Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 1. In vitro and in vivo studies of nitrogen utilization[1]

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ABSTRACT: Two experiments were carried out to determine the effects of feeding grass silages differing in their water-soluble carbohydrate content, with or without red clover silage, on the efficiency of nutrient use. High-sugar grass, control grass, and red clover were ensiled in laboratory silos for use in an in vitro experiment (Exp. 1). For an in vivo experiment (Exp. 2), the same forage types were baled and ensiled. All silages were well preserved; within experiments the grass silages had similar composition, except for greater ($P < 0.05$) water-soluble carbohydrate concentrations in the high-sugar than the control grass silage. In Exp. 1, high-sugar grass, control grass, and red clover silages were fed alone or as mixtures (30:70, 50:50, or 70:30 on a DM basis, respectively) of each grass with the red clover silage to a simulated rumen culture system. There were no significant differences in microbial N flow or efficiency of microbial protein synthesis between individual forages. However, the corresponding values for the 70:30 ratio of high-sugar grass:red clover silage were greater ($P < 0.05$) than for the red clover silage. The value for the efficiency of N use (g of microbial N/g of feed N) was greater (0.86; $P < 0.05$) for high-sugar grass silage than the control grass silage. In addition, the high-sugar grass:red clover silage mixtures all gave greater ($P < 0.05$) values for the efficiency of N use than red clover silage alone; this difference was not achieved with the control grass mixture. Experiment 2 was an incomplete Latin square design conducted with 6 Hereford × Friesian steers (163 ± 5.9 kg of BW) with rumen and duodenal cannulas fed the following 5 silage diets: high-sugar grass silage; control grass silage; high-sugar grass and red clover silage (50:50 DM basis); control grass and red clover silage (50:50 DM basis); and red clover silage. Rumen NH_3-N concentration was lowest ($P < 0.05$) with the high-sugar grass silage. Microbial N flows to the duodenum and efficiency of microbial protein synthesis were greater ($P < 0.05$) for steers fed the high-sugar grass silage than for control grass and red clover silages, and mixing red clover with grass silages increased ($P < 0.05$) these values compared with red clover silage alone. In both experiments, the efficiency of incorporation of silage N into microbial N was more than 20% greater ($P < 0.05$) for high-sugar grass than for control grass silage. These data suggest that grass silage with high-sugar content provides a forage-based strategy for balancing N and energy supply and improving the efficiency of use of grass silage N in the rumen.

Key words: nitrogen use efficiency, perennial ryegrass silage, red clover silage, rumen function, water-soluble carbohydrate

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INTRODUCTION

The relatively low level of readily fermentable energy in grass silage is a limiting factor in the utilization of N by ruminants (Chamberlain and Choung, 1995). Grass breeders have addressed this problem by producing ryegrasses (Humphreys, 1989) with greater water-soluble carbohydrate content (high-sugar grasses). This approach increased the efficiency of N use by grazing dairy cows (Miller et al., 2001), increased BW gain of grazing lambs (Lee et al., 2001), and increased intake by beef cattle (Lee et al., 2002). With appropriate ensiling technology, the same approach could be applied to grass silage.

The target in producing good quality silage has been to maximize conversion of water-soluble carbohydrate into lactate (Weinberg and Muck, 1996), but little con-

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sideration has been given to retaining water-soluble carbohydrate with the specific objective of increasing microbial efficiency in the rumen (Jaakola et al., 1993). Ensiling high-sugar grasses results in silage with greater residual water-soluble carbohydrate content (Davies et al., 2002).

Legume silages have high intake characteristics (Thomas et al., 1985) and increase milk production compared with grass silage (Dewhurst et al., 2003a). However, they have lower water-soluble carbohydrate concentrations than grass, which, coupled with their high N content, means that less than 20% of feed N is often converted to product (Dewhurst et al., 2003a). A complementary effect of mixing grass and red and white clover silages was indicated by Dewhurst et al. (2003a,b). High-sugar grass silages may have an additive effect.

The objective of this work was to determine the effects of feeding high-sugar grass silage, with or without red clover silage, on DMI, rumen function, and efficiency of nutrient use by rumen microbes. The hypothesis tested was whether or not content of high-sugar grasses in the diet can increase the efficiency of use of silage N for microbial protein synthesis in the rumen.

MATERIALS AND METHODS

For these studies, all animal procedures and the care for the animals were carried out under strict regulations described in the Animals (Scientific Procedures) Act 1986 issued by the Home Office of Her Majesty's Britannic Government.

Experiment 1

Primary growths of 2 perennial ryegrass varieties: 1) an experimental cultivar (IGER var. Ba11353) bred for high-sugar content, or 2) AberElan, a control grass, and also a red clover (cv. Milvus), were cut in June 2000, wilted for 24 h, and chopped to 2- to 4-cm lengths using an electrically powered stationary forage harvester. Both grasses had similar heading dates and were harvested at midbloom. The herbages were all treated with an inoculant (Powerstart; Genus plc, Crewe, Cheshire, UK) containing *Lactobacillus plantarum* and *Lactococcus lactis* (cultured, diluted with water, and applied to give an application rate of 10^6 cfu/g of fresh matter), and ensiled in glass jars (Weck, Wehr Offingen, Germany; capacity of approximately 1 kg of fresh matter) for 90 d.

The resultant silages were evaluated individually or as mixtures using an 8-vessel in vitro rumen simulated culture system (RUSITEC) as described by Czerkawski and Breckenridge (1977). Jars of the 3 silages described above were emptied and mixed before each experimental period. The following treatments were prepared: high-sugar grass, control grass, and red clover; high-sugar grass:red clover that was mixed by hand on a plastic sheet to give ratios of 70:30, 50:50, or 30:70 (DM basis); or control grass:red clover that was likewise mixed to give ratios of 70:30 and 30:70, respectively. The 50:50 control grass:red clover treatment was omitted because the culture system used in this experiment only had 8 vessels, and a decision was taken to go with the 2 extremes. The mixed silages were frozen at –20°C in aliquots (11.1 g of DM) required for daily feeding of cultures. Samples (100 g) of each silage and mixture were taken and also stored at –20°C to await analysis of DM, OM, fermentation characteristics, N, water-soluble carbohydrate, and fiber fractions.

The experiment consisted of three 10-d runs, with each treatment randomly allocated to 1 vessel in each run. At the beginning of each experimental run (d 0), approximately 2 L of digesta were taken from the ventral sac of the rumen of a fistulated dairy cow just before its morning meal of grass and red clover silage (50:50 on a DM basis). Digesta was transported to the laboratory (within 1 h) in a preheated vacuum flask. The rumen fluid was strained and squeezed through a double layer of muslin into a CO_2-filled beaker, and each vessel was charged with 500 mL of strained rumen liquor and 200 mL of artificial saliva (McDougall, 1948).

One nylon bag (pore size 40 μm) containing approximately 11 g of DM of rumen digesta solids (fibrous fraction remaining after straining of rumen contents) and another containing 11.1 g of DM of the test silage were placed in a polythene bottle attached to a motor-driven arm located in the lid of the vessel. The vessel was topped up with artificial saliva, sealed with the lid assembly, and artificial saliva was pumped continuously into the culture vessels at 0.5 mL/min by a peristaltic pump (202U, Watson-Marlowe Ltd., Falmouth, Cornwall, UK) to give an overall liquid turnover rate of 2.92%/h. The vessels were maintained at a temperature of 39°C in a water bath.

The motor-driven arm moved the bottle containing the bags vertically through the rumen fluid/buffer mixture. The infusion of artificial saliva continuously displaced effluent from the vessel, which was collected in a container cooled with ice. After 24 h, the vessels were opened and the rumen digesta solids were removed, squeezed through a double layer of muslin, and washed with artificial saliva. The washings were returned to the vessel, and a bag containing fresh silage was inserted. On subsequent days the feed bag that had been in the vessel for 48 h was replaced with a bag containing fresh silage, as described above for the rumen digesta solids.

Samples of the liquid phase of the vessel contents were taken using a 10-mL syringe on d 9 and 10 at 0, 1, 2, 3, 4, 6, 14, and 24 h from the addition of the new feed bag. The pH of these samples was measured using a Hydrus 400 pH probe (Fisher Scientific UK, Loughborough, Leicestershire, UK), 1 mL being taken and acidified with 100 μL of 2 *M* HCl and used for NH_3-N analysis, and another 1 mL being acidified with 100 μL of 15 *M* orthophosphoric acid and analyzed for VFA concentrations.

Ammonium ^{15}N sulfate was added to the artificial saliva from d 3 of each run and infused as a microbial marker (Durand et al., 1988). On the last day of the experiment, the vessel contents and effluent collected over the previous 24 h were combined to produce a sample from which to harvest the liquid-associated bacteria. The solid-associated bacteria (**SAB**) were harvested from the silage residues collected from the nylon bags on d 10 of each run. To achieve this, the silage residues were suspended in 100 mL of saline (9 g of NaCl/L) in a polythene bag and pummeled by 2 alternating pistons for 5 min in a Colworth Stomacher (Colworth 400, A. J. Seward, Bedford, Bedfordshire, UK) to detach bacteria adhering to the fiber. The liquid washings were retained, and the residue was processed again in the same manner, the combined washings providing a sample for harvesting SAB.

The SAB and liquid-associated bacteria samples were then combined to provide a total bacterial sample, and 200 mL were centrifuged at 1,500 × g for 10 min. The supernatant was centrifuged at 30,000 × g for 25 min, followed by a distilled water wash and a further 25 min spin (30,000 × g), to obtain a pure bacterial pellet. A total solids sample was produced in the same way but without the initial 1,500 × g spin. All pellets obtained after the final wash and spin were freeze-dried before analysis. These samples were analyzed for N and ^{15}N to determine microbial N (**MN**) enrichment using a mass spectrometer (ANCA/SL 20/20, PDZ Europa Ltd., Crewe, Cheshire, UK). Values for MN flow and efficiency were calculated as described by Carro and Miller (1999).

Chemical Analysis. Water-soluble carbohydrate in the forages was determined as described by Thomas (1977), whereas the OM content of forages and effluents was analyzed by combusting at 550°C for 6 h in a muffle furnace. Volatile fatty acids in the effluent liquor and silages were determined by GLC using Chrompack CP 9002 (CP-Sil 5CB column 10 m × 0.25 mm ID; Varian Inc., CA) following the method of Zhu et al. (1996). Ammonia-N was assessed enzymatically using glutamate dehydrogenase on a discrete analyzer (FP-901M Chemistry Analyzer, Labsystems Oy, Helsinki, Finland; Test kit No. 66-50, Sigma-Aldrich Co. Ltd., Poole, Dorset, UK). Nitrogen was determined by a microKjeldahl technique using Kjeltec equipment (Perstorp Analytical Ltd., Maidenhead, Berkshire, UK). Neutral detergent fiber was determined as described by Van Soest et al. (1991), and ADF was analyzed according to the method of Van Soest and Wine (1967), using the Tecator Fibretec System equipment (Tecator Ltd., Thornbury, Bristol, Somerset, UK).

Statistical Analysis. The silage treatments were constructed from silage samples taken from each crop. Given that there were only 8 vessels, replicates were not possible. However, to introduce animal variation, 3 experimental runs were made with inoculum from the same donor animals. The implicit assumption was that environmental and biological variation is reflected in the rumen contents at any given time. Treatments were compared by 2-way ANOVA with treatment × run interaction as the error term. Where the overall treatment effect was significant ($P < 0.05$), individual treatments differences were determined using the Student-Newman-Keuls test. All statistical analyses were carried out using Genstat 7 (Lawes Agricultural Trust, 2003).

Experiment 2

Secondary growths (midbloom) of 2 intermediate perennial ryegrass swards, Ba11353 (high-sugar grass) and Fennema (control grass), with similar heading dates, were harvested on 5 June 2001, 6 wk after grazing by sheep, using a precision-chop forage harvester (J. Haldrup, Løgstør, Denmark). Harvested forages were ensiled in round bales (ca. 0.5 t) after wilting for 24 h to 209 and 210 g of DM/kg of fresh matter, respectively. A monoculture red clover (cv. Milvus) sward was cut on 9 August 2001 as a third cut (6-wk regrowth), wilted for 48 h to 261 g of DM/kg of fresh matter, and ensiled in round bales as described above. An inoculant (Powerstart; Genus plc, Crewe, Cheshire, UK) was cultured and applied to each forage during harvesting at 10^6 cfu/g of fresh matter.

Six Hereford × Friesian steers, initial BW of 163 ± 5.9 kg, surgically prepared with a rumen cannula and a simple T-piece cannula in the proximal duodenum (immediately after the pylorus and before the common bile duct; Jarrett, 1948), were used to evaluate the forages. Animals were housed in individual pens (5.10 × 5.12 m), with a 14-d adaptation period before the beginning of each experimental period and transferred to metabolism crates (1.31 × 1.83 m) for each 10-d measurement period. The building was well ventilated and continuously illuminated. Animals had free access to fresh water and mineral blocks (Baby Red Rockies, Tithebarn Ltd., Winsford, Cheshire, UK). The mineral licks included: 380 g/kg of Na, 5,000 mg/kg of Mg, 1,500 mg/kg of Fe, 300 mg/kg of Cu, 300 mg/kg of Zn, 200 mg/kg of Mn, 150 mg/kg of I, 50 mg/kg of Co, and 10 mg/kg of Se.

There were 4 experimental periods with 6 animals allocated to 1 of the 5 diets: high-sugar grass silage; control grass silage; high-sugar grass and red clover silage (50:50 DM basis); control grass and red clover silage (50:50 DM basis); and red clover silage. Although the 30% grass:70% red clover mixture of silages had given slightly lower values for the efficiency of N use in Exp. 1 than the corresponding 50:50 mixture, a decision was taken to be more cautious in this experiment (where less control was possible under in vivo conditions) and use a 50:50 mixture to reduce dilution of water-soluble carbohydrate when mixing with red clover silage.

Sufficient quantities of silages were offered at 0900 and 1600 to ensure ad libitum consumption, which was predetermined in the adaptation periods. Refusals were removed daily at 0845, weighed, and analyzed for DM to accurately predict DMI. Separate samples of fresh

Table 1. Chemical composition of the experimental silages[1] used in Exp. 1 (n = 4; g/kg of DM, unless stated otherwise)

Item	HG	R	HG:R 70:30	HG:R 50:50	HG:R 30:70	CG	CG:R 70:30	CG:R 30:70
DM, g/kg of fresh matter	243.7	233.5	241.3	238.2	235.3	236.8	234.1	232.9
OM	963.8	935.8	956.7	953.2	948.2	955.4	955.8	943.3
pH	3.58	3.77	3.63	3.68	3.70	3.80	3.78	3.77
ADF	264.8	264.4	264.5	265.6	258.1	300.0	292.5	273.6
NDF	420.0	325.9	399.0	383.2	363.0	481.7	442.8	374.2
WSC[2]	85.7	30.1	71.1	59.2	47.0	30.1	28.6	29.1
N	22.1	32.9	25.3	27.2	26.5	24.6	26.9	30.4
NH_3-N, g/kg of N	62.6	50.2	54.5	51.2	56.4	98.3	85.1	64.2
Fermentation acid								
Lactate	119.7	99.0	114.0	111.7	106.4	108.6	111.6	105.3
Acetate	8.2	11.4	9.7	10.1	11.9	11.0	12.9	12.9
N-Butyrate	1.9	1.9	1.8	1.8	1.8	3.7	3.5	2.5

[1] HG = high-sugar grass silage; CG = control grass silage; HG:R = high-sugar grass and red clover grass silage; CG:R = control grass and red clover silage; R = red clover silage; 70:30, 50:50 and 30:70 = ratios of respective grass silages to red clover silage in mixtures (DM basis).

[2] Water-soluble carbohydrate.

silage (1 kg) were taken daily during the digestion periods. Subsamples of silage and digesta (100 g) were either stored frozen or freeze-dried, ground with a Cyclotec 1093 grinder with a fixed 1-mm mesh screen (Foss Tecator, Hoganas, Sweden), and stored at –20°C before analysis of fiber, OM, N, and crude fat.

Digesta flow at the duodenum was estimated using a dual-phase marker system, with YbAc and Cr-EDTA as the particulate and liquid phase markers, respectively (Faichney, 1975). Ytterbium acetate (244 mg of Yb/d) and Cr-EDTA (1454 mg of Cr/d) were infused intraruminally via separate lines at a rate of 19 mL/h for 7 d. Duodenal digesta (100 mL) was collected every 4 h over two 24-h periods on d 20 to 21, and each collection for each day was bulked with the previously collected sample in a container maintained at 4°C, with each day kept as a separate sample. Rumen fluid (1 L) was sampled 2 h after feeding on d 24 for microbial isolations, as described by Lee et al. (2002).

Accumulated daily samples (n = 7) for each day (20 and 21) of duodenal digesta were thoroughly mixed in a bucket by hand, and a 200-g subsample was weighed and freeze-dried to represent whole digesta. A separate 200-g portion of duodenal digesta was weighed and centrifuged at $3{,}000 \times g$ for 25 min to provide the centrifuged solid digesta. These were subsequently freeze-dried, ground with a Cyclotec 1093 grinder with a fixed 1-mm mesh screen (Foss Tecator, Hoganas, Sweden), and stored at –20°C before analysis of OM, NDF, Yb, Cr, and N. A duodenal microbial fraction was obtained as described by Lee et al. (2002). Rumen pH was measured with a Hydrus 400 pH probe (Fisher Scientific UK, Loughborough, Leicestershire, UK) immediately after the samples (10 mL) were taken, and the samples were then acidified with 100 μL of 2.5 *M* sulfuric acid. Samples were stored at –20°C before analysis of NH_3-N and VFA.

Chemical Analysis. Water-soluble carbohydrate, N, VFA, IVDMD, ADF, NDF, and NH_3-N were analyzed as described for Exp 1. Chromium and Yb concentrations of digesta and infusate solutions were prepared as described by Williams et al. (1962) and analyzed using a Pye Unicorn SP9 Atomic Absorption Spectrophotometer (Spectronic Unicam, Cambridge, Cambridgeshire, UK). Purine and pyrimidine bases were used as microbial markers and were determined using the HPLC method of Cozzi et al. (1993).

Statistical Analysis. Digesta, nutrient, and MN flows were calculated after mathematical reconstitution of true digesta as described by Faichney (1975), with flows calculated for the 2 sampling days (20 and 21), and then a mean value was calculated per animal. Endogenous N was assumed to be 2.8 g/kg of DMI for the calculation of feed N degradability (Bartram, 1987). All measurements other than silage composition were subjected to GLM-ANOVA with DMI as a covariate based on a 5×5 Latin square design with an extra column (animal) added and 1 row (period) omitted (Cochran and Cox, 1957). Thus, there were 6 animals fed over 4 periods. In each period, 2 animals were fed the same diet. Where the overall treatment effect was significant ($P < 0.05$), individual treatment differences were determined using the Student-Newman-Keul's test. Due to the unbalanced nature of the design, SEM was calculated based on 4 replications. All analyses were conducted using Genstat 7 (Lawes Agricultural Trust, 2003).

RESULTS

Experiment 1

Before ensiling, the high-sugar grass, control grass, and the red clover had water-soluble carbohydrate contents of 244, 126, and 118 and N contents of 19.0, 22.1, and 30.3 g/kg of DM, respectively. The chemical characteristics of the silages or silage mixtures prepared for this experiment are shown in Table 1. The silages were

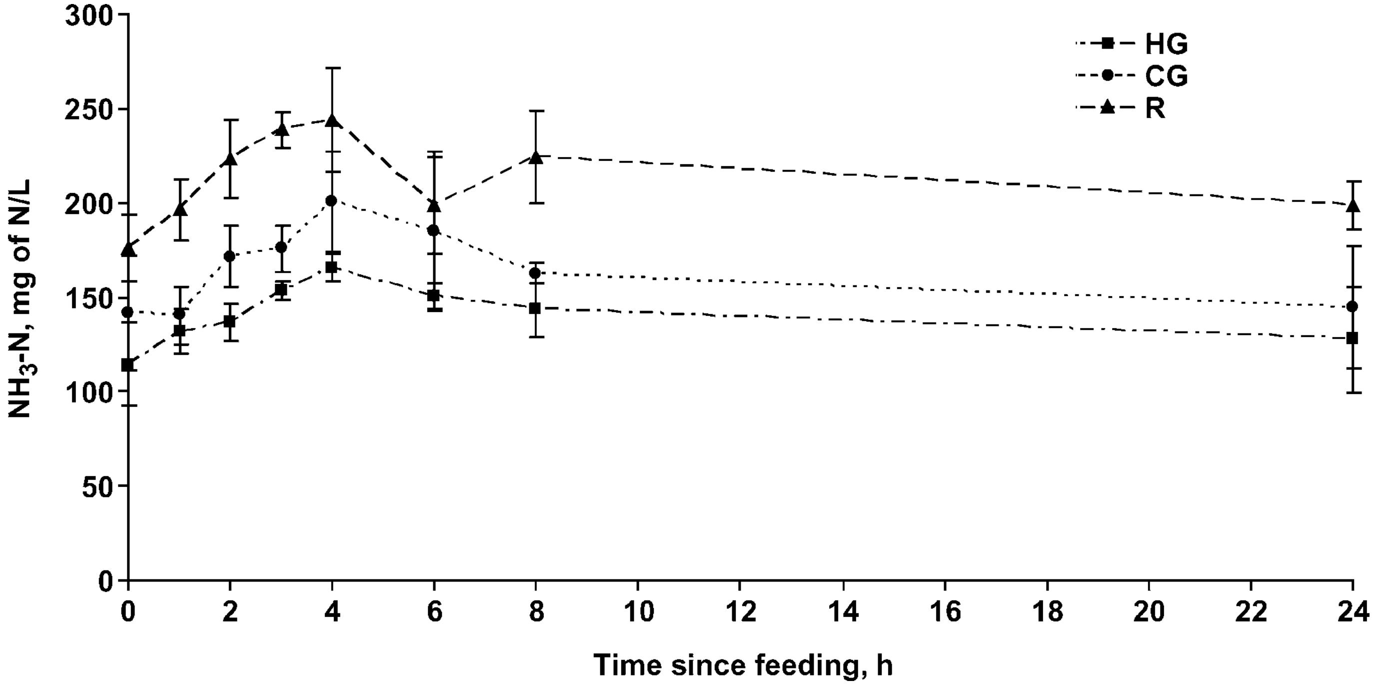

Figure 1. Changes in NH_3-N concentrations during incubation of high-sugar grass silage = HG; control grass silage = CG; red clover silage = R, in an in vitro, simulated culture system (Exp. 1). Values are means ± SEM.

all well preserved with pH values lower than 4.0, high lactic acid contents, and NH_3-N concentrations of less than 10% of total N. Ammonia-N concentrations were lower in the high-sugar grass silage than in the control grass silage. The water-soluble carbohydrate content of the high-sugar grass silage was approximately 3 times greater than in the control grass silage, which had a similar content to the red clover silage. Lactic acid concentration was greater in the high-sugar grass silage compared with the red clover silage, with the control grass silage having an intermediate value. When the silages were inserted into the RUSITEC, total VFA concentrations in the vessel contents for high-sugar grass silage reached 95 m*M* with red clover silage and control grass silage lower at approximately 80 m*M*. All maintained levels of >80 m*M* up to the next feed, which was offered 24 h later. Ammonia-N concentrations for the grass and red clover silages (Figure 1) increased up to 4 h after feeding and then declined, red clover silage values being greatest with a peak value of 240 mg of N/L and with respective peaks of approximately 200 and 165 mg of N/L for control grass and high-sugar grass silages, all values declining thereafter. Intermediate concentrations and similar patterns were observed for the mixed silages except for the 30:70 high-sugar grass/red clover mixture, which gave similar patterns and levels to those for red clover silage (Figure 2). There was a consistent trend for decreased NH_3-N with the highest level of grass in the mixtures, the smallest values being with high-sugar grass silage.

Concentrations of NH_3-N and VFA in the effluents and values for daily OM input, OM apparently digested (**OMAD**), MN flow and efficiency of microbial protein synthesis (**EMPS**; g of MN/kg of OMAD and g of MN/kg of OM truly digested, **OMTD**) are presented in Table 2. Differences in NH_3-N concentration were observed ($P < 0.05$) among the different silages, with lower values for the high-sugar grass than for the red clover silages. Mixing of either high-sugar or control grass silage with red clover silage did not decrease NH_3-N concentration for any of the mixing ratios. Values for OMAD were greater ($P < 0.05$) for high-sugar than for control grass and red clover silages, and only the 70% high-sugar grass: 30% red clover silage mixture had a greater efficiency value than red clover silage.

There were no significant differences in values for MN flow or EMPS between the silages made from the 3 individual forages when fed alone. Mixing of red clover silage with high-sugar grass silage increased ($P < 0.05$) MN flows and EMPS values compared with red clover silage alone, the greatest values were observed when high-sugar grass silage was included at the 30% level. The effect of mixing is more evident when EMPS was calculated on an OMTD basis with both grass silages being significantly greater than the red clover silage when mixed at a ratio of 30% grass silage to 70% red clover. In addition, all mixing ratios for the high-sugar grass had greater ($P < 0.05$) EMPS than the red clover silage alone. The efficiency of conversion of feed N into MN was greater ($P < 0.05$) for high-sugar grass silage than for control grass silage. On the other hand, values for red clover were less ($P < 0.05$) than those of the grass silages. Mixing high-sugar grass silage with red clover silage increased ($P < 0.05$) the efficiency of N use at all mixing ratios by > 40% compared with red clover

Table 2. Fermentation variables, microbial N production, and OM input and flows in rumen cultures fed the experimental silages[1] used in Exp. 1

Item	HG	R	HG:R 70:30	HG:R 50:50	HG:R 30:70	CG	CG:R 70:30	CG:R 30:70	SEM n = 4	P^2
NH_3-N, mg/L	99.4^{a}	175.0^{b}	127.4^{ab}	151.2^{b}	155.4^{b}	127.4^{ab}	147.0^{ab}	175.0^{b}	6.73	0.001
VFA, mM	88.3	77.8	89.9	80.9	81.1	87.2	81.1	86.7	3.23	NS[3]
OM input, g/d	10.7^{b}	10.4^{a}	10.6^{ab}	10.6^{ab}	10.5^{ab}	10.6^{b}	10.6^{ab}	10.5^{ab}	0.04	0.002
OMAD,[4] g/d	9.2^{c}	8.5^{ab}	9.1^{c}	8.9^{bc}	8.8^{abc}	8.3^{a}	8.3^{a}	8.5^{ab}	0.11	0.001
Microbial N g/d	0.22^{abc}	0.20^{ab}	0.24^{bc}	0.24^{bc}	0.25^{c}	0.18^{a}	0.20^{ab}	0.21^{abc}	0.009	0.001
EMPS,[5] g of MN/kg of OMAD	23.6^{abc}	23.1^{ab}	25.8^{abc}	27.3^{bc}	28.7^{c}	21.4^{a}	23.6^{abc}	25.3^{abc}	0.96	0.002
EMPS,[6] g of MN/kg of OMTD	17.8^{ab}	17.8^{ab}	19.2^{cd}	19.9^{de}	20.7^{e}	16.5^{a}	17.9^{b}	18.7^{bc}	0.67	0.002
NUE,[7] g of MN/g of feed N	0.86^{c}	0.54^{a}	0.82^{bc}	0.80^{bc}	0.77^{bc}	0.65^{ab}	0.67^{ab}	0.64^{ab}	0.032	0.001

[a–e]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1] HG = high-sugar grass silage; CG = control grass silage; HG:R = high-sugar grass and red clover grass silage; CG:R = control grass and red clover silage; R = red clover silage; 70:30, 50:50 and 30:70 = ratios of respective grass silages to red clover silage in mixtures (DM basis).

[2] Overall model *P*-value.

[3] Not significant at $P < 0.05$.

[4] Organic matter apparently digested.

[5] Efficiency of microbial protein synthesis.

[6] Organic matter truly digested.

[7] Nitrogen use efficiency.

silage, compared with approximately 20% ($P < 0.05$) for the control grass silage mixtures, respectively.

Experiment 2

Silage pH values ranged from 4.06 to 4.14, with lesser values for high-sugar grass and control grass silages compared with red clover silage and high-sugar grass:red clover silages (Table 3). All silages had undergone a predominantly lactate fermentation. The red clover silage had lower OM, NDF, crude fat, and water-soluble carbohydrate and greater DM, NH_3-N, and N than the 2 grass silages. Values for the mixtures of grass and red clover silages were intermediate to those for the red clover with high-sugar grass silages or control grass silages. The high-sugar grass silage had a greater content of water-soluble carbohydrate than the control grass silage but otherwise was similar in chemical composition.

Rumen pH values were similar for all silage treatments at approximately 6.8. Concentrations of VFA and their relative proportions were also similar across treatments (Table 4). Ruminal NH_3-N concentrations ranged from 73 with high-sugar grass silage to 117 mg of N/L with red clover silage, only these 2 treatments differed ($P < 0.05$). Diurnal rumen NH_3-N concentrations are shown in Figure 3. The value for high-sugar grass silage was consistently less than for the control grass silage over a 24-h period and only reached 100 mg of N/L just after the morning of feed and before feeding on the next day and fell to below 50 mg of N/L 5 h after both daily feedings. Red clover silage and the mixtures with the greatest proportion of red clover silage had the greatest concentrations of NH_3-N, the mixture with high-sugar

Table 3. Chemical composition of the experimental silages[1] (n = 4; g/kg of DM, unless stated otherwise) used in Exp. 2

Item	HG	CG	HGR	CGR	R
DM, g/kg	276.7	260.0	287.2	273.5	310.9
OM	918.8	923.6	907.7	914.5	894.6
pH	4.06	4.03	4.12	4.09	4.14
ADF	329.5	330.4	308.4	321.1	317.2
NDF	549.9	583.8	463.6	498.7	402.4
Crude fat	37.3	39.0	32.1	31.6	27.4
Water-soluble carbohydrate	90.9	55.0	65.8	42.7	30.8
GE, MJ/kg of DM	19.6	19.3	19.1	18.9	18.5
N	24.4	25.6	29.5	29.2	31.9
NH_3-N, g/kg of N	104.2	109.0	113.2	109.4	123.1
Fermentation acid					
Lactate	79.5	72.4	77.8	83.4	77.6
Acetate	8.9	9.0	11.7	12.6	13.8
Propionate	0.4	0.7	0.3	0.2	0.01
N-Butyrate	5.6	5.5	4.6	4.4	2.9

[1] HG = high-sugar grass; CG = control grass; HGR = high-sugar and red clover; CGR = control grass and red clover; R = red clover.

Table 4. Rumen characteristics of steers fed the experimental silages[1] in Exp. 2

Item	HG	CG	HGR	CGR	R	SEM n = 4	P^2
pH	6.67	6.76	6.80	6.82	6.82	0.021	NS[3]
NH_3-N, mg/L	73.4^b	90.4^a	96.3^a	88.4^{ab}	117.2^a	4.73	0.015
VFA, m*M*							
Acetate	54.8	55.4	56.9	57.6	57.5	2.78	NS
Propionate	15.0	14.8	15.2	14.2	15.3	0.90	NS
Iso-Butyrate	0.86	0.87	0.99	0.93	0.93	0.052	NS
N-Butyrate	6.71	6.49	6.18	6.19	5.67	0.342	NS
Iso-Valerate	1.16	1.42	1.20	1.12	1.00	0.033	NS
Total VFA	79.3	81.5	83.5	83.4	83.7	3.673	NS
P/(A+B)[4]	0.22	0.24	0.24	0.23	0.24	0.009	NS

[a,b] Within a row, means without a common superscript letter differ ($P < 0.05$).

[1] HG = high-sugar grass; CG = control grass; HGR = high-sugar and red clover; CGR = control grass and red clover; R = red clover.

[2] Overall model *P*-value.

[3] Not significant at $P < 0.05$.

[4] Propionate/(acetate + butyrate).

grass silage reducing NH_3-N compared with the control grass silage.

Mean BW of the animals on each treatment were not significantly different among treatments (Table 5). In comparison with control grass silage, DMI was greater ($P < 0.05$) for steers fed high-sugar grass silage. No difference was observed in nutrient flow between red clover and either of the grass:red clover silage mixtures. Flows of nonNH$_3$-N (**NAN**) were greater ($P < 0.05$) for steers fed high-sugar grass than control grass silages, but there was no difference in values for either of the silage mixtures compared with red clover silage alone. Microbial N flows reflected purine and NAN values, being greater ($P < 0.05$) for steers offered the high-sugar grass silage than for the control grass silage. The mixed red clover:grass silages both promoted greater ($P < 0.05$) MN flows than were observed for red clover silage, but there was no difference between the 2 mixtures. NonNH$_3$, nonmicrobial-N (**NANMN**) flows, as an indicator of feed N flow, were greater ($P < 0.05$) for animals fed the red clover silage than the grass silages with the mixtures intermediate.

Efficiency of microbial protein synthesis when calculated on an OMAD and an OMTD basis in steers fed high-sugar grass silage were greater ($P < 0.05$) than in steers fed control grass and red clover silages, whereas the grass:red clover mixtures were greater than the red clover silage alone.

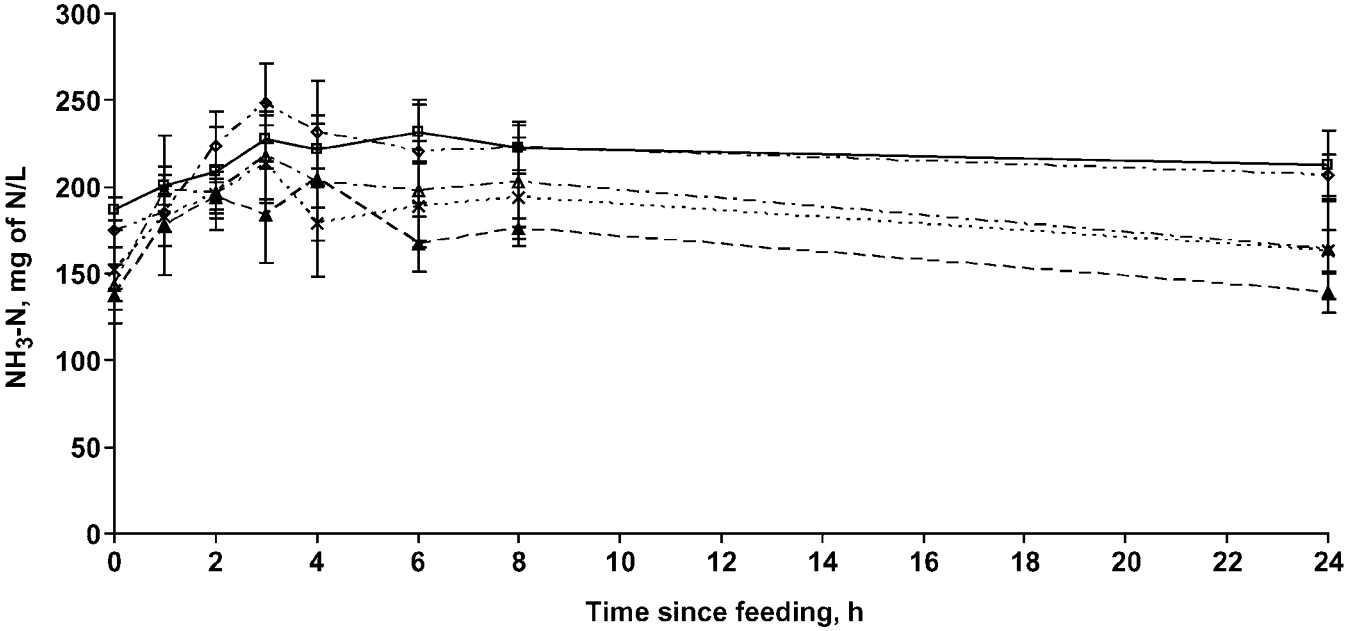

Figure 2. Changes in NH_3-N concentrations during incubation of mixtures of high-sugar grass and red clover silages at ratios of 70:30 ▲; 50:50 X; and 30:70 ◇ (DM basis) and control grass and red clover silages at ratios of 70:30 △ and 30:70 □ (DM basis) in an in vitro, simulated culture system (Exp. 1). Values are means ± SEM.

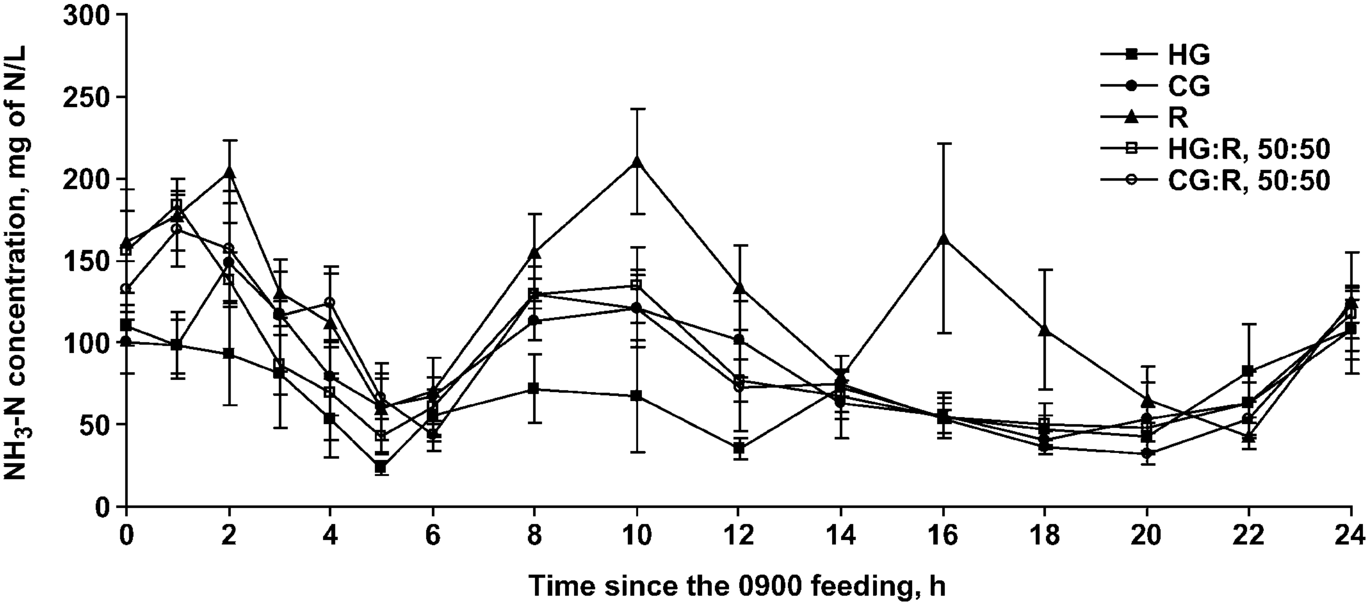

Figure 3. Changes in rumen NH_3-N concentration of beef cattle fed high-sugar grass = HG; control grass = CG; red clover = R; HG:R, 50:50; or CG:R, 50:50. Silage mixtures were prepared on a DM basis in the ratios given. Values are means ± SEM.

The efficiency of conversion of feed N into MN was greater ($P < 0.05$) for high-sugar grass silage than for control grass silage. The red clover silage had the smallest conversion factor, but this increased ($P < 0.05$) when it was mixed with the high-sugar and control grass silages, but the latter values were not significantly dif-

Table 5. Mean BW, intake, and duodenal flow of steers fed the experimental silages[1] (g/d unless otherwise stated) used in Exp. 2

Item	HG	CG	HGR	CGR	R	SEM n = 4	P^2
BW, kg	195.4	191.4	207.4	197.7	190.5	9.42	NS[3]
Intake							
DM, kg/d	4.29^a	3.60^b	4.68^a	4.47^a	4.78^a	0.195	0.050
OM, kg/d	3.95^a	3.33^b	4.25^a	4.08^a	4.28^a	0.179	0.010
N	93.1^a	91.3^a	136.4^b	130.7^b	150.9^c	4.63	0.001
Duodenal flow							
DM, kg/d	2.14^a	1.82^b	2.37^a	2.28^a	2.29^a	0.081	0.009
OM, kg/d	1.67^a	1.34^b	1.82^a	1.78^a	1.81^a	0.074	0.007
N	96.2^a	74.9^b	107.9^a	101.8^a	110.4^a	3.07	0.001
NH_3-N	3.31	3.10	3.82	3.51	3.43	0.256	NS
NonNH_3-N	92.9^a	71.8^b	104.1^a	98.3^a	107.0^a	3.82	0.001
Microbial N	63.2^a	41.7^b	58.2^a	58.3^a	46.8^b	2.85	0.005
NonNH_3, nonmicrobial-N	31.6^{cd}	30.4^d	45.5^b	39.4^{bc}	59.3^a	2.10	0.001
Purine and Pyrimidine	8.46^a	5.34^b	6.96^{ab}	6.93^{ab}	5.29^b	0.403	0.004
N use efficiency, g of MN/g of feed N	0.72^a	0.46^b	0.42^b	0.44^b	0.28^c	0.023	0.001
EMPS,[4] g of MN/kg of OMAD[5]	28.9^a	20.8^c	23.6^b	25.3^b	18.9^c	0.75	0.001
EMPS, g of MN/kg of OMTD[6]	19.4^a	15.2^c	17.0^{bc}	17.3^b	13.7^d	0.69	0.001
Rumen degradability of N,[7] %	79.2^a	77.8^a	75.7^a	77.5^a	68.3^b	1.90	0.001
Microbial N:Purine, g/g	8.08	7.68	8.34	8.54	7.75	0.243	NS
N:Purine, g/g	12.2^d	14.0^c	15.5^b	14.8^{bc}	19.7^a	0.35	0.001

[a–d] Within a row, means without a common superscript letter differ ($P < 0.05$).

[1] HG = high-sugar grass; CG = control grass; HGR = high-sugar and red clover; CGR = control grass and red clover; R = red clover.

[2] Overall model P value.

[3] Not significant at $P < 0.05$.

[4] Efficiency of microbial protein synthesis.

[5] Organic matter apparently digested in the rumen.

[6] Organic matter truly digested in the rumen.

[7] Assuming endogenous-N = 2.8 g per kg of DM intake (Bartram, 1987).

ferent. Rumen degradability of N did not differ among steers fed high-sugar grass, control grass, and the mixed silages, but was less ($P < 0.05$) in steers fed red clover silage. Microbial N:purine ratio was not significantly different across treatments, whereas the N:purine ratio was greater ($P < 0.05$) in steers fed red clover silage than the mixtures and grass silages, with high-sugar grass silage greater ($P < 0.05$) than the control grass silage.

DISCUSSION

The principle underlying the benefits of water-soluble carbohydrate on the efficiency of ruminal N use in cattle and sheep grazing grasses (Miller et al., 2001; Lee et al., 2001) applies equally, or perhaps more so to silage, where available sugar is often in short supply (Rooke et al., 1987; Chamberlain and Choung, 1995). Water-soluble carbohydrate is generally fermented to lactic acid and other products so that the residual water-soluble carbohydrate concentration is often as low as 20 g/kg of DM (McDonald et al., 1991), except where extensive wilting has occurred (Davies et al., 2002) or high levels of formic or sulfuric acid have been added to restrict the fermentation (Carpintero et al., 1979; Jaakola et al., 1993). The availability of high-sugar grass cultivars, which express high (150 to 200 g/kg of DM) water-soluble carbohydrate contents, has stimulated us to reassess approaches for controlling silage fermentation and describing silage quality in terms of environmental sustainability and product quality (Davies et al., 2005). Both from environmental and safety standpoints, strong acids, although very effective, are now less popular. We have taken the environmentally benign approach of inoculating forages with homo-fermentative lactic acid bacteria to manipulate end point water-soluble carbohydrate content and silage quality.

Composition of Experimental Silages

The more extensive fermentations in the laboratory silages (Exp. 1) compared with the baled silages (Exp. 2) probably reflected less DM in the ensiled herbages (McDonald et al., 1991). On the other hand, reduced compaction and lesser damage to forage during the baling process also lead to a slower and less intensive fermentation (Field and Wilman, 1996).

Success was achieved in preserving water-soluble carbohydrate at the laboratory and field scale, with 3.5 and 5.7 percentage units (absolute) greater values, respectively, in high-sugar grass silages compared with the control grass silages. Thus, at both scales, suitable experimental material was prepared to test the proposed hypothesis. In unwilted silages water-soluble carbohydrate is generally fermented to give low residual concentrations of < 20 g/kg of DM in grass silage (McDonald et al., 1991), but in these experiments the absolute values of water-soluble carbohydrate in the high-sugar grass silages approached 100 g/kg of DM. To obtain such high levels of water-soluble carbohydrate in the high-sugar grass silages a rapid and very efficient, homolactic fermentation must have occurred, which suggests that the silage inoculant bacteria applied to both forages dominated the fermentation. It was accompanied by high ratios of lactate:acetate in these silages, a characteristic of silages treated with homo-fermentative inoculants (Muck and Kung, 1997). Our data and that of other workers supports the premise that high levels of residual water-soluble carbohydrate (> 20 g/kg of DM) can be retained in silages prepared from grass that has been wilted (Carpintero et al., 1979), treated with homo-fermentative silage inoculants (Davies et al., 2002), or high levels of formic acid (Jaakola et al., 1993; Davies et al., 2002).

Effect of High Sugar Grass Silage on Rumen Fermentation and the Efficiency of N Use

There has been much effort to test the theory that microbial protein synthesis is maximized by synchronizing the availability of fermentable energy and degradable N in the rumen (Dewhurst et al., 2000). Chamberlain and Choung (1995) reviewed the case for asynchrony with conserved forage-based feeds, where an extreme imbalance can arise; little readily available energy is available at a time when there is an abundance of protein degradation products (McDonald et al., 1991), and concluded that it was difficult to attribute effects to synchronization of energy and N supply to the rumen because of confounding effects of changes in dietary ingredients. Dewhurst et al. (2000) concluded that absolute synchrony was not possible but proposed that, particularly in the case of silage, the term balance may be more appropriate when describing ruminal N and energy supply. In the current study, water-soluble carbohydrate supply was manipulated without changing the basic feed class (i.e., grass silage), so it was not an attempt to synchronize energy supply absolutely but to provide energy in the early stages of the fermentation when silage soluble N is often in excess and energy is limiting (MacRae and Theodorou, 2003). Likewise, Huhtanen and Shingfield (2005) argued, “manipulating the ratio of degradable protein and fermentable energy supply in the rumen provides the best opportunity to enhance N efficiency of milk production.” Any improvement will be related to, in part, increases in the efficiency of ruminal N use (Jaakola et al., 1993).

Lower NH_3-N concentrations and greater MN production have been shown to be a typical response to available carbohydrate (Henning et al., 1993; Kolver et al., 1998). In the current study EMPS when calculated on an OMTD basis were significantly lower than on an OMAD basis, to a similar magnitude as reported by Beever et al. (1990), and both were greater in steers fed on the high-sugar grass silage as opposed to the control grass silage. Other authors found the EMPS (g of MN/kg of OMAD), whereas being greater and similar to accepted estimates of EMPS (AFRC, 1992), was not significantly different for high-sugar grass silage than control grass silage (Lee et al., 2002). On the other hand the efficiency of N use of converting of feed N into MN (g/g of feed N) in both the current study and Lee et al. (2002) was greater for high-sugar grass silage than the control grass silage. Although this figure was not corrected for rumen undegraded N, it represents a large increase in efficiency that could potentially translate into a significant improvement in overall efficiency of N use in the animal.

Previous studies have shown that rumen function or the efficiency of N use in dairy cows fed pure legume silages can be modified by mixing them with grass silages (Auldist et al., 1999; Dewhurst et al., 2003b). This may in part be driven by ruminal responses, but the lack of response at the greater levels (70 and 50%) of high-sugar grass silage inclusion and the overall lack of significant difference of the high-sugar grass mixtures in comparison to the control mixture in terms of MN flow and EMPS suggests that sugar was not the sole driving force, unlike when the 2 grass silages were compared. These observations may be related to the dilution effect on water-soluble carbohydrate when the silages are mixed. Despite these findings, mixing of red clover silage with high-sugar grass silage increased the efficiency of N use at all levels of inclusion by > 40% compared with red clover silage.

Rumen conditions in these steers were stable, with high pH (6.8 mean) and low total VFA concentrations (81.8 m*M*/L mean). There were no significant differences between rumen pH for any of the diets fed to steers in Exp. 2. Also, despite the greater water-soluble carbohydrate concentration in high-sugar grass silages and silage mixtures, the VFA concentrations and molar proportions were not significantly different across all diets. This contrasts with results from previous studies in which silages were supplemented with readily available carbohydrate (Rooke et al., 1987; Kim et al., 1999).

Experiment 2 confirmed that animals tend to consume more red clover silage compared with grass silage (Thomas et al., 1985; Dewhurst et al., 2003a; Lee et al., 2003a), though DMI of high-sugar grass silage was similar to that of the red clover silage. Lee et al. (2002) also observed increased intakes when harvested high-sugar grass was offered to beef cattle. Greater DMI of high-sugar grass in previous studies have been related to differences in NDF content (Thornton and Minson, 1972; Lee et al., 2001, Miller et al. 2001); in contrast, in the current study there was no difference in NDF content between the grass silages. However, forages containing high levels of water-soluble carbohydrate have been shown to reduce rumen NH_3 concentrations in earlier studies (Lee et al., 2002) and are highly correlated to water-soluble carbohydrate concentration in continuous batch culture (Lee et al., 2003b). This may be expected to prolong the onset of satiety (Davidovich et al., 1977) and may partly explain the greater DMI by steers fed high-sugar grass silage compared with steers fed the control grass silage.

The decrease in rumen NH_3-N concentration and increase in MN production observed with high-sugar grass compared with the control grass silage was probably due to a combination of lower DMI on the control grass silage and to the extra readily available energy in the high-sugar grass silage because the main difference observed between the compositions of the 2 silages was in water-soluble carbohydrate content. Chamberlain et al. (1993) reported similar trends for NH_3-N concentration and MN production when soluble sugars were given as supplements to a basal diet of silage. The above changes were accompanied by a 1.3 times increase in flow of NAN to the small intestine, similar to that observed by Rooke et al. (1987) when glucose syrup was infused into the rumen of grass silage-fed cows. The fact that NAN flows in our experiment were of a similar order of magnitude to those for N and NANMN flows for high-sugar and control grass silages suggested that the increase in MN was largely due to an increase in the efficiency with which silage-N was incorporated into microbial protein in the rumen (Rooke et al., 1987; Chamberlain et al., 1993; Chamberlain and Choung, 1995). This was confirmed by the increase in the efficiency of N use with high-sugar grass silage and agrees with the results obtained in Exp. 1. The increase in MN flow with the high-sugar grass silage compared with the control grass silage was reflected by a 20% increase in microbial growth efficiency supply. This represents a shift from 21 g of MN/kg of OMAD, a fairly low value for unsupplemented silage (AFRC, 1992), in steers fed the control grass silage to a more acceptable value of 29 g of MN/kg of OMAD in steers fed high-sugar grass silage. The same relationship is shown when reporting EMPS as a function of grams of MN/kilogram of OMTD increasing from 15 to 19 in steers fed the control grass silage and high-sugar grass silage, respectively. This gives a more accurate assessment of EMPS because it is only the OMTD that truly contributes to the growth of microorganisms. This finding supports the conclusions of Chamberlain and Choung (1995) who reviewed data on the output of microbial protein for silages in response to supplementation with different carbohydrate sources.

In the silage used in Exp. 2 the ratios for rumen degradable N:water-soluble carbohydrate ratio for the control and high-sugar grass silages were 0.36 and 0.21, respectively, which were similar to corresponding values for the silages used in Exp. 1. Although these values were at the lower end of the range (8.7 to 0.28) examined by Jaakola et al. (1993), similar trends for increasing N flow and efficiency were observed by those authors. Both studies suggest the potential for increasing ruminal microbial protein synthesis by increasing water-soluble carbohydrate supply and manipulating the ratio of rumen degradable N:fermentable energy (Huhtanen and Shingfield, 2005).

The significantly lower rumen NH_3-N for the high-sugar grass silage compared with the control grass silage with comparable N intakes suggests a greater incorporation of silage N into microbial cells or a reduced breakdown of gluconeogenic AA to provide energy for microbial protein synthesis, and thus resulting in a greater efficiency of N use. The greater rumen NH_3- N values for red clover than the high-sugar grass silage reflects the greater N intake of the red clover silage but may also in part reflect the effect of residual water-soluble carbohydrate on MN use in the rumen. Similar effects, but with greater concentrations, were observed by Dewhurst et al. (2003b) when mixtures of grass silage and red clover silage (50:50 on a DM basis) were fed to dairy cows, compared with red clover silage alone.

The effects of mixing grass and red clover silages on EMPS have not been consistent across the literature (Lee et al. 2003a; Dewhurst et al. 2003b), though differences in concentrate supplementation as well as the limitations of in vitro systems may explain some of the discrepancies. There were marked differences in patterns of EMPS when comparing results from steers with those from dairy cows. Efficiency of microbial protein synthesis was greatest with red clover silage for dairy cows (Dewhurst et al., 2003b), but lowest with red clover silage for steers (Lee et al., 2003a, and Exp. 2). This probably reflects the effects of the supplementary concentrates (8 kg/d) given to the cows. Red clover silage has a faster rumen passage rate than grass silage (Dewhurst et al., 2003b), and this potentiates an increase in EMPS; but only when the supply of available carbohydrates is adequate (Lee et al., 2003b). Efficiency of microbial protein synthesis with steers was greatest with either high-sugar grass silage or forage mixtures of grass silage and red clover silage, suggesting that issues relating to substrate availability dominated microbial activity and function when forage-only diets were fed. It seems that providing available carbohydrates, through concentrates or in the forage (grass silage), can overcome the limitations of red clover silage as a microbial substrate.

Values for microbial efficiency of N use were comparable between the 2 experiments. Greater microbial efficiency of N use could possibly be translated into an increase in the overall efficiency of N use for BW gain and milk production if high-sugar grass silages or legume-grass silage mixtures are used in practical feeding systems. Auldist et al. (1999) showed that mixing maize silage with white clover silage improved the efficiency of use of dietary N for BW gain and milk production, although other factors such as differences in particle outflow rate between diets could have played a role. Bertilsson et al. (2005) added sucrose to grass:red clover silage to simulate a high-sugar silage mixture and did not observe a significant effect on efficiency of feed N into milk N compared with nonsupplemented silage. However, in contrast to the present experiments, a moderate level of supplementary concentrate containing available sugar was fed, which may have masked the effect of the added sugar.

Rumen N degradability estimates were significantly less for red clover silage than for both of the grass silages. The results of Dewhurst et al. (2003b) with dairy cows showed a similar trend, but the difference was not significant, perhaps because their diets included concentrates. The lower ruminal degradability of N was probably due, in part at least, to the activity of polyphenol oxidase in red clover and is supported by the greater flow of feed N predicted by the flow of NANMN and the N:purine ratio. This enzyme is active during ensiling and converts complex phenolics into quinones, which bind to proteins and decrease their degradability (Albrecht and Muck, 1991; Lee et al., 2004).

IMPLICATIONS

Our results suggest that grass silage with high residual sugar content can provide a forage-based strategy for balancing nitrogen and energy supply for rumen microbial growth and thereby improve the efficiency of nitrogen use in the rumen of ruminants fed silage as the sole diet. This could potentially lead to an improvement in the overall efficiency of nitrogen use by the ruminant and a reduction in nitrogen loss in urine and feces.

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