

# Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation

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## Abstract

Twelve multiparous Holstein–Friesian dairy cows in early lactation were used to investigate the potential of using perennial ryegrass (*Lolium perenne*) with a high concentration of water-soluble carbohydrates (WSC) to increase the efficiency of milk production. *Ad libitum* access to one of two varieties of zero-grazed herbage was given continuously for 3 weeks: treatment High Sugar (HS), an experimental perennial ryegrass variety (Ba11353) bred to contain a high concentration of WSC, harvested in the afternoon; or Control, a standard variety of perennial ryegrass (cv. AberElan), harvested in the morning. All dairy cows also received 4 kg d<sup>-1</sup> of a standard dairy concentrate. Dairy cows given the HS diet treatment consumed 2.8 kg dry matter (DM) d<sup>-1</sup> more than Control dairy cows ( $P < 0.01$ ), and the DM digestibility of the diet on the HS treatment was significantly greater than that of the diet on the Control treatment (0.75 vs. 0.72; s.e.d. 0.010;  $P < 0.05$ ). Excretion of urinary purine derivatives (PD) tended ( $P < 0.1$ ) to be higher from dairy cows on the HS treatment, implying increased microbial protein flow to the duodenum, although there was no significant difference in the apparent efficiency of rumen fermentation of either dietary nitrogen (N) or DM expressed as a ratio to urinary PD. Milk yields and milk composition were not significantly affected by dietary treatment, although true protein yields of milk were higher ( $P < 0.05$ ) from dairy cows given the HS treatment. The proportion of dietary N excreted in urine was significantly lower from HS cows, although the values were low for both treatments (0.20 g g<sup>-1</sup> vs. 0.27 g g<sup>-1</sup>; s.e.d. 0.020;  $P < 0.05$ ). It is concluded that

increased DM intakes by dairy cows given the HS treatment led to increased milk protein outputs. With a proportional decrease in urinary N excretion, the use of perennial ryegrass with a high WSC concentration, in the context of the harvesting regime used in this study, may help to reduce N pollution from dairy systems into which it is incorporated.

**Keywords:** milk composition, nitrogen balance, nitrogen use efficiency, purine derivative

## Introduction

Current economic conditions in the UK dairy industry suggest that milk producers must choose between increasing the intensity of production through increased concentrate feed inputs, or reducing inputs and relying more heavily on grazed pastures. Miller *et al.* (2001) showed that milk yields of dairy cows in late lactation can be increased by using an experimental variety of perennial ryegrass (*Lolium perenne* L.) that expresses increased concentrations of water-soluble carbohydrates (WSC). The environmental impact of farming in Europe is becoming increasingly important (Tamminga, 2003), and Miller *et al.* (2001) also showed that the partitioning of feed nitrogen (N) was increased, with more feed N being secreted in milk, and less being excreted in urine, in dairy cows offered the high WSC concentration grass. Lee *et al.* (2002) found ammonia-N concentrations in rumen fluid to be significantly lower in steers offered the same high WSC concentration perennial ryegrass variety compared with steers offered the control perennial ryegrass variety, which helps to explain reduced urine N excretion.

The physiology of dairy cows in late lactation is quite different to that of dairy cows in early lactation, with a greater use of nutrients for foetal development and body condition replenishment than in dairy cows in early lactation. Milk production by dairy cows in early lactation is increased with the provision of spring grass herbage, compared with the feeding of grass silage (Dillon *et al.*, 2002). Therefore, milk yield increases and

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favourable N partitioning responses to a high WSC concentration ryegrass may not be the same in early lactation as they were found to be in late lactation of dairy cows (Miller *et al.*, 2001).

The objective of this study was to test the hypothesis that increased WSC concentrations in grass can lead to an increased efficiency of utilization of dietary protein for milk production by dairy cows in early lactation. The work was achieved by using a novel experimental 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

### Dairy cows and their management

Twelve dairy cows in early lactation (42, s.e. 2.4, days in milk) from the IGER Trawsgoed Research Farm herd started the experiment, using the methods described by Miller *et al.* (2001). Briefly, the experiment was of a short-term (3 weeks) continuous design, with diets offered as zero-grazed grass with a concentrate supplement. The experiment commenced on 8 May 2000. Before the start of the experiment all the dairy cows grazed together on a common perennial ryegrass-dominated pasture, and during the week before the start of the experiment covariate measurements of milk yield (7 d) and composition (from four consecutive milkings) were taken. The dairy cows 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 experiment were used for adaptation and the third week was used for measurements. The dairy cows were milked twice daily, at c. 08:00 and 16:00 hours. They had constant free access to fresh water and a mineral lick (red Baby Rockies mineral blocks for cattle; Tithebarn Ltd, Winsford, Cheshire, UK).

### 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) which was harvested in the afternoon; 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 varieties of perennial ryegrass, and harvested in the morning.

The two perennial ryegrass varieties were sown at the Trawsgoed Research Farm (52°25'N, 4°05'W) in the spring of 1997, as described by Miller *et al.*

(2001), 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. The plots were managed as described by Lee *et al.* (2002). Briefly, the sward was fertilized at the end of March 2000 at a rate of 250 kg ha<sup>-1</sup> of a 25:5:5 (N:P:K) compound fertilizer (Norsk Hydro, Sluiskil, the Netherlands), and divided into three subplots of each variety for use in each of the 3 weeks of the experiment. During the experiment, fresh material was harvested each day, as described by Miller *et al.* (2001), using a Haldrup 1500 plot harvester (J. Haldrup, Løgstør, Denmark), with grass herbage being cut at 10:00 hours (Control) or 14:00 hours (HS), and chilled to reduce respiration losses, and offered in two portions: either on the day of cutting at c. 16:30 hours, or the next morning at c. 09:00 hours following storage overnight at 4°C.

All dairy cows received enough fresh forage for the measurement of *ad libitum* intakes with refusals of at least 0.1 of the forage offered. All dairy cows also received a standard concentrate feed offered at the rate of 4 kg d<sup>-1</sup> in two equal portions, 2 kg at each milking. The concentrate was a commercial pelleted product containing (in decreasing proportion) wheat, soya bean meal, molasses, rapeseed meal, sugar beet pulp, maize gluten, palm kernel meal, sunflower meal, vegetable oil, and a mineral and vitamin mix.

### Measurements

Milk yields were recorded daily, and milk samples were collected throughout the last 6 d of the experiment, during the N-balance measurement period. Milk was sampled by collecting 5 mL of milk per kg of milk yield from each animal at each milking, preserved with a LacTab milk preservative tablet (Thompson and Capper Ltd, Runcorn, Cheshire, UK) and bulked over the course of the N-balance measurement period.

To obtain information about the WSC concentration of the grasses before the start of the experiment, snip samples of grass (c. 100 g) were collected on five different dates from each subplot of each grass variety from the beginning of April 2000 to the end of May 2000, before the start of the measurement period of the experiment, for prediction of WSC concentration by near-infrared spectroscopy. Approximately forty small snip samples were collected from each subplot along a 'W'-shaped transect, and bulked to give one sample per subplot. These samples were freeze-dried and ground as described below, and scanned at 2 nm intervals over the wavelength range from 1100 to 2498 nm in reflectance mode, using a NIRSystems 6500 spectrophotometer (FOSS UK, Warrington, UK).

The calibration model used for the prediction of the concentration of WSC was developed as described by Lister and Dhanoa (1998) using modified partial least squares regression. The model was based on WSC data determined using the method described by Thomas (1977), modified for use on an AA3 Continuous Flow Analyser (Bran + Luebbe, Northampton, UK).

Grass herbage samples were collected from each of the feed containers before being offered to the cows (morning and afternoon) throughout the measurement week of the experiment. Samples were bulked to give one sample per treatment per day and were stored frozen before freeze-drying and grinding for analysis. Concentrate samples were similarly collected and bulked during the measurement week.

Whole body nitrogen partitioning was measured by collecting the total production of urine, faeces and milk over a 6-d period during the measurement week of the experiment from eight of the twelve experimental animals using externally applied urine and faeces separators as described by Miller *et al.* (2001). It was not practically possible to carry out N partitioning and feed digestibility measurements on all twelve animals, so those which were used for these measurements were chosen on the basis of temperament for use with the urine and faeces collection equipment. On the last day of the measurement week, blood samples were collected from all animals onto ice from the tail vessels into evacuated blood collection tubes containing lithium heparin (Vacuette; Greiner Labor-technik, Kremsmünster, Austria). Plasma was immediately separated by centrifugation (1700 g for 25 min at 4°C) and decanted in 1.5-mL aliquots into micro-centrifuge tubes which were frozen at -18°C until later analysis.

The analyses of milk, feed and faeces samples were carried out as described by Miller *et al.* (2001), with feed and faeces being freeze-dried and ground to pass through a 1-mm dry mesh screen. The metabolizable energy (ME) concentration of the diet was estimated from digestible organic matter (OM) kg<sup>-1</sup> DM (DMOD) by the equation  $ME = 0.16 \times g \text{ DOMD}$  (Agricultural and Food Research Council, 1993). Urine purine derivative (PD; allantoin and uric acid) concentrations were measured as described by Dewhurst *et al.* (1996). Plasma metabolite concentrations were determined using a Cobas Mira S chemistry analyser (Hoffman-LaRoche, Nutley, NJ, USA) and Sigma (Sigma-Aldrich Company Ltd, Poole, Dorset, UK) reagents and procedures for glucose (glucose reagent HK 20, procedure 17), urea-N (Infinity<sup>TM</sup> BUN reagent, procedure 63-UV) and  $\beta$ -hydroxybutyrate ( $\beta$ -HBA reagent no. 310-3 and  $\beta$ -HBDH reagent no. 310-4, procedure 310-UV).

## Statistical analysis

Milk production data were analysed statistically by analysis of covariance, with grass variety as a treatment factor and data from the pre-experimental grazing period as covariates, using 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.

## Results

Two of the experimental dairy cows (both on treatment HS) were removed from the experiment due to lameness; neither condition was related to treatment and both dairy cows subsequently recovered. The data from these cows were excluded from the statistical analyses.

### Diet composition

The nutritional composition of the two perennial ryegrasses and the concentrate feed, as offered during the measurement week, is presented in Table 1. There was a mean difference of 82 g kg<sup>-1</sup> DM in the WSC concentrations between the HS and Control herbage, which was associated with a similar but opposite difference in neutral-detergent fibre (NDF) concentrations. Mean WSC concentrations of snip samples collected at the same time of day at each sampling over the 6 weeks before the start of the experiment tended to be higher in the HS than in the Control herbage (Figure 1). Crude protein (CP) concentrations were similar, but rather low, at about 100 g kg<sup>-1</sup> DM for the two herbage. The composition of the concentrate was as expected for a standard high protein concentrate for lactating dairy cows (Table 1).

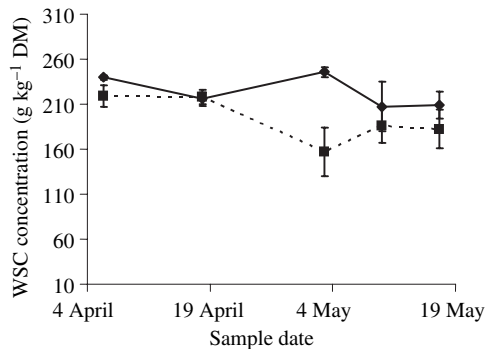
### Feed intake and milk production

Dairy cows on the HS treatment consumed 2.2 kg DM d<sup>-1</sup> more of the herbage than dairy cows offered the Control treatment herbage (Table 2), which represents a 0.17 increase in herbage intake on a proportional basis. There were significant increases in apparent digestibilities of DM and OM on the HS treatment (Table 3), although the apparent digestibilities of nitrogen and NDF were similar for both treatments. The predicted ME concentration of the diet on the HS treatment was higher than that of the Control treatment, with values of 11.1 and 10.8 MJ kg<sup>-1</sup> DM for diets on the HS and Control treatments respectively (s.e.d. 0.15;  $P = 0.051$ ).

**Table 1** Nutritional composition of the High Sugar (HS) and Control grasses (mean of seven samples) and concentrate (one sample bulked over 7 d) as offered to dairy cows during the measurement week of the experiment; all values in  $\text{g kg}^{-1}$  DM unless otherwise stated.

	Treatment				Concentrate
	HS	Control	s.e.d.	Significance	
Dry matter ( $\text{g kg}^{-1}$ )	192	174	12.1	NS	862
Organic matter	936	943	2.2	*	919
Water-soluble carbohydrate	243	161	7.4	***	107
Crude protein ( $\text{N} \times 6.25$ )	104	99	3.8	NS	225
Neutral-detergent fibre	480	563	6.6	***	288
Acid-detergent fibre	251	296	5.1	***	123
Acid-hydrolysis ether extract	–	–	–	–	47.7

NS, not significant; \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ .



**Figure 1** Mean (of three bulked samples) water-soluble carbohydrate (WSC) concentrations in plots of High Sugar (solid line) or Control (dotted line) perennial ryegrass in the 6 weeks leading up to the start of the experiment; error bars represent standard errors of the mean values.

Dairy cows offered the HS treatment yielded  $2.3 \text{ kg d}^{-1}$  of milk more than those on the Control treatment (Table 2), although this effect was not statistically significant ( $P = 0.159$ ). The concentrations of milk constituents were unaffected by treatment, apart from the concentration of milk NPN, which tended to be slightly higher on the Control treatment. The yields of milk fat and lactose were similarly unaffected by treatment, although the yields of milk true protein and whey were significantly increased from animals given the HS treatment (Table 2). Milk protein fractions were only measured in the milk collected from dairy cows undergoing the N-partitioning measurements, while fat, CP and lactose concentrations were measured in the milk of all the dairy cows on the experiment. Differences in CP yields were not significantly affected by treatment (Table 2), although there was a statistical difference in the CP yields of the dairy cows on the

**Table 2** Milk yields and composition of dairy cows during the measurement week of the experiment offered zero-grazed High Sugar (HS) and Control herbage-based diets.

	Treatment			
	HS	Control	s.e.d.	Significance
Grass herbage DM intake ( $\text{kg d}^{-1}$ )	15.3	13.1	0.78	*
Total DM intake ( $\text{kg d}^{-1}$ )	18.8	16.6	0.78	*
Milk yield ( $\text{kg d}^{-1}$ )	32.7	30.4	1.48	NS
Milk constituent concentrations ( $\text{g kg}^{-1}$ )				
Fat	39.0	40.1	2.60	NS
Crude protein	28.8	28.2	0.64	NS
True protein‡	25.6	24.3	1.12	NS
Casein‡	20.4	20.1	0.71	NS
Whey‡	5.2	4.2	0.47	NS
Non-protein nitrogen‡	0.21	0.24	0.015	†
Lactose	48.1	48.0	0.87	NS
Milk constituent yields ( $\text{g d}^{-1}$ )				
Fat	1272	1212	46.5	NS
Crude protein	940	855	45.4	NS
True protein‡	824	711	39.1	*
Casein‡	658	589	33.4	†
Whey‡	166	123	11.3	*
Non-protein nitrogen‡	6.7	7.1	0.27	NS
Lactose	1573	1456	73.7	NS

NS, not significant; †,  $P < 0.10$ ; \*,  $P < 0.05$ .

‡Values for milk protein fractions from cows that underwent N-partitioning measurements only.

**Table 3** Feed intake, apparent digestibilities of diets, and nitrogen intake and whole body partitioning of dairy cows ( $n = 4$  per treatment) during the N-balance measurement period offered zero-grazed High Sugar (HS) and Control herbage-based diets.

	Treatment			Significance
	HS	Control	s.e.d.	
Grass herbage DM intake (kg d <sup>-1</sup> )	15.3	12.5	0.76	**
Total DM intake (kg d <sup>-1</sup> )	18.8	16.0	0.76	**
Digestible DM intake (kg d <sup>-1</sup> )	14.1	11.5	0.69	**
Apparent digestibility of				
Dry-matter	0.75	0.72	0.010	*
Organic-matter	0.76	0.73	0.010	*
Nitrogen	0.64	0.62	0.010	†
Neutral-detergent fibre	0.74	0.72	0.019	NS
Nitrogen intake (g d <sup>-1</sup> )	376	320	12.4	**
Nitrogen output				
Urine (g d <sup>-1</sup> )	75	87	7.3	NS
Proportion of N intake	0.20	0.27	0.020	*
Faeces (g d <sup>-1</sup> )	137	123	4.0	*
Proportion of N intake	0.36	0.38	0.010	†
N intake				
Milk (g d <sup>-1</sup> )	136	119	5.6	*
Proportion of N intake	0.36	0.37	0.021	NS
Total output (g d <sup>-1</sup> )	348	329	8.8	†
Nitrogen balance (g d <sup>-1</sup> )	28.2	-8.4	11.95	*
Proportion of N intake	0.07	-0.03	0.029	*

NS, not significant; †,  $P < 0.10$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

N-partitioning measurements (868 g d<sup>-1</sup> vs. 759 g d<sup>-1</sup>, s.e.d. 35.7,  $P < 0.05$ ; Table 3).

### Nitrogen partitioning and purine-derivative excretion

Nitrogen intake was significantly higher in dairy cows consuming the diet on the HS treatment compared with those offered the Control treatment (Table 3), but there was no significant increase in the daily output of N in urine. Nitrogen concentration tended to be lower in the urine of the cows on the HS treatment compared with the Control treatment (3.39 g kg<sup>-1</sup> vs. 4.59 g kg<sup>-1</sup> fresh

**Table 4** Purine-derivative (PD, allantoin + uric acid) excretion in urine and PD excretion per unit of feed intake of dairy cows offered zero-grazed High Sugar (HS) and Control herbage-based diets (N-balance cows only).

	Treatment		s.e.d.	Significance
	HS	Control		
Urinary PD excretion (mmol d <sup>-1</sup> )				
Allantoin	468	290	77.8	†
Uric acid	34.4	20.8	6.92	†
Total PD	502	311	83.9	†
Total PD excretion per unit intake (mmol kg <sup>-1</sup> )				
PD/DM intake	26.7	19.4	4.44	NS
PD/OM intake	28.6	20.7	4.74	NS
PD/N intake	1.33	0.97	0.224	NS

NS, not significant; †,  $P < 0.10$ .

weight; s.e.d. 0.505;  $P = 0.055$ ). Urine N output as a proportion of N intake was significantly lower from dairy cows given the HS treatment compared with the Control treatment. Treatment had no effect on N digestibility, so faeces N output was significantly higher in those cows with higher N intakes. Milk N output was higher on the HS treatment than the Control treatment, although the efficiency of dietary N use for milk protein production (milk N/N intake) was similar for both treatments. There was a significant ( $P < 0.05$ ) difference in the use of body protein, with dairy cows given the HS treatment being in positive N balance, accreting 0.07 of consumed N, while dairy cows given the Control treatment were in negative N balance and lost the equivalent of 0.03 of the N consumed ( $P < 0.05$ ).

Purine-derivative excretion in urine tended to be higher from dairy cows on the HS treatment (Table 4). Despite large numerical differences in the mean values, individual variation in PD excretion meant that the difference between treatments failed to obtain statistical significance ( $P = 0.063$ ). When total PD excretion in the urine was expressed as a ratio of intakes of DM, OM and N, there were no significant differences between treatments.

There were no significant treatment effects on plasma concentrations of glucose (means of 4.9 mM and 3.7 mM for HS and Control treatments respectively; s.e.d. 1.08), urea N (9.3 mM vs. 7.9 mM; s.e.d. 1.08) or  $\beta$ -hydroxybutyrate (0.76 mM vs. 0.70 mM; s.e.d. 0.151).

### Discussion

The differences in WSC concentration between the two perennial ryegrass varieties in the 6-week period before the experiment were less than that previously found by Miller *et al.* (2001) who used the same perennial

ryegrass plots 2 years earlier. However, the mean difference in WSC concentration ( $84 \text{ g kg}^{-1} \text{ DM}$ ) between varieties of the herbage offered during the experimental period was greater ( $39 \text{ g kg}^{-1}$ ) than that found by Miller *et al.* (2001) and the same ( $82 \text{ g kg}^{-1}$ ) as that reported by Lee *et al.* (2002) who used herbage of the same grass varieties harvested at the same times, although from different plots. Diurnal variation in WSC concentrations was exploited to increase the difference between the two treatments – cutting the HS herbage several hours after the Control grass gave more time for photosynthesis to occur. A similar strategy of cutting at different times of the day has been shown to lead to differences in non-structural carbohydrate concentrations in other forages such as cocksfoot (*Dactylis glomerata* L.; Griggs *et al.*, 2005), tall fescue hay (*Festuca arundinacea* Schreb.; Fisher *et al.*, 1999) and lucerne hay (Burns *et al.*, 2005). The CP concentrations of the herbage were very low for early-summer grass, which is likely to be the result of the low rates of fertilizer application in the spring (Miller *et al.*, 2001; Lee *et al.*, 2002).

### Feed intake and milk production

Dry matter intake of herbage was significantly higher in cows on the HS treatment compared to those on the Control treatment, which agrees with the results of Lee *et al.* (2002) in which growing steers were offered very similar herbage zero-grazed. As discussed by Miller *et al.* (2001), it is possible that the difference in DM intake between the two treatments in the present study was caused by differences in NDF concentration of the herbage. No significant difference in DM intake was found by Miller *et al.* (2001), although the difference in the NDF concentrations of the two grass diets ( $45 \text{ g kg}^{-1} \text{ DM}$ ) in that study was approximately half the difference in NDF concentrations between the two grasses in the current study ( $81 \text{ g kg}^{-1} \text{ DM}$ ). When given sufficient forage to allow diet selection, dairy cows are able to select more digestible diets containing higher CP and lower NDF concentrations than the mean concentrations in the fresh forage on offer (Dalley *et al.*, 1999; Danelón *et al.*, 2002), but in the present study the opportunity for selection was limited by zero-grazing. The positive relationship between forage DM intake and forage DM content is well established (John and Ulyatt, 1987) and in the present study the fresh matter intakes of both grass treatments were the same (*c.*  $75 \text{ kg d}^{-1}$ ). However, the DM contents of the two treatments were not significantly different and therefore DM content of the fresh grass is unlikely to have played a large role in determining feed intakes in the current study.

Small but significant increases in apparent digestibilities of DM and OM, which were presumably a direct

consequence of the higher WSC concentration and lower NDF concentration in the herbage of the HS treatment compared with the Control treatment, led to significantly increased digestible DM intakes by the cows on the HS treatment. Despite this, milk yields were not significantly different between treatments. This is not in agreement with results from late-lactation cows offered similar treatments in which significantly higher milk yields were achieved from HS grass without significant differences in DM intakes (Miller *et al.*, 2001), although higher digestible DM intakes were found on the HS herbage in that study. Yields of true protein in the milk were higher on the HS treatment in the present study, while the concentrations of the major milk components were unaffected, which agree with the results of Miller *et al.* (2001).

Urinary PD excretion was used in this study as a marker for microbial protein production, as urine excretion of PD is well correlated with the duodenal flow of purines (Vagnoni *et al.*, 1997; González-Ronquillo *et al.*, 2003), most of which is associated with microbial protein, and is degraded and absorbed from the small intestine (McAllan, 1982). In this experiment total PD excretion tended to be higher on the HS treatment. However, there were no differences between treatments in the ratios of PD excretion to intakes of DM, OM or N, suggesting no difference in the efficiency of rumen fermentation, which would agree with the rumen fermentation studies in steers (Lee *et al.*, 2002) and was despite a large difference in the WSC concentrations in the grasses. Lee *et al.* (2002) reported relatively low yields of microbial protein per unit of OM apparently digested in the rumen and suggested that low dietary protein concentrations may have led to low rumen ammonia concentrations which limited microbial protein production. However, the steers in that study received no supplementary concentrate feed, unlike the cows in the present study, and, although the cows' CP intakes were relatively low, the milk yields and milk protein concentrations from them were as expected for the stage of lactation. Most of the non-protein N in milk would have been present as urea. This suggests reduced ammonia absorption from the rumen in cows on the HS treatment (Oltner and Wiktorsson, 1983; Gustafsson and Palmquist, 1993; Roseler *et al.*, 1993) because milk non-protein N concentrations tended to be lower on the HS treatment. Plasma urea-N concentrations were not significantly different between treatments, but the shorter half-life of urea in plasma, compared with that of urea in milk, suggests that concentrations in plasma are subject to greater variation than in milk. Rumen ammonia concentration of HS-fed steers was significantly lower than that of control steers (Lee *et al.*, 2002), which supports the hypothesis of reduced ammonia absorption from the

rumen, although it is unknown what effect the additional concentrate supplement received by the dairy cows in the present study would have had on their rumen ammonia concentrations.

The concentrations and yields of milk fat and lactose were unaffected by treatment, which implies that the precursors for these milk constituents were supplied in similar quantities from each of the two diets (Sutton, 1989), despite the difference in the DM intake between treatments. There was no difference in fatty acid flow to the duodenum and absorption from the small intestine in steers offered the same two perennial ryegrass varieties even when DM intake was higher, as in the present study, for steers offered the HS grass than that of steers on a control grass (Scollan *et al.*, 2003). Statistically significant but numerically small differences in rumen concentrations of individual volatile fatty acids (but not total concentrations) were found by Lee *et al.* (2002) and, therefore, significant changes in milk fat and lactose synthesis, and hence changes in milk yields, might not be expected. Plasma  $\beta$ -hydroxybutyrate concentrations, which are influenced by the absorption of butyrate from the rumen, were not significantly affected by treatment, and indicate a lack of substantial treatment difference in absorption of butyrate from the rumen.

### Nitrogen partitioning

The apparent efficiency of utilization of dietary protein for milk protein production was extremely high in both treatments, with a mean of 0.365 g milk N g<sup>-1</sup> feed N. This is likely to be because of the low rates of fertilizer-N applied to the swards and the inverse relationship between rates of fertilizer-N application and WSC concentration of grass herbage (Peyraud and Astigarraga, 1998). In addition to overall rates of N-utilization efficiency for milk production, cows on the HS treatment tended to have a higher total N balance compared with Control cows, with cows on the HS treatment being in positive N balance while cows on the Control treatment were in negative N balance. The N content of the body of the lactating dairy cow tends to be at its lowest at about 6 weeks of lactation (Belyea *et al.*, 1978; Gibb *et al.*, 1992) so the cows were expected to be at approximately zero N balance at the time of this experiment. As milk protein output was increased on the HS treatment, it is not surprising that the same cows were in positive N balance, and this is likely to be due to their higher N intakes.

Perhaps of greater importance than N balance is the significantly reduced proportional excretion of N in the urine of the cows on the HS treatment. Urea in urine is the most important source of ammonia in terms of N emissions from dairy systems (Monteny

and Erisman, 1998), and the rate of ammonia volatilization depends on the concentration of urea in urine. Therefore, assuming a constant proportion of urea N in total N in urine in the present study, the lower N output in the urine of cows on the HS treatment, associated with a reduction in the proportion of dietary N being excreted in urine, implies a reduction in the potential environmental burden of dairy systems utilizing a perennial ryegrass with elevated concentrations of WSC.

In conclusion, cows given the HS treatment consumed more DM and produced more milk protein than cows given the Control treatment, which appears to have been a result of increased flows of microbial protein to the duodenum. Milk yields and the concentrations of milk constituents were not affected by the treatment HS. Whole body N balance was increased, and the proportion of dietary N excreted in urine was reduced, in cows offered the HS treatment. Dairy systems which incorporate HS grasses may have improved whole-farm N balance, with more dietary N being used for productive purposes, and less being lost to the environment through volatilization.

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