

## Rumen metabolism and nitrogen flow to the small intestine in steers offered *Lolium perenne* containing different levels of water-soluble carbohydrate

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### Abstract

Eight Hereford × Friesian steers were used to investigate the effect of feeding *Lolium perenne* (L) forage containing elevated levels of water-soluble carbohydrate (WSC) on rumen metabolism and nitrogen (N) absorption from the small intestine. The steers were offered ad libitum access to one of two varieties with matched heading dates (Ba11353, high WSC, HS; AberElan, intermediate WSC, control) cut at different times of the day to accentuate WSC differentials, zero-grazed for 21 days. This was followed by a 14-day period where the animals were on grass silage to provide a covariate intake. Although the total N concentration was similar for the two grasses, all other measured values were significantly different. The dry matter (DM) concentration of HS was greater than that of the control (202 v. 167 g DM per kg;  $P < 0.01$ ). WSC and in-vitro dry matter digestibility (IVDMD) were 243 and 161 g/kg DM, and 0.61 and 0.56 for HS and control, respectively. In contrast, acid- and neutral-detergent fibre were 251 and 296 g/kg DM and 480 and 563 g/kg DM for HS compared with control, respectively. DM intake was increased (9.3 v. 6.7 kg/day;  $P < 0.001$ ) for HS animals and this contributed significantly towards higher flows of non-ammonia N to the duodenum as well as increased absorption of amino acids from the small intestine. This DM intake response was partly due to the elevation in DM concentration of HS. However fresh weight intake was increased proportionately by ca. 0.15 ( $P < 0.05$ ) in animals on HS compared with control. Rumen ammonia levels were lower (14.0 and 26.4 mg N per l;  $P < 0.001$ ) and concentrations of rumen propionate higher ( $P < 0.01$ ) and acetate lower ( $P < 0.01$ ; increasing the glucogenic : lipogenic volatile fatty acid ratio) in animals on HS compared with control. However, the efficiency of microbial protein synthesis (15.9 and 17.8 g microbial nitrogen per kg organic matter apparently digested) and flow of N to the duodenum per unit N intake (0.84 and 0.93) for HS and control, respectively, were similar across both diets.

**Keywords:** *Lolium perenne*, nitrogen, rumen digestion, steers, water-soluble carbohydrate.

### Introduction

It has been reported that *Lolium perenne* selected for elevated levels of water-soluble carbohydrate (WSC) can improve lamb performance in terms of live-weight gain (Lee *et al.*, 2001). MacRae *et al.* (1985) reported a greater absorption of amino acids from the small intestine when sheep were given spring-compared with autumn-harvested dried grass. It was postulated that the greater amino acid absorption

was related to higher WSC and lower fibre concentration in the spring-harvested dried grass. The greater live-weight gains of lambs, noted by Lee *et al.* (2001), were achieved when the differential between the varieties was approximately 38 g WSC per kg DM; however when the differential was reduced no differences in live-weight gain were observed. Previous studies (Lee, 2001) have shown that WSC concentrations in grasses fluctuated

throughout the day, rising during the morning and then falling in the afternoon. Grasses were harvested at different times of the day in the current work in order to ensure a greater WSC differential between the grasses.

The objective of this study was to determine effects on rumen function and amino acid absorption in zero-grazed beef steers on *Lolium perenne* with different WSC concentrations.

## Material and methods

### *Experimental site and fertilizer application*

On 30 March 2000, 1.5 ha plots of two varieties of *Lolium perenne*, the high WSC variety Ba11353 (HS) and the control AberElan were each divided into three subplots to provide grass for the 3-week experimental period. Subplots were cut to a height of 5 cm and fertilized with 250 kg/ha of 25:5:5; N:P:K (nitrogen, phosphorus, potassium; Norsk Hydro, Sluiskil, Netherlands) on a week stagger starting from 30 March. A relatively low level of fertilizer application was adopted as McGrath *et al.* (1988) noted that the addition of fertilizer-N reduced the concentration of WSC in grass through an elevation in growth rate. The low fertilizer-N application rate adopted in this experiment ensured a higher WSC concentration in the grasses offered to the experimental animals. Subplots were allowed to regrow for 6 weeks before the start of the experiment with the aim of using material before it reached 50% ear emergence.

### *Animals and experimental design*

Eight Hereford × Friesian steers, initial live weight 430 (s.e. 9.0) kg, each prepared with a rumen cannula and simple 'T'-piece cannulae in the proximal duodenum and terminal ileum (Jarrett, 1948) were used. They were housed in individual pens, and transferred to metabolism crates for the measurement period. The building was well ventilated and animals had free access to fresh water. A vitamin/mineral pre-mix (Rumins Cattle Regular, Rumenco, Burton, Staffordshire) was offered at 100 g per head per day, sprinkled on top of the morning meal. This pre-mix included macro-minerals Ca, P, Mg, and Na at 200, 40, 50 and 80 g/kg, respectively; micro-minerals Se, Co, I, Mn, Zn, Fe and Cu at 0.02, 0.15, 0.25, 3.5, 3.5, 3 and 1.8 g/kg, respectively and vitamins (retinol, cholecalciferol and alphatocopherol at 90, 1.5 and 667 mg/kg, respectively).

The experiment consisted of one main 21-day experimental period during which the animals were allocated at random to receive either control (no. = 4) or HS (no. = 4) zero-grazed at *ad libitum*. The 21-day period consisted of 14 days for adaptation to the diet

and 7 days for collection of rumen, duodenal and ileal digesta. Faeces were not collected so measurement of whole tract digestibility of dietary components was not made. Following the end of the experimental period, animals were gradually changed onto a standard experimental silage for a 14-day period after which daily food intakes were monitored for a further 5 days as a covariate measurement. The single period design with a covariate measurement on silage was chosen to minimize the effects of the large seasonal variations observed in grass chemical composition (Pollock and Jones, 1979).

### *Feeding and weighing*

Control and HS grasses were cut at approximately 10:00 and 14:00 h, respectively. The grass was then chilled for 2 h in a blast freezer (-20°C) and stored at 4°C prior to feeding the following day. Daily samples were taken for accurate estimates of dry matter (DM) concentration and chemical analyses. Animals received their food as two equal meals, at 09:00 and 16:00 h. Daily samples (100 g) were taken at these times for chemical analysis of the food as given during the 7-day collection of digesta; food intakes were also recorded over this period. Refusals were recorded and sampled at 08:45 and 15:45 h.

### *Experimental procedures*

Digesta flow at the duodenum and ileum were estimated using a dual-phase marker system with ytterbium acetate (YbAc) and chromium ethylene diamine tetra-acetic acid (CrEDTA) as the particulate and liquid markers, respectively (Faichney, 1975). The markers (YbAc: 50 mg Yb per kg DM intake; CrEDTA: 3700 mg/day) were infused continuously into the rumen for 6 days before digesta sampling by a peristaltic pump (202U, Watson-Marlow Ltd, Falmouth, Cornwall) at a rate of 30 ml/h. On day 15 of the experiment ileal digesta was collected manually every 4 h over a 24-h period. This was followed by a rest day before duodenal digesta was collected for 2 days consecutively (days 17 and 18) in the same manner. On day 20, samples of rumen fluid were taken hourly over a 24-h period. On day 21 a 1-l sample of rumen fluid was taken for separation of rumen micro-organisms. Diet change-overs commenced for the covariate period after completion of sampling.

### *Sample preparation and analysis*

Subsamples of digesta were either stored frozen (-20°C) or freeze-dried, ground and retained for chemical analysis. Accumulated samples of daily duodenal and ileal digesta were thoroughly mixed and a 200-g subsample representing whole digesta was freeze-dried. A separate 200-g portion was

centrifuged at 3000  $\times$  g for 25 min to provide the centrifuged solid digesta. These were subsequently freeze-dried, ground and retained frozen for analysis. Microbial fractions of both duodenal and rumen digesta were obtained by centrifuging the supernatant from the original centrifuged sample at 30 000  $\times$  g for 25 min followed by a distilled water wash and a further spin (30 000  $\times$  g) repeated three times, to minimize contamination from whole rumen contents. The pellet was then freeze-dried prior to analysis.

WSC of the grass was determined spectrophotometrically using anthrone in sulphuric acid on a Technicon Autoanalyser (Technicon Corporation, New York, USA; Thomas, 1977). Ash and by mass difference organic matter (OM) was analysed by combusting the ground samples at 550°C for 6 h in a muffle furnace. Volatile fatty acid (VFA) components in the rumen liquor were determined by gas chromatography using Chrompack CP 9002 (CP-Sil 5CB column 10 m  $\times$  0.25 mm i.d.; Chrompack, UK) following the method of Zhu *et al.* (1996). *In-vitro* dry matter digestibility (IVDMD) was determined following the method of Jones and Hayward (1975). Ammonia-N was assessed enzymatically using glutamate dehydrogenase on a discrete analyser (FP-901 M Chemistry Analyzer, Labsystems Oy, Helsinki, Finland; Test kit no. 66-50, Sigma-Aldrich Co. Ltd, Poole, Dorset). Ammonia concentration of digesta was determined immediately after extraction in water. Total nitrogen was determined by a micro-Kjeldahl technique using 'Kjeltec' equipment (Perstorp Analytical Ltd, Maidenhead, Berkshire). Neutral-detergent fibre (NDF) was determined as described by Van Soest *et al.* (1991) and acid-detergent fibre (ADF) was analysed according to the method of Van Soest and Wine (1967) using the Tecator Fibretec System equipment (Tecator Ltd, Thornbury, Bristol). Chromium and ytterbium concentrations of digesta and infusate solutions were determined using a Pye Unicor SP9 atomic absorption spectrophotometer (Spectronic Unicam, Cambridge, Cambridgeshire) as described by Williams *et al.* (1962). Purine and pyrimidine bases were used as microbial markers and were determined using the high-performance liquid chromatography (HPLC) method of Cozzi *et al.* (1993). Total and individual amino acids were also determined using HPLC with an initial performic acid hydrolysis used prior to the acid hydrolysis to convert the sulphur amino acids to their stable form. The HPLC system was a Dionex (Surrey) advanced computer interface, Spectromonitor III reader cell and pump (LDC/Milton Roy, Florida, USA) a GINA 50 Autosampler (Gynkoteck, Munich, Germany) and a

Partisil 100 im SCX 4.0  $\times$  250 mm column (Waters Ltd, Hertfordshire).

#### Calculations and statistical analysis

Digesta flows were calculated after mathematical reconstitution of true digesta as described by Faichney (1975). Means of digesta flow parameters and intake for each treatment, blocked according to steer were subjected to a general analysis of variance (HS *v.* control) corrected for covariate intake (Genstat 5: Lawes Agricultural Trust, 1995). The chemical composition of the foods was subjected to a general analysis of variance (Genstat 5: Lawes Agricultural Trust, 1995). The hourly estimates of rumen pH, ammonia and VFA concentrations were analysed using a repeated measure analysis of variance (Genstat 5: Lawes Agricultural Trust, 1995).

## Results

### Herbage chemical characteristics

Herbage chemical characteristics as given to the steers are shown in Table 1. Although the total N concentration was similar for the two grasses, all other measured values were significantly different. The DM concentration of HS was greater than that of the control (202 *v.* 167 g DM per kg;  $P < 0.01$ ). WSC and IVDMD were approximately 83 g/kg DM and 0.05 higher while ADF and NDF were approximately 44 and 83 g/kg DM lower in HS compared with the control, respectively.

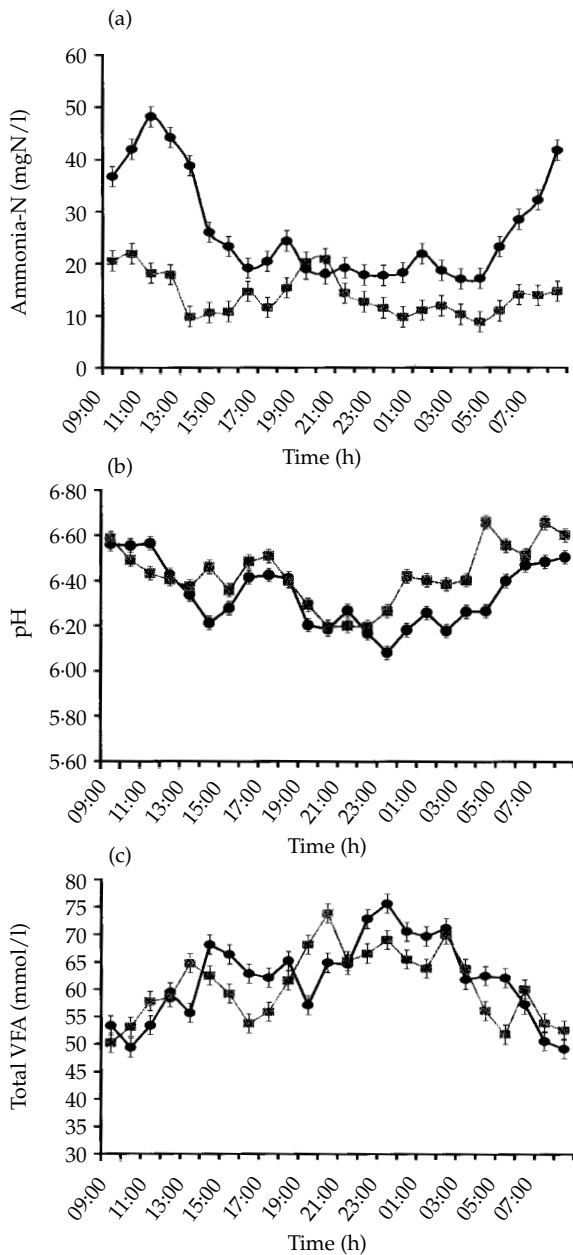
### Rumen parameters

Hourly rumen ammonia-N concentration, pH and VFA concentration are shown in Figure 1a to c, and mean daily concentrations for these parameters are presented in Table 2. Rumen ammonia-N levels in animals offered the control *Lolium perenne* increased post feeding (09:00 h) until 11:00 h, before declining until a second less discernible increase after the afternoon meal (16:00 h) occurred. The level then declined and plateaued until 04:00 h, when a sharp increase was observed (Figure 1a). Animals on HS

**Table 1** Chemical composition (g/kg DM) of the two grass diets Ba11353 high water-soluble carbohydrate (WSC) (HS) and the control (AberElan; g/kg DM) (residual degrees of freedom = 11)

	Control	HS	s.e.d.
Dry matter (g DM per kg)	167.0	202.0**	1.23
Organic matter	942.9	936.1**	2.01
WSC	160.7	243.2***	8.72
Total nitrogen	15.9	16.6	0.66
Acid-detergent fibre	295.7	251.4***	7.16
Neutral-detergent fibre	562.5	479.6***	12.24
IVDMD†	0.56	0.61**	0.014

† *In-vitro* dry matter digestibility.



**Figure 1** Hourly rumen parameters of fistulated steers offered the two grass diets Ba11353 high WSC (HS; ■) and the AberElan (control; ●). (a) ammonia nitrogen (b) pH and (c) total volatile fatty acids (no. = 4,  $\pm$  s.e.d.).

showed a relatively constant lower level of rumen ammonia-N throughout the day at approximately 10 to 20 mg N per l. Averaged across the 24-h sampling period, rumen ammonia-N levels were significantly higher on the control compared with HS ( $P < 0.001$ )

**Table 2** Volatile fatty acids (VFA; mmol/l), ammonia-nitrogen (mg N per l) concentrations and pH in the rumen of fistulated steers offered the two grass diets Ba11353 high water-soluble carbohydrate (WSC) (HS) and AberElan (control) (residual degrees of freedom = 6)

	Control	HS	s.e.d.
Total VFA	61.9	60.7	1.74
Acetate	40.4	37.7**	1.10
Butyrate	6.9	7.2	0.31
Propionate	12.4	13.7**	0.42
Ratio (P/A + B)†	0.26	0.30***	0.006
Molar proportions			
Acetate	0.65	0.62***	0.004
Butyrate	0.11	0.12**	0.003
Propionate	0.20	0.23***	0.004
Ammonia-N	26.4	14.0***	1.95
pH	6.3	6.4**	0.03

† Ratio of propionate: (acetate + butyrate).

diet. The pattern of pH change (Figure 1b) on both treatments was similar, showing a decline after the morning meal, a rise after the second meal at 16:00 h followed by a second decline and a gradual rise after 23:00 h. Mean rumen pH values were greater on HS ( $P < 0.001$ ), although the difference was numerically small. No significant difference in the total VFA or butyrate concentrations were noted (Figure 1c, Table 2). However, acetate concentration was greater on control ( $P < 0.01$ ) and propionate concentration greater on HS ( $P < 0.01$ ), resulting in a greater glucogenic:lipogenic VFA ratio on HS ( $P < 0.001$ ) (propionate:acetate + butyrate). When VFAs were expressed as molar proportions, animals offered the HS diet had greater proportions of butyrate and propionate ( $P < 0.01$ ,  $P < 0.001$ , respectively) and lower proportions of acetate ( $P < 0.001$ ) than animals offered the control diet.

#### Nitrogen intake, flow and absorption from the small intestine.

N intakes and the flow of N components entering and leaving the small intestine as well as rumen digestibility of these components are shown in Table 3. Animals offered HS had higher intakes of DM, OM and nitrogen ( $P < 0.001$ ). Duodenal flows of non-ammonia nitrogen (NAN) and microbial nitrogen ( $P < 0.05$  and  $P < 0.01$ , respectively) were also higher. Since flow of nitrogen at the duodenum is elevated on the HS diet and the ileal flows are similar between the two diets this may suggest a higher apparent absorption from the small intestine on the HS diet. Rumen digestibility of DM, OM and N were not significantly different between treatments. The proportion of MN in the duodenal NAN flow and the proportion of NAN absorbed from the small intestine were not significantly different between

**Table 3** Nitrogen flow and apparent absorption from the small intestine of fistulated steers offered the two grass diets Ba11353 high water-soluble carbohydrate (WSC) (HS) and AberElan (control) (residual degrees of freedom = 6)

	Control	HS	s.e.d.
Intake			
Dry matter (kg/day)	6.7	9.3***	0.02
Organic matter (kg/day)	6.3	8.7***	0.17
Nitrogen (g/day)	105.9	153.9***	2.86
Duodenal flows (g/day)			
Organic matter (kg/day)	1.8	2.3**	0.15
Nitrogen	99.6	129.9*	11.80
Ammonia nitrogen	1.1	1.0	0.22
Non-ammonia nitrogen (NAN)	98.5	128.9*	11.91
Microbial nitrogen (MN)	79.9	101.4*	11.36
Ileal flows (g/day)			
Nitrogen	48.2	58.8	10.73
Ammonia nitrogen	0.5	0.6	0.11
Non-ammonia nitrogen	47.8	58.2	10.63
Rumen digestibility			
Dry matter	0.55	0.60	0.041
Organic matter	0.71	0.74	0.015
Nitrogen	0.07	0.11	0.085
Duodenal MN : NAN	0.81	0.79	0.042
Apparent absorption (gNAN per day)	50.7	70.7**	4.07
Duodenal NAN absorbed (%)	0.52	0.55	0.044
EMPS† (gMN per kg OMAD‡)	17.8	15.9	2.24
Ratio duodenal MN : N intake	0.75	0.66	0.083
Ratio duodenal NAN : N intake	0.93	0.84	0.080

† Efficiency of microbial protein synthesis.

‡ Organic matter apparently digested.

treatments averaging 0.8 and 0.54, respectively. The efficiency of NAN and MN transfer to the duodenum, defined as the duodenal NAN flow and MN flow, respectively, as a function of N intake were not different, nor was the efficiency of microbial protein synthesis (EMPS; averaging 16.9 g MN per kg OM apparently digested (OMAD)).

*Amino acid flow to and absorption from the small intestine.*

Duodenal flows of individual amino acids (Table 4), with the exception of arginine, glycine and histidine, were greater ( $P < 0.05$ ) on HS. There was a trend ( $P < 0.1$ ) for ileal flow and apparent absorption from the small intestine of total amino acids to be greater on HS (Table 5). Ileal flows of aspartate, leucine,

**Table 5** Total amino acid (TAA) flow to and absorption from the small intestine of fistulated steers offered the two grass diets Ba11353 high water-soluble carbohydrate (HS) and AberElan (control)

	Control	HS	s.e.d.
TAA† intake (g/day)	474.1	676.6***	12.78
TAA duodenal flow (g/day)	513.0	677.0*	68.5
TAA ileal flow (g/day)	189.0	233.0†	20.3
Apparent absorption‡ (g/day)	324	441†	65.3
Absorption coefficients§	0.63	0.65	0.040
Ratio duodenal TAA : TAA intake	1.10	0.99	0.126

†  $P < 0.1$ .

‡ Duodenal TAA flow – ileal TAA flow.

§ Absorption coefficient = (apparent absorption/TAA duodenal flow).

**Table 4** Mean quantities of individual amino acid flow (g/day) to duodenum and ileum of fistulated steers offered the two grass diets Ba11353 high water-soluble carbohydrate (HS) and the AberElan (control) and the individual amino acid composition (g/day) of the total amino acid fraction apparently absorbed from the small intestine (no. = 15)

	At duodenum			At ileum			Apparently absorbed		
	Control	HS	s.e.d.	Control	HS	s.e.d.	Control	HS	s.e.d.
Alanine	37.9	48.9*	4.22	15.7	19.2†	1.66	22.2	29.7†	3.69
Arginine	20.9	22.7	6.17	4.8	3.5	1.82	16.1	19.3	5.27
Aspartate	61.9	80.3*	6.7	21.2	26.8*	1.77	40.8	53.5†	6.24
Cysteine	6.5	8.3*	0.61	4.9	5.6	0.46	1.6	2.7	0.61
Glutamate	68.6	89.3*	7.54	29.5	38.1	4.79	39.1	51.5	6.89
Glycine	32.1	44.6	7.02	11.0	13.1	1.22	21.1	31.5	7.25
Histidine	9.4	11.9†	1.21	3.9	4.2	0.45	5.53	7.69†	0.95
Isoleucine	25.9	33.1*	3.02	10.4	12.7	1.51	15.5	20.4	2.53
Leucine	41.7	53.8*	4.24	12.7	15.8*	1.27	29.0	38.0†	3.49
Lysine	39.6	51.9*	4.42	10.8	13.4*	1.05	28.8	38.5†	4.29
Methionine	9.3	14.2*	1.54	3.6	4.7	0.61	5.7	9.5†	1.62
Phenylalanine	24.2	31.3*	2.65	8.9	11.3*	0.93	15.3	20.0†	2.29
Proline	18.01	23.22*	1.70	8.3	10.1	0.98	9.7	13.1†	1.39
Serine	26.3	33.9*	2.98	11.0	14.6**	0.74	15.3	19.2	2.64
Threonine	24.7	31.2*	2.87	10.2	12.6†	1.07	14.5	18.6	2.24
Valine	33.1	41.6*	3.47	14.5	19.4**	1.28	18.6	22.2	2.75

†  $P < 0.1$ .

lysine, phenylalanine, serine and valine were greater ( $P < 0.05$ ) and there was a trend ( $P < 0.1$ ) for alanine and threonine to be greater on the HS diet. The amino acid absorption coefficients ranged from 0.76 for arginine to 0.24 for cysteine and were not different between diets. Total intake and duodenal flow of amino acids were greater on HS compared with the control. There were no differences in apparent absorption of total amino acids or the efficiency of transfer to the duodenum, defined as duodenal total amino acid flow : total amino acid intake. However, there was a trend ( $P < 0.1$ ) for higher absorption of amino acids on HS compared to the control (Table 5).

## Discussion

### *Herbage chemical composition and intake*

In this study a greater differential between the WSC concentration of control and HS was obtained by exploiting the diurnal changes in WSC concentration. By cutting the control in the morning (10:00 h) and HS in the afternoon (14:00 h) a differential of 82.5 g WSC per kg DM ( $P < 0.001$ ) between the two grasses as given to the steers was achieved. Fibre concentrations of the two grasses were also significantly different. Typically this is a genotypic rather than an environmental effect and therefore the differences observed in this study were likely to be an additive effect between genotype and environmental/diurnal factors. The combined higher WSC and lower fibre concentrations on HS resulted in a greater IVDMD. However, the method of grass harvesting also resulted in a significant difference in DM concentration of the two grasses with HS having 35 g DM per kg fresh weight greater than control. The total N concentrations of the grasses offered in this trial were extremely low (*ca.* 16.3 g/kg DM). This was related to the relatively low quantities of fertilizer-N applied to the swards.

Numerous authors have reported the relationship between 'sweet' foods and increased intake (Chiy and Philips, 1999; Siever-Kelly *et al.*, 1999). These relationships appear to be related to the WSC concentration and the reduced fibre concentration or increased digestibility of the food. The relationship between fibre concentration and herbage intake is well established. Thornton and Minson (1972) suggested that voluntary intake of forages by ruminants was largely dependent on retention time in the rumen, which was principally affected by the fibre components of the diet. However, intake may also be regulated by ammonia proliferation in the rumen from the rapid degradation of plant protein. Symonds *et al.* (1981) noted that ammonia levels in the systemic circulatory system above 0.8 mmol/l might become toxic to the animal. This mechanism

for the control of intake was concluded as a matter for debate (Buttery, 1977). Indeed Moorby and Theobald (1999) noted no significant drop in intake when ammonium acetate was infused into the duodenum of dairy cows. In the present study and in a similar study carried out with dairy cows given the same grasses (Moorby *et al.*, 2001) DM intake was 1.39 and 1.17 times higher, respectively, in animals on HS than the control. The higher DM intake may be related to the higher DM concentration of the afternoon cut HS grass in these experiments. However, the fact that fresh weight intakes were also elevated (were *ca.* 1.15 times greater in animals on the HS diet) suggests that changes in composition of DM (increase in WSC concentration; decrease in fibre concentration) also affected DM intake.

### *Rumen parameters*

The addition of sugar-based supplements to a basal diet has been shown to decrease rumen ammonia levels (Obara and Dellow, 1994; Carruthers and Neil, 1997). In the present study mean daily rumen ammonia concentration was lower ( $P < 0.001$ ) on HS. The reduction in ammonia concentrations could be explained by greater utilization of ammonia produced by the micro-organisms with access to a readily available energy source increasing microbial protein synthesis (Rooke *et al.*, 1987); or by reductions in the use of amino acids as an energy source by micro-organisms (Nocek and Russell, 1988). The mean daily rumen ammonia concentrations in this study were low for a forage-based diet, 26.4 and 14.0 mg N per l on control and HS, respectively. Satter and Slyter (1974) suggested that a minimal ammonia-N concentration of 20 to 50 mg N per l was required to maintain efficient microbial production in the rumen of steers given concentrate-based diets. However, they concluded that minimum levels of ammonia-N in ruminants on fresh forage vary. Nevertheless, such low rumen ammonia levels may indicate that the animals in this study were in a nitrogen-limiting situation, due to the low levels of N in the grass offered. The ability to reduce the level of ammonia production in the rumen may have significant effects on the efficiency of amino acid utilization. Lobley *et al.* (1995) suggested that the liver may utilize amino acid-N (aspartate) in the conversion of ammonia to urea (ureagenesis). Consequently a reduction in ammonia absorption may increase amino acid supply to the commercially important peripheral tissues, such as skeletal muscle and the mammary glands. However, more recent work has shown that a sustained increase in the basal ammonia supply to the liver, *via* a direct infusion of  $\text{NH}_4\text{HCO}_3$  into the mesenteric vein, did not impair amino acid supply to peripheral tissues and indicates that amino acid utilization

during ureagenesis is insignificant (Milano *et al.*, 2000). This work, however, was with sheep and therefore their conclusions may not apply to cattle.

Corbett (1987) suggested that rumen acetate : propionate ratios above 3 : 1 indicated that herbage WSC levels were sufficiently low to limit the supply of readily available energy for microbial protein synthesis. In this study, values of 2.77 and 3.23 were observed for HS and control, respectively. These results compare favourably with those of Beever *et al.* (1978) who reported values of 2.57 and 3.15, on a high WSC spring-harvested grass and a lower WSC autumn-harvested grass, respectively. In this trial, however, no increase in the efficiency of microbial protein synthesis in animals on the HS diet was observed. This may have been due to the low ammonia levels in the rumen as a result of the low N concentration of the grass offered. Significant increases in the molar proportions of propionate and butyrate and reductions in molar proportions of acetate were noted in animals on the HS diet. As well as decreasing rumen ammonia levels, increasing the molar proportions of propionate may also have a positive effect on amino acid utilization. Potentially fewer amino acids may have to be used to provide energy to convert acetate, absorbed from the rumen, into storage triglycerides in the liver, as propionate acts as a glucogenic precursor (MacRae and Lobley, 1982 and 1986).

Animals offered HS had a statistically significantly higher rumen pH than those on the control diet, although differences were numerically small and probably biologically insignificant. This contrasts with the findings of Obara *et al.* (1991) and Carruthers and Neil (1997) who observed a significant decrease in rumen pH on a sugar-supplemented diet compared with the unsupplemented control. Obara *et al.* (1991) related this decrease in rumen fluid pH on a rapidly fermented diet to an increase in the production of VFA and lactic acid.

#### *Nitrogen flow and absorption from the small intestine*

The efficiencies of N transfer to the small intestine were high in comparison to the literature for steers on fresh ryegrass, 0.93 and 0.84 for control and HS, respectively, compared with values of 0.76 reported by MacRae and Ulyatt (1974) for sheep given Ruanui ryegrass. Ulyatt *et al.* (1980) demonstrated that as the amount of N in the crop increases considerable losses of N across the rumen may occur, with losses in the order of 0.3 to 0.4 of N intake on fresh forage. However, Beever and Siddons (1986) reported similar values (0.84) for cattle grazing ryegrass. The relatively small losses in the current work may reflect

the lower levels of nitrogen in the grasses. Values were below the level (25.6 g N per kg OM) at which Poppi (1990) suggested that significant losses of N would occur across the rumen.

N intakes and rumen digestible OM were higher in animals on HS, relating to the higher DM intake, compared with those on the control diet. This resulted in higher non-ammonia N and MN flow to and apparent absorption from the small intestine. There was, however, no significant increase in the efficiency of nitrogen transfer to the duodenum or EMPS. This is in contrast to what has been predicted by numerous authors with respect to supplying extra readily available energy to the rumen microbial population (Nocek and Russell, 1988; Rooke *et al.*, 1987).

The EMPS in this study were low in comparison to the Agricultural Research Council (1980) recognized value of 30 g MN per kg OMAD. Robinson *et al.* (1998) and Henning *et al.* (1993) also reported low values of 17.0 and 15.0 g MN per kg OMAD for dairy cows on grass and whole-crop barley silage and sheep on wheat straw and fish meal, respectively. A deficiency in N may have been expected to apparently increase the EMPS (expressed as g N per kg OMAD), provided that sufficient N was being recycled. A higher proportion of the OMAD will be carbohydrate (and less will be protein) and fermentation of carbohydrate will generate more ATP than fermentation of protein thus increasing EMPS. However, if there is insufficient N from the diet or recycling, which appears to be the case in this study, carbohydrate fermentation and microbial growth will become 'uncoupled' leading to a futile cycle of bacterial energy metabolism and a consequent reduction in EMPS. It may therefore be of interest to speculate a potential increase in EMPS if the HS diet had provided extra degradable N. Moorby *et al.* (2001), when feeding the same material to dairy cows, found that milk protein levels were low, 26.0 and 26.8 g crude protein per kg for control and HS, respectively. These results may substantiate the argument that N supply was limited. However, it was interesting to note that despite similar milk yields, cows on HS retained more body N. This was entirely explained by a reduction in urinary N excretion (as a proportion of N intake). This may relate to the greater availability of protein (as a result of a greater intake, flow and absorption of protein from the small intestine as demonstrated in this study) and perhaps an increase in the efficiency of utilization of absorbed amino acids or simply a reduced load of ammonia on the liver. MacRae *et al.* (1995) suggested that an increase in the readily available energy supply on a forage-based diet may

increase the efficiency of utilization of absorbed amino acid mediated by a reduction in the requirement for glucogenic amino acids to provide glucose to facilitate the utilization of acetate.

#### *Amino acid flow and absorption from the small intestine*

The patterns of individual amino acid flow to the duodenum are similar to those determined by Armstrong and Hutton (1972). There were significantly greater flows of individual amino acids in animals on HS compared with those on control, with the exception of arginine, glycine and histidine, related to the higher DM intakes in animals on HS. There was a trend ( $P < 0.1$ ) for a greater absorption of total and individual amino acids on HS, but due to large residual errors no significant differences were found between the two grasses.

MacRae *et al.* (1985) accredited the greater absorption of amino acids from the small intestine when sheep were given spring- compared with autumn-harvested dried grass, to the elevated level of WSC and reduced fibre concentration of the spring-harvested grass. This may induce a greater synchrony between the rapidly degradable plant proteins and elevated readily available energy fraction of the cell (WSC) made available to the rumen micro-organisms (Rooke *et al.*, 1987; Nocek and Russell, 1988). However, numerous authors have reported little effect of inducing synchrony; Newbold and Rust (1992) reported that an asynchronous supply of N and energy to *in-vitro* micro-organisms did not significantly alter final bacterial yield compared with synchronous provision of these nutrients. Any changes in rumen function which occurred within these experiments may have been due to a change in the overall daily ratio of N and fermentable OM or short-term changes between N and fermentable OM within the day, deemed synchrony. In this experiment the higher levels of WSC in the HS grass did not result in changes to the overall daily ratio (21.9 and 20.8 g N per kg OMAD for control and HS, respectively). Synchrony was probably improved within the day but may have had no effect on EMPS due to the N-limited rumen environment (low N concentration in the diet and insufficient N recycling). Indeed, the reduction in the fibre concentration of these high WSC grasses may have just as an important rôle to play in increasing animal performance through an increase in DM intake (Thornton and Minson, 1972).

In this study, the amino acids entering the small intestine per unit amino acid intake were not significantly different, 1.10 and 0.99 for animals offered the control and HS, respectively. The greater NAN absorption from the small intestine appears to

be a result of the greater DM intake on HS rather than an improved efficiency of amino acid/NAN transfer to the duodenum. The results provide little evidence that an increase in the synchrony between energy and N release in the rumen, which may be achieved by feeding grasses of higher WSC concentration, would improve microbial protein synthesis in the rumen.

In conclusion the feeding of a variety of *Lolium perenne* selected for elevated levels of WSC compared with a control resulted in an increased DM intake leading to increased flows of N to the small intestine, a reduction of rumen ammonia and an enhancement of the propionate : acetate VFA ratio. However, there was no change in the efficiency of microbial protein synthesis on the high-sugar grass diet.

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