily available in the rumen, amino acids taken up by the microorganisms can be incorporated into microbial protein. However, if there is insufficient energy, in the form of adenosine triphosphate (ATP), which is derived mainly from carbohydrate fermentation, amino acids are used as an energy source; this leads to

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ammonia accumulation in the rumen (for reviews, see Nocek and Russell, 1988; Kingston-Smith and Theodorou, 2000). Limited supplies of energy in the rumen also mean that the ammonia that is produced is not assimilated into microbial protein, but is absorbed across the rumen wall and largely lost from the animal after hepatic conversion into urea. When the availability of water-soluble carbohydrate (WSC) is relatively low, structural components of the plant (cellulose and hemicellulose) are used by rumen microorganisms for the bulk of their energy supply and, as a result, both the balance and the temporal release of N and energy-yielding components can be out of phase (Nocek and Russell, 1988). One of the reasons for the increased nutritive value of spring grass compared with autumn grass is the increase in amino acid absorption brought about mainly because the WSC concentration in the dry matter (DM) of the spring grass is twice that in the autumn grass (MacRae *et al.*, 1985).

The objective of this study was to test the hypothesis that increased WSC concentrations in grass can lead to increased efficiency of utilization of dietary protein for milk production by dairy cows. The work was achieved using a novel variety of perennial ryegrass that was bred by conventional techniques at the Institute of Grassland and Environmental Research (IGER) to express increased concentrations of WSC.

Materials and methods

An experiment was carried out at the Trawscoed Research Farm (52°25'N, 4°05'W) of IGER to test the effect of grasses with normal and high WSC concentrations on milk production from dairy cows. To investigate potential differences in rumen function from the two grasses, two further experiments were carried out: an *in situ* degradation experiment and an *in vitro* gas production experiment.

Animals and their management

Eight multiparous Holstein–Friesian dairy cows (628, s.e. 9.4, kg live weight) in mid- to late lactation (176, s.e. 3.6, days in milk) were used in a short-term (3-week), zero-grazing experiment. Before the start of the experiment, all animals grazed together on a perennial ryegrass (*Lolium perenne* L.) pasture and, during the week before the start of the experiment, covariate measurements of milk yield (7 days) and composition (from four consecutive milkings) were taken. Animals were then assigned to treatment at random, balanced for milk yield and housed in individual stalls fitted with neck yokes, where they remained for the rest of the experiment. The first 2 weeks of the 3-week experiment were used for

adaptation and the third week for measurements. All animals had free access to fresh water and a mineral lick (red Baby Rockies mineral blocks for cattle; Tithebarn, Winsford, Cheshire, UK) at all times.

Diets

Two dietary treatments were imposed: high sugar (HS), an experimental variety (Ba11353) of perennial ryegrass that had been bred to express elevated concentrations of WSC (Humphreys, 1989); and control, a commercially available ryegrass (cv. AberElan) with a similar intermediate heading date (National Institute of Agricultural Botany, 1997) and with concentrations of WSC typical of perennial ryegrass varieties.

The two perennial ryegrass varieties were sown at the Trawscoed Research Farm in the spring of 1997 as monoculture plots of 1.5 ha each, separated by a guard plot in the same field of well-drained valley soil, consisting of a silty loam over silurian shale. In early 1998, each plot was split into three subplots that were subsequently managed to provide sufficient fresh grass to be offered zero grazed for each week of the experiment. Primary growth of each variety was grazed with sheep in February 1998. A compound fertilizer (25:5:5, N:P:K) was applied to the plots at a rate of 62.5 kg N ha⁻¹, 12.5 kg P₂O₅ ha⁻¹ and 12.5 kg K₂O ha⁻¹ on 23 March 1998, and the plots were cut for a silage crop on the 21 May 1998. Fertilizer was again applied to the plots at the same rates on 28 May 1998. Grass was harvested for *in situ* measurement of degradation in mid-May 1998 and used fresh. Some of the grass cut at this time was stored frozen (–15°C) for later use in the gas production experiment. The milk production experiment was carried out using a 6-week regrowth harvested in July 1998.

During the production experiment, fresh grass was harvested daily in the early afternoon using a Haldrup 1500 plot harvester (J. Haldrup, Løgstør, Denmark). Forage was cut to a height of 5 cm above soil level with a reciprocating finger bar and collected into a storage compartment using a conveyor belt. The cut forage was immediately transported from the field and placed in large weld-mesh containers (120 × 80 × 100 cm, length × depth × height), which were transferred to a blast freezer kept at –15°C for 2 h to chill the grass, without freezing, and prevent depletion of WSC as a result of continued plant respiration. A short piece of perforated drainage tube (20 cm diameter × 80 cm long) was placed in the middle of the grass in each basket to allow cold air to circulate and reach the middle of the material. After 2 h in the blast freezer, approximately half the forage was offered to the dairy cows, at 17.00 h, and the remainder of the fresh forage was transferred to a cold room kept at 4°C, where it stayed

until it was offered to the cows the following morning at 09.00 h. Forage refusals were removed and weighed before fresh material was offered to the animals.

All animals received enough fresh forage for the measurement of *ad libitum* intakes with refusals of at least 0.1 of the forage offered. All animals also received a standard concentrate offered at the rate of 4 kg day⁻¹ in two equal portions, 2 kg at each milking at approximately 08.00 h and 16.00 h. The concentrate was a commercial product containing wheat, soybean meal, molasses, rapeseed meal, sugar beet pulp, maize gluten, palm kernel meal, sunflower meal, vegetable oil and a mineral and vitamin mix.

Herbage monitoring

To monitor seasonal changes in grass WSC concentrations, snip samples were collected from the grass plots weekly from the beginning of March until the end of August. Several snip samples were cut at a height of ≈5 cm above soil level from across each plot along a 'W' transect and immediately frozen in liquid nitrogen and bulked to give a sample of 200–300 g of fresh material from each plot. Bulked samples were stored frozen at -15°C before being freeze dried, ground to pass through a 1-mm dry mesh screen and analysed for WSC concentration. On three consecutive days in August 1998, similar bulked snip samples were collected in the same way from the plots every hour between 06.00 h and 20.00 h to monitor diurnal changes in grass WSC concentration.

To monitor changes in WSC concentration during storage and feeding, representative samples of each grass variety were collected from each of the feed containers and bulked within each grass variety at five sampling times on four consecutive days during the second adaptation week. Samples were taken: (i) immediately after cutting/collection; (ii) at the afternoon feeding time; (iii) when refusals were removed in the morning; (iv) at the morning feeding time; and (v) when refusals were collected in the afternoon. Samples were immediately frozen before being freeze dried, ground to pass through a 1-mm dry mesh screen and analysed for WSC concentration.

Animal measurements

Milk yields were recorded daily, and milk samples were collected throughout the last six days of the experiment (during the N balance measurement period). Five millilitres of milk kg⁻¹ milk yield was collected from each milking and bulked over the course of the N balance measurement period. The samples were preserved with a LacTab milk preservative tablet (Thompson and Capper, Runcorn, Cheshire, UK).

Grass samples were collected from the feed containers when offered to the cows both morning and afternoon and were bulked during the N balance measurement period. Samples were stored frozen before analysis. Concentrate samples were taken similarly and bulked during the measurement week.

Nitrogen balance was measured by collecting the total production of urine and faeces from each animal, using externally applied urine and faeces separators, for a period of 6 days (Aston *et al.*, 1998). Urine was preserved by acidification (using 1.5 l of 2 mol l⁻¹ sulphuric acid for each day's collection), and subsamples were collected daily, cooled to 4°C and bulked on a weight basis (0.01 of each day's collection) with collections from the previous days, before storage at -15°C at the end of the collection week. Daily subsamples of faeces were collected after thorough mixing and bulked on a weight basis using 0.05 of the total daily collection over the course of each period, during which it was stored at 4°C. After the collection week, the bulked faecal samples were thoroughly mixed, and approximately 500 g was collected and frozen for later analysis. Nitrogen balance was calculated as the mean quantity of N consumed over the N balance measurement period minus the mean of total N excreted in faeces and urine and N secreted in milk over the same period. No correction factors were imposed for losses of N through skin and hair.

In situ degradation of grass DM in polyester bags

Fresh grass was collected and bulked from the experimental plots in mid-May 1998, cutting at 5 cm above ground level from five sites within each plot using a 'W' transect design. The grass was immediately placed in a blast freezer kept at -15°C, where it was left to chill for ≈5 min, without freezing, to reduce respiration. The grass was then lightly bruised for 2 min to simulate the effect of chewing using a Linhakker rotating blade (model GH5232; Georg Hansen, Copenhagen, Denmark) and then returned to the blast freezer to chill for a further 5 min before being placed in polyester incubation bags (pore size 40 µm). The bags of grass were incubated in the rumens of two multiparous late-lactation Holstein-Friesian dairy cows fitted with established rumen cannulae. Single bags containing ≈4 g of DM of each grass variety were incubated in each cow for 0, 1, 6, 12, 24, 48 or 72 h. During the incubation period, the cows grazed on a mixed grass pasture dominated by perennial ryegrass and were given 4 kg day⁻¹ dairy concentrate in two equal portions at milking. Initial DM solubility was determined by washing the 0-h incubation samples in a domestic washing machine on a 20-min cycle using cold water

and without spinning. After removal from the rumen after their designated incubation times, the remaining polyester bags were rinsed under cold running tap water for 2 min and then washed in a domestic washing machine, as described for the 0-h incubation samples. The residues were freeze dried to a constant weight to measure the DM content. Degradability kinetics were described by fitting the model described by Ørskov and McDonald (1979): $y = a + b(1 - e^{-cx^t})$ using the FITNON-LIN procedure from GENSTAT 5 (Lawes Agricultural Trust, 1998).

Gas production and DM degradation

A portion of the grass harvested for the *in situ* degradation experiment was frozen immediately after collection and later freeze dried and ground to pass through a 1-mm dry mesh screen. Triplicate samples of ≈ 1 g DM were accurately weighed into gas-tight culture bottles with 75 ml of an anaerobic digestion medium (Longland *et al.*, 1995) and inoculated with 10 ml of rumen fluid taken from a Holstein-Friesian dairy cow, fitted with a permanent rumen cannulae, that grazed grass. The samples were incubated at 39°C, and the time course of cumulative gas production was measured, using the automated pressure evaluation system of Davies *et al.* (2000). Apparent DM loss was estimated after 72 h of incubation by filtering the residue remaining in the culture bottles through sintered crucibles (porosity 1).

All gas produced over the 72-h period was automatically measured from each bottle. Cumulative gas volume (y ; ml) was obtained using the model of France *et al.* (1993):

$$y = A \left\{ 1 - e^{[-b(t-T) - c(\sqrt{t} - \sqrt{T})]} \right\} \quad (1)$$

where A = predicted asymptotic value for gas pool size (ml), b = rate constant (h^{-1}), c = rate constant (0.5 h^{-1}), t = incubation time (h) and T = lag time (h).

Data were fitted to the equation using the maximum likelihood program (Ross, 1987). The fractional rate of degradation (μ) was calculated using the equation (France *et al.*, 1993):

$$\mu = b + (c/2 \times \sqrt{t}) \quad (2)$$

The extent of rumen degradation was predicted, assuming a rumen passage rate of 0.033 h^{-1} , as described by France *et al.* (1993).

Sample analyses

Bulked milk samples were analysed in duplicate for concentrations of total N, non-casein N and non-

protein nitrogen (NPN) by the methods of Rowland (1938). Samples from four consecutive milkings taken during the pre-experimental grazing period and the N balance measurement period were analysed for fat and lactose by the National Milk Records laboratory (Yeovil, Somerset, UK).

The analyses of feed and faeces samples were carried out using freeze-dried material ground to pass through a 1-mm dry mesh screen. Dry matter content was determined by drying at 100°C for 24 h. Organic matter (OM) was determined as DM minus ash by combustion at 550 °C. Total N content was measured by combustion using a Leco FP-428 analyser (Leco Corporation, St Joseph, MI, USA); feed crude protein (CP) was calculated as $N \times 6.25$, and milk crude protein was calculated as $N \times 6.38$. Neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) analyses were carried out according to the methods of Van Soest *et al.* (1991). Concentrations of WSC in the grass samples taken to monitor seasonal and diurnal changes were estimated using near infrared (NIR) spectroscopy (NIR Systems; Perstorp Analytical, Berkshire, UK) as described by Lister and Dhanoa (1998). Concentrations of WSC in the feed samples taken at feeding were measured using the anthrone technique (Thomas, 1977). Digestibilities of DM, OM, CP, ADF and NDF in the diet were calculated from the quantities in feed and faeces. The ME content of the concentrate was predicted using the E3 equation using the neutral cellulase gamanase digestibility and acid hydrolysis ether extract content as described by Thomas *et al.* (1988). The metabolizable energy content of the diet was estimated from digestible OM kg^{-1} DM (DOMD) (metabolizable energy = $0.016 \times \text{g of DOMD}$; Agricultural and Food Research Council, 1993).

Statistical analysis

Milk production data were analysed statistically by analysis of covariance, using individual animals as blocks, grass variety as a treatment factor, and data from the pre-experimental grazing period as covariates, with the GENSTAT 5 statistical software package (Lawes Agricultural Trust, 1998). Feed intake, digestibility, liveweight change and nitrogen partitioning data were all subjected to analysis of variance in a similar way but without covariate adjustment. Water-soluble carbohydrate concentrations in samples of grass taken during storage and at feeding were subjected to analysis of variance using day of collection as a blocking factor and sample time, grass variety and their interaction as factors. Gas production data were subjected to analysis of variance using grass variety as a factor and replicate (i.e. gas production bottle) as a blocking factor.

Results

Concentrations of WSC in the two grasses varied considerably with both season (Figure 1) and time of day (Figure 2). From the beginning of March, the WSC concentrations of the HS grass were always higher than those of the control grass and, by the beginning of May, the concentrations of WSC in HS grass peaked at over 350 g kg⁻¹ DM, whereas in the control grass, the WSC concentrations peaked at about 240 g kg⁻¹ DM. After the plots were cut for silage at the end of May, there was a period of ≈5 weeks before the difference in WSC concentration again became substantial, although the WSC concentrations were much lower in regrowth material than in primary growth, with maximum

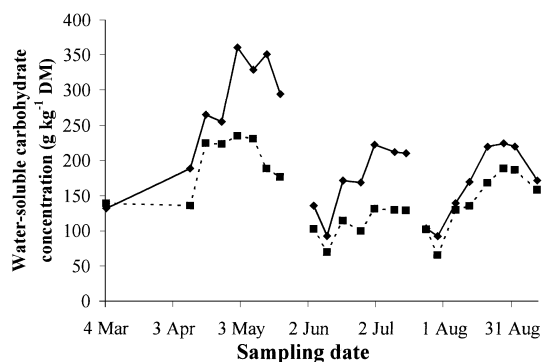


Figure 1 Mean water-soluble carbohydrate concentrations of snip samples of high sugar (—◆—) and control (---■---) grasses from March to September 1998. Lines are broken after cutting for silage in May and for the production experiment in July.

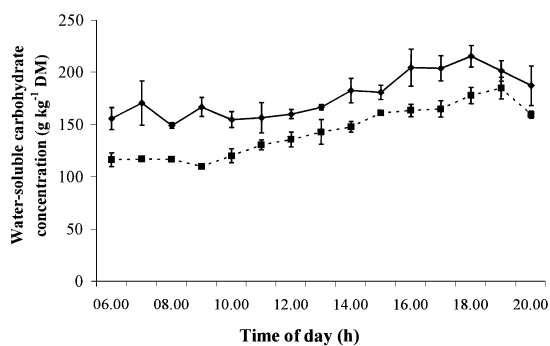


Figure 2 Mean water-soluble carbohydrate concentrations of snip samples of high sugar (—◆—) and control (---■---) grasses (with s.e.m.) from 06.00 to 20.00 h over three consecutive days in August 1998. The three days were overcast, sunny and overcast, with a minimum temperature of 13°C and a maximum temperature of 19°C.

concentrations of about 220 and 140 g WSC kg⁻¹ DM in HS and control grasses respectively. After cutting the plots for the production experiment in July, the difference in WSC concentration between the two grass varieties remained small until the end of August, when sampling ceased. The WSC concentration increased in both grass varieties as the day progressed. However, the concentration of WSC in the HS grass was always higher than that in the control grass.

The concentration of WSC in the fresh grasses offered to the animals or used in the DM degradation experiments differed considerably between the HS and control ryegrass varieties (Table 1). Differences in nutritional composition of the two grasses used in the degradation experiment compared with the *in vivo* production experiment are attributed to environmental effects (i.e. season) and the different stages of grass growth.

In both experiments, WSC concentrations were significantly higher in the HS grass, and the concentrations of CP, NDF and ADF were significantly lower. The nutritional composition of the concentrate feed is also presented in Table 1. The WSC concentrations of the grasses used *in vivo*, when measured at different stages during cutting, storage and feeding, were found not to be significantly affected by storage, either when kept at 4 °C or when on offer to the animals at ambient temperatures. There was no significant effect on WSC concentrations of time at which the samples were taken during storage (as cut, at the afternoon feeding, at morning collection of refusals, at morning feeding after overnight storage or at afternoon collection of refusals; s.e.d. 14.8 g kg⁻¹ DM), although there was a significant effect of grass variety (grand means of 191 and 145 g kg⁻¹ DM for HS and control grasses, respectively, as sampled over the 4 days; s.e.d. 9.4; $P < 0.001$); there was no interaction between variety and time of sampling.

The intake of forage DM was not significantly different between the two treatments (Table 2). However, the DM digestibility of the HS grass-based diet was significantly greater than that of the control diet, leading to significantly increased intakes of digestible DM. Digestibility of NDF and ADF was also significantly higher on the HS diet, but N digestibility was not significantly different. The estimated ME values of the diets were significantly different at 10.5 and 9.5 MJ kg⁻¹ DM (s.e.d. 0.19; $P < 0.01$) for the HS and control diets respectively.

Milk yields from animals offered the HS diet were significantly increased by ≈2.5 kg day⁻¹ (Table 3) and, although milk constituent concentrations were unaffected by dietary treatment, the yields of milk crude protein, true protein and casein all increased significantly as milk yield increased. Treatment differences between milk fat and lactose yields nearly reached

Table 1 Dry-matter content, concentration of chemical entities and metabolizable energy values of the high sugar (HS) and control grasses (mean of seven samples) and concentrate (mean of three samples) fed to dairy cows during the production experiment, and of the grasses used for the degradation studies (*in vitro* and *in situ*; one bulked sample).

Grass variety	Production experiment						Degradation experiments	
	HS	s.e.	Control	s.e.	Concentrate	s.e.	HS	Control
Dry matter (g kg ⁻¹)	213	8.8	198	5.1	880	2.2	190	170
Organic matter	930	1.5	931	0.8	895	4.9	912	923
Water-soluble carbohydrate	165	16.8	126	10.0	57	2.5	351	189
Crude protein (N × 6.25)	92	1.5	106	2.8	193	2.0	108	122
Metabolizable energy (MJ kg ⁻¹ DM)	–	–	–	–	11.9	0.5	–	–
Neutral-detergent fibre	544	4.4	589	2.9	380	8.7	380	488
Acid-detergent fibre	300	1.8	330	2.7	195	2.7	–	–
Ether extract	20	0.5	18	0.4	–	–	–	–
Acid hydrolysis ether extract	–	–	–	–	43	1.9	–	–

Units: g kg⁻¹ DM (unless stated otherwise).

	Treatment		s.e.d.	Significance†
	HS	Control		
Grass DM intake (kg day ⁻¹)	11.6	10.7	0.95	NS
Total DM intake (kg day ⁻¹)	15.1	14.2	0.95	NS
Digestible DM intake (kg day ⁻¹)	10.7	9.1	0.58	*
Digestibility of:				
Dry matter	0.71	0.64	0.014	**
Nitrogen	0.61	0.58	0.023	NS
Neutral-detergent fibre	0.70	0.63	0.019	**
Acid-detergent fibre	0.68	0.60	0.018	**

†NS, not significant; **P* < 0.05; ***P* < 0.01.

	Treatment		s.e.d.	Significance†
	HS	Control		
Milk yield (kg day ⁻¹)	15.3	12.6	0.87	*
Milk constituent concentrations (g kg ⁻¹)				
Fat	48.2	48.2	4.00	NS
Crude protein	34.5	34.5	1.21	NS
True protein	33.5	33.1	1.12	NS
Casein	27.7	26.9	1.10	NS
Whey	5.86	6.18	0.253	NS
Non-protein nitrogen	0.16	0.22	0.033	NS
Lactose	44.8	43.6	0.73	NS
Milk constituent yields (g day ⁻¹)				
Fat	737	606	51.5	+
Crude protein	526	437	20.2	**
True protein	510	420	20.4	**
Casein	421	341	18.5	**
Whey	88.8	78.9	4.06	NS
Non-protein nitrogen	2.47	2.68	0.362	NS
Lactose	692	546	57.1	+

†NS, not significant; +*P* = 0.051; **P* < 0.05; ***P* < 0.01.

Table 2 Feed intake and digestibilities of the diet of dairy cows offered zero-grazed high sugar (HS) and control grass-based diets.

Table 3 Milk yield and composition data from dairy cows offered zero-grazed high sugar (HS) and control grass-based diets.

statistical significance ($P = 0.051$ for both variables), with increased yields from animals offered the HS diet. The ratio of milk yield to digestible DM intake was not significantly different between the two treatments (1.44 vs. 1.38 kg milk kg^{-1} digestible DM; s.e.d. 0.074).

Despite similar intakes of dietary N, there was a marked reduction in the amount of N excreted in the urine of cows offered the HS diet compared with the control diet (Table 4). There was also an increase in the amount of N secreted in milk by animals offered the HS diet, but there was no effect of diet in the amount of N excreted in the faeces nor on overall N balance.

In situ dry-matter degradation

The model of Ørskov and McDonald (1979) fitted the *in situ* DM disappearance data well (Figure 3). The data in Table 5 indicate that there was little difference between the grass varieties in the 'truly' soluble (plus escaped insoluble) fraction (*a*) nor in the potentially degradable and less soluble fraction (*b*). The fractional rate of degradation of *b* (*c*) and the potential degradability of the DM (*a* + *b*) tended to be higher for the HS grass than for the control grass.

Gas production

There were no differences between the two grass varieties in the lag time (0.93 and 0.93 h; s.e.d. 0.099, for HS and control grass respectively), nor in the time taken to produce 0.50 and 0.95 (T95: 26.2 and 30.6 h; s.e.d. 2.57) of the total gas produced. Similarly, cumulative gas production and the amount of gas produced per gram of substrate DM apparently degraded was not

different between the two grasses at T50 or T95 (251 and 266 ml g^{-1} DM at T95; s.e.d. 41.2). However, the fractional rates of degradation (μ) were considerably higher throughout the time course (Figure 4) with mean rates of degradation of 0.0476 vs. 0.0217 h^{-1} for HS and control respectively (s.e.d. 0.00507; $P < 0.001$). Despite an increased fractional rate of degradation, the predicted extent of rumen DM degradation was similar for both grasses, with values of 0.52 vs. 0.50 g g^{-1} DM (s.e.d. 0.016) for HS and control grasses respectively.

Discussion

Seasonal changes in the WSC concentrations of the two varieties of grass used in the current study followed similar trends to those observed previously by others

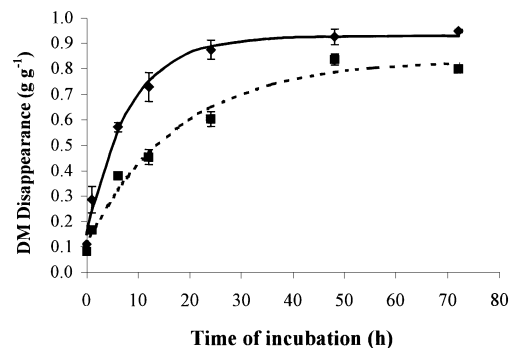


Figure 3 Mean *in situ* dry matter disappearance curves ($n = 2$) for high sugar (—◆—) and control (---■---) grasses (with s.e.m.) incubated in the rumens of two late-lactation dairy cows.

Table 4 Nitrogen intake and partitioning in dairy cows offered zero-grazed high sugar (HS) and control grass-based diets.

	Treatment		s.e.d.	Significance†
	HS	Control		
Nitrogen intake (g day^{-1})				
Grass	171	181	7.3	NS
Concentrate	109	109	—	—
Total nitrogen intake (g day^{-1})	280	290	15.1	NS
Nitrogen output				
Urine (g day^{-1})	71.3	100	5.0	***
Proportion of N intake	0.25	0.35	0.020	**
Faeces (g day^{-1})	110	121	10.7	NS
Proportion of N intake	0.40	0.42	0.023	NS
Milk (g day^{-1})	83.3	67.5	4.12	**
Proportion of N intake	0.30	0.23	0.012	**
Total output (g day^{-1})	265	289	15.8	NS
Nitrogen balance (g day^{-1})	15.1	1.3	8.83	NS
Proportion of N intake	0.05	0.00	0.033	NS

†NS, not significant; ** $P < 0.01$; *** $P < 0.001$.

	$\text{g g}^{-1} \text{ DM}$			h^{-1}	s.e. observations	r^2
	<i>a</i>	<i>b</i>	<i>a + b</i>	<i>c</i>		
HS	0.150	0.771	0.927	0.124	0.0496	0.97
Control	0.115	0.715	0.831	0.058	0.0472	0.97

Table 5 *In situ* dry-matter degradability characteristics (mean of two samples) of high sugar (HS) and control grass varieties derived from the model of Ørskov and McDonald (1979).

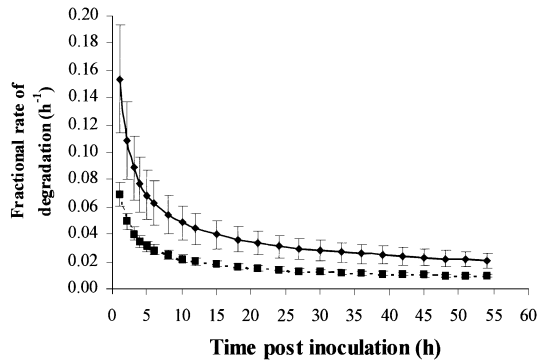


Figure 4 Mean ($n = 3$ for each data point) fractional rates of degradation produced using the *in vitro* gas production technique for high sugar (—◆—) and control (—■—) grasses (with s.e.m.).

(McGrath, 1988; Humphreys, 1989; Givens *et al.*, 1993; Radojevic *et al.*, 1994). Concentrations increased from the start of the main growing season, reached a peak in late May as heading progressed and declined towards the end of the growing season. After cutting for silage in late May, and again after the cutting of the grasses for the production experiment in July, WSC concentrations decreased, and there was little difference in the sugar content between the two grasses until after ≈ 5 weeks of regrowth. This can be attributed to the use of WSC for protein synthesis and plant growth, which is influenced in turn by the levels of N available to the plant. Thus, concentrations of WSC are negatively correlated with N fertilizer application (Peyraud and Astigarraga, 1998; Keating and O'Kiely, 2000). The quantity of fertilizer applied in the preparation of the plots for this study was relatively low (Agricultural Development and Advisory Service, 1983). This level of fertilizer application was chosen to ensure that varietal differences in WSC were not diminished by the effects of adding fertilizer N. However, it is important to note that the milk yield responses in the present study were obtained at a time when WSC concentrations, despite being some 1.3 times greater in the HS grass than in the control grass, were not as great as they had been earlier in the year when harvested for the degradation experiment. Similarly, later in the season, WSC concentrations were hardly different between varieties. Speculation about milk yield responses at different stages of lactation, and

at different times during the growth season, is beyond the scope of this discussion and is a subject for further research.

Diurnal changes in grass WSC concentration were similar for the two varieties used, with the HS grass containing higher WSC concentrations than the control grass throughout the day. Greater variability in WSC concentrations was found in the HS grass compared with the control grass, and it is not known why this occurred. However, it is possible that this is caused by differences in the physiology of the HS grass variety compared with the control, which make the plants more sensitive to environmental light intensity (e.g. changes in cloud cover).

Feed intake and milk production

In the grazing ruminant, DM intake is influenced by sward structural characteristics (Burlison *et al.*, 1991; Laca *et al.*, 1992), sward availability (Dalley *et al.*, 1999; Vazquez and Smith, 2000) and grazing behaviour (Gross *et al.*, 1993). Furthermore, current methodologies only allow the estimation of feed intake at grazing, rather than its accurate measurement (Mayes and Dove, 2000). Zero-grazing methodology was therefore used in the current study, thus eliminating the effect of sward structural characteristics and allowing the response in intake to the nutritional composition of the grasses offered to be quantified. However, it is accepted that, because of the factors listed above, the results from a zero-grazing experiment may not be directly applicable to the grazing situation. Also, by cutting before feeding, the nutritional composition of the grasses was maintained constant throughout each 24-h period, despite the WSC concentration of the uncut grasses changing over the course of each day. Orr *et al.* (1998) found that dairy cows consumed the greatest proportion of their total daily DM intake after being moved to fresh pasture, and when fresh pasture was offered after afternoon milking instead of after the morning milking. Under the circumstances of that study, the cows produced significantly more milk at the same level of DM intake, although the grass consumed by the cows offered fresh pasture in the afternoon contained more WSC than the pasture offered in the morning. Milk yields were similarly increased in the current study without a significant

increase in DM intake when cows were offered ryegrass with a higher WSC concentration than the control.

Despite there being no significant difference in DM intake, there were significant differences in digestibility between the two diets, leading to an increase of $>1.5 \text{ kg day}^{-1}$ digestible DM intake by HS cows. Digestibilities of N and NDF were substantially lower than those reported by van Vuuren *et al.* (1992) for perennial ryegrass, although they reported that the digestibilities were lower for grasses produced at lower rates of N fertilizer application; the rates of N fertilizer applied by these authors were, however, still higher than that used in the present study. Peyraud and Astigarraga (1998) reviewed the effects of altering the nutritional composition of herbage by altering the rate of N fertilizer application and concluded that herbage intake is generally unaffected by the level of fertilizer application. The CP concentrations of both grasses offered in the current study were rather low, which was probably the result of moderately low rates of fertilizer N applied, and were similar to the values at the lower end of fertilizer N application rates found by de Visser *et al.* (1997) and Keating and O'Kiely (2000). Voluntary intake of forages by ruminants is largely dependent on feed retention time in the rumen (Thornton and Minson, 1972), and this is principally affected by the fibre components of the diet. Nandra *et al.* (1993) also found that organic matter intake was affected by the fibrous components of the diet and not by readily soluble fractions, such as WSC; given a choice, cattle will select forage diets that maximize the digestible organic matter intake (Lippke, 1986). In the current study, offering the grass as zero-grazed material severely restricted the selection opportunities of the cows, and differences in the NDF concentration of the diets were apparently not large enough to cause significant differences in DM intake. However, differences in the WSC concentrations of the diet, together with differences in the digestibility of the fibre fractions of the diet, which is not necessarily indicative of the degradation of fibre in the rumen (Berzaghi *et al.*, 1996), led to the increased digestible DM intake by animals offered the HS diet. The increased intake of fresh grass in the present study contrasts with the work of Rooke *et al.* (1987) with ensiled grass, who reported a reduction in forage intake, caused by a reduction in rumen fibre fermentation, when exogenous sugars were infused.

Milk yield and composition is largely controlled by nutrient supply to the udder (Sutton and Morant, 1989; DePeters and Cant, 1992), which in turn is influenced by digestible DM intake. The major effect of increased digestible DM intake by animals offered the HS diet in the present study was that of an increase in milk yield without any effect on milk composition, and thus the

ratio of milk yield to digestible DM intake was the same for animals on both treatments. Other work investigating the effect of altering the CP:WSC ratio of grass by different rates of N fertilizer application has shown that, at high levels of application, the CP concentration of the grass increases concomitantly with a decrease in the WSC content. Under these circumstances, large quantities of ammonia can be absorbed from the gut (O'Mara *et al.*, 1997), but the flow of amino acids to the duodenum can remain unaffected (van Vuuren *et al.*, 1992; O'Mara *et al.*, 1997). We conclude that, in the present study, when WSC concentrations in the grass were increased with no difference in the concentration of CP, the increase in milk yield was achieved through an increase in the supply of nutrients to the udder, without significant differences in the relative supplies of the various nutrients needed to produce the constituents of milk. For this to have occurred, there must have been a significant improvement in the efficiency of utilization of rumen-degradable nitrogen, principally by the rumen microbial population, although this can only be inferred as no direct measurements were made. However, gross differences in the partitioning of feed N towards milk and urine suggest that this is the case, and further experimentation is required to investigate this.

Nitrogen utilization

There was no difference in the intake of N by animals offered the diets. However, the partitioning of digestible dietary N was significantly different, with more dietary N being partitioned into milk and less into urine in animals offered the HS diet. A 0.30 proportional increase in dietary N in the milk of HS cows compared with that in the control cows was balanced by the 0.26 proportional decrease in N excretion in urine. The gross efficiency of use of dietary N for milk production by animals offered the control diet (0.23) was similar to values quoted in the literature (Astigarraga *et al.*, 1993; Kolver *et al.*, 1998), although the value can be much lower (Kolver and Muller, 1998). The differences in the partitioning of digestible N between milk protein and N excreted in urine suggest that the efficiency of utilization of N within the rumen was increased. After the rapid degradation of proteins in the rumen, a large proportion of feed N may be absorbed from the gut as ammonia and, in some cases, the quantities of ammonia N can equal or even exceed the amount of non-ammonia N that is absorbed by the animal (Huntington, 1984). Absorbed ammonia is extracted from the portal vein by the liver, where it enters the urea (ornithine) cycle. A substantial proportion of the resulting urea is recycled back to the gut, either through the saliva or directly across the gut walls (Nolan and Leng, 1972;

Huntington, 1989), but the remainder is excreted in urine and thus lost from the animal.

Increases in the partitioning of dietary N to milk protein are also of practical importance because they reduce the excretion of N into the environment. This is becoming an increasingly important issue, particularly in Europe (Smith and Frost, 2000), and the use of grass varieties with high WSC concentrations may offer a means of reducing urine-N excretion.

***In vitro* and *in situ* dry-matter degradation**

Two different techniques were used to study the fermentation of the two grasses *in vivo* and *in vitro*. The disappearance of DM of the HS grass measured from polyester bags was slightly higher than that of the control grass, but no significant differences in the extent of degradation were observed between the two grasses when the gas production technique was used. López *et al.* (1998) showed clearly that the gas production technique was more suitable than gravimetric techniques, including *in situ* methods, for assessing the fermentation of the water-soluble pool. However, the fractional rate of DM degradation was consistently higher for the HS grass compared with the control when measured in both gas production and *in situ* degradation experiments.

Although the greater degradability of DM could be attributable to the lower fibre fraction within the HS grass, the fact that this grass was degraded at a faster rate suggests that the additional WSC induced a microbial effect. This result is in agreement with previous research in which the WSC concentration of grasses has been shown to be highly correlated with the rate of gas production and the rate of degradation (Davies *et al.*, 1994).

The WSC fraction of grass is released over a period of 6–8 h in the rumen (L. A. Miller, unpublished results) and, therefore, increases in the fibre digestibility of the HS grass may have been a result of a shift in the microbial population towards genera that are both sugar and fibre fermenters. This is in contrast to the work of Rooke *et al.* (1987), who found that rumen fibre degradation of ensiled grass was reduced when additional sugars were infused into the rumen. Further research is needed to elucidate the exact nature of the mechanisms giving these results.

Increases in the utilization of rumen-degradable protein have been observed when ensiled and dried grasses have been supplemented with sugars (Rooke *et al.*, 1987; Heldt *et al.*, 1999). In the present study, additional sugars were provided as an integral part of the HS diet, and increased rates of fermentation of the energy-yielding components of the diet may have led to improved balance or synchrony in the use of protein

and energy-yielding components of the diet, which could in turn have led to reduced absorption of ammonia from the gut (and thus reductions in urinary N excretion) and increased microbial protein flow to, and absorption from, the duodenum (MacRae *et al.*, 1985).

In conclusion, an increase in the WSC concentration of the diet led to an increase in the digestible DM intake, which in turn led to increased milk yields. Increased milk protein production was achieved at the same time as a reduction in urine-N excretion, leading to significant overall increases in the efficiency of utilization of dietary N for milk production. It is likely that these responses resulted from increased rumen utilization of the diets.

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