



The Effect of Energy Supplementation on Intake and Utilisation Efficiency of Urea-treated Low-quality Roughage in Sheep

I. Rumen Digestion and Feed Intake

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ABSTRACT : Inefficient rumen microbial fermentation is a major factor limiting intake of low quality roughage in ruminants. In this study, the effect of energy supplementation on rumen microbial fermentation, absorption of balanced digestion products and voluntary feed intake in sheep was investigated. A basal diet of a urea-treated mixture of wheaten chaff and barley straw (3:1 DM) containing 22.2 g N/kg DM was used. Four Merino-cross wethers weighing 45±4.38 kg and fitted with permanent rumen and abomasal cannulae were allocated to four treatments in a 4×4 Latin square design. The dietary treatments were basal diet (E₀), or basal diet supplemented with sucrose (112.5 g/d) administered to the animals intra-ruminally (E_R), abomasally (E_A), or through both routes (50:50) (E_{RA}). Feed intake (basal and dietary) was increased (p<0.05) by sucrose supplementation through the rumen (E_R) or abomasum (E_A). However, there was no difference (p>0.05) in intake between animals on the control diet and those supplemented with sucrose through both intraruminal and abomasal routes (E_{RA}). The digestibility of DM and OM was highest in E_R and E_A supplemented animals. Although the rumen pH was reduced (p<0.001) in animals supplemented with sucrose entirely intra-ruminally (E_R), the *in sacco* degradation of barley straw in the rumen was not adversely affected (p>0.05). Intra-ruminal sucrose supplementation resulted in a higher concentration of total VFA, acetate and butyrate, while the pattern of fermentation showed a higher propionate: acetate ratio. Intra-ruminal supplementation also increased (p<0.05) the glucogenic potential (G/E) of the absorbed VFA. However, there was no difference (p>0.05) in microbial protein production between the four dietary treatments. Protozoa numbers were increased (p<0.05) by intra-ruminal supplementation of sucrose. (**Key Words :** Roughage, Energy Supplementation, Intake, Glucogenic Potential, Microbial Protein, Purine Derivatives)

INTRODUCTION

Low quality roughages such as cereal straw and stover are generally high in fibre but low in key nutrients such as nitrogen (N), minerals such as sulphur and readily fermentable carbohydrates (Preston and Leng, 1987). As a result, they are poorly utilized by ruminants mainly due to a combination of high cell wall contents (CWC), low microbial growth and fermentation activity in the rumen (Ferrell et al., 1999). Energy supplementation has been reported as being variably successful in enhancing digestibility and intake of basal roughage (Ørskov 1986; Lee et al., 1987; Fonseca et al., 2001). However, there are indications that the response in digestibility and intake of

poor quality basal roughage to energy supplementation has been variable and appears to be influenced by a wide range of factors that include; the type, form and amount of energy supplement used, and the relative proportion and quality of the basal roughage (Iwanyanwu et al., 1990; Obara et al., 1991). Use of readily digestible carbohydrate supplements in low quality roughage also poses a risk of lowering cellulolytic activity in the rumen and may impact negatively on digestibility of the basal roughage and therefore reduce intake (Royes et al., 2001). However, the fermentation of energy supplement in the rumen may promote propionate absorption into the body tissues besides enhancing microbial protein synthesis when the ruminal ammonia-N level is not limiting. In this study, it is hypothesized that the enhanced propionate absorption and improved microbial protein amino acid supply to the body tissues may increase the body's glucogenic potential and/or protein to energy ratio (P:E), thereby boosting the ability of the animal to metabolise rumen-absorbed acetate which may stimulate higher intake of low quality roughage. It has long been

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Table 1. The four dietary treatments used in the feeding trial

Treatments		
T1	Basal diet (11% CP) (Control)	E ₀
T2	Basal diet+Energy* (100% intraruminal)	E _R
T3	Basal diet+Energy (100% intra-abomasal)	E _A
T4	Basal diet+Energy (50% intraruminal+50% intra-abomasal)	E _{RA}

* Energy supplied as sucrose dissolved in warm water.

established that inability to completely metabolize absorbed acetate is a major factor constraining intake of fibrous roughage by ruminants, especially in the tropics (Leng and Preston, 1987). The net effect of carbohydrate supplementation when rumen NH₃-N is not limiting is to remove this constraint and therefore enhance both dietary intake and feed utilization efficiency, though this may vary with the circumstances of the diet.

The main objective of the present study was to investigate the effect of *in vivo* supplementation with sucrose as a means of improving rumen fermentation so as to maximize the absorption of fermentation and digestion products into the body tissues. It is hypothesized that this will enhance the glucogenic potential and/or P:E ratio at the tissue metabolism level and therefore remove a major hurdle that limits voluntary intake of fibrous roughage in ruminants. It is anticipated that increasing dietary intake is the key to improving efficiency of utilization of high fibre-low protein basal roughage in ruminants and therefore higher production of animal products.

MATERIALS AND METHODS

Animals, their management and experimental design

Four (4) Border Leicester×Merino crossbred wethers weighing 45.0±4.38 kg, each fitted with permanent rumen and abomasal cannulae, were re-located to the animal house and housed in individual pens with a slatted floor. A feeding trial with the four wethers and four treatments was carried out in four periods in a balanced 4×4 Latin square design (Table 1).

Sheep were allocated at random to the four dietary treatments so that each animal completed a schedule of activities that included the determination of voluntary

intake of feed dry matter (DM) and organic matter (OM), a digestibility and N balance trial, *in sacco* study of rumen kinetics, and acetate clearance test. The results on rumen kinetics and acetate clearance are reported in Part II of this study.

Diets and the application of treatments to animals

The basal diet consisted of wheaten chaff (91% DM, 0.71% N) and barley straw (93% DM, 0.55% N) (3:1 DM basis) treated with urea at 2.5% DM (2.5 kg urea in 20 L warm water), and placed in synthetic gunny bags, compacted and stored at room temperature until the time of feeding. The animals were offered the basal diet *ad lib.* alone or supplemented with an energy supplement providing equal amounts of metabolisable energy (ME) in the form of sucrose. Fresh supplement was prepared daily by dissolving sucrose (375 g) in 750 ml of warm tap water to make 1 L of sugar solution. About 300 ml of this solution was administered to each animal on the three dietary treatments except the control (i.e. E_R, E_A, and E_{RA}) in two doses of 150 ml at 09.00 and 16.00 h each day with each animal receiving about 112.5 g/d of sucrose (Table 3). Every morning before offering the basal roughage diet, the animals were provided with a mixture of 50 g cottonseed meal (CSM) and 5 g complete mineral supplement. The mixture was heaped on the basal roughage so that it was eaten before the animals commenced eating the basal diet. The composition of the ingredients used in the basal roughage and their proportions in the basal diet are shown in Table 2 and 3.

Voluntary feed intake, digestibility and N balance

Voluntary intake of the basal roughage and total diet was determined during the 14-d adaptation period when

Table 2. The composition of basal roughage and the ingredients used in the basal diet

Parameter	Ingredients					
	Basal roughage	Wheaten chaff	Barley straw	Cottonseed meal	Mineral supplement	Sucrose
DM (g/kg)	772	910	932	918	963	999
OM (g/kg DM)	931	927	924	924	67	1,000
N (g/kg DM)	22.2 (139)	7.1(44.6)	5.5(34.6)	73.6(460)	ND	ND
Ash (g/kg DM)	69.5	73.4	75.7	75.9	932.8	0.0

DM = Dry matter; OM = Organic matter; N = Nitrogen; CP = Crude protein (N×6.25); Figures in parentheses refer to the CP (g/kg DM); ND = Not determined.

Table 3. The proportion and quantities of the dietary ingredients and treatments

Ingredients	Treatments			
	Basal+E ₀	Basal+E _R	Basal+E _A	Basal+E _{RA}
Wheaten chaff (%)	73.1	73.1	73.1	73.1
Barley straw (%)	24.4	24.4	24.4	24.4
Urea (%)	2.50	2.50	2.50	2.50
Total basal diet (%)	100	100	100	100
Sucrose (g/d)	0	112.5	112.5	112.5
Cottonseed meal (g/d)	50	50	50	50
Complete mineral mix (g/d)	5	5	5	5

E₀, no sucrose supplementation (control), or sucrose supplement administered through the rumen (E_R), abomasum (E_A) or both routes in equal amounts (E_{RA}).

animals were offered the basal diet once at 09.00 h so as to leave about 15-20% refusals. The refusals were collected every morning before the daily feeding, weighed, and a 10% sample collected. The data for the last 7 d was used in the determination of voluntary intake. The digestibility and N balance trial was conducted during the 3rd week (7 d) with animals being offered 95% of the voluntary intake established during the 2nd week. Urine was collected into plastic buckets containing 50 ml of 10% (v/v) H₂SO₄ to stop microbiological or enzymatic activity, preserve the purine metabolites, and to prevent loss of ammonia-N. The daily urine collection was transferred to a 2-litre measuring cylinder and the volume recorded, and then diluted with cold tap water to 2 L after which a 50 ml sub-sample was drawn for bulking and stored at -20°C. After the 7-day collection period the bulked urine was thawed, mixed thoroughly and three 20 ml sub-samples taken and stored at -20°C awaiting total N and purine derivatives (PD) analyses. The daily faecal output was also collected in the morning and a 10% aliquot taken and bulked for the entire collection period. The faecal samples stored at 4°C were thawed and dried (60°C/72 h) in a forced draught oven, ground in a Wiley Mill (1 mm) and stored awaiting chemical analysis.

Estimation of microbial nitrogen supply

The amount of microbial purine absorbed from the intestines (X, mmol/d) was predicted from the quantity of purine derivatives (PD) excreted in urine (Y, mmol/d) using the relationship for sheep derived by Chen et al. (1990) and Chen and Gomez (1992):

$$Y = 0.84X + (0.150 \text{ kg}^{0.75} e^{-0.25x}).$$

The supply of microbial nitrogen (MN) (g/d) based on total PD excreted in urine was then estimated as follows:

$$\text{MN (g/d)} = 70X / (0.116) (0.83) (1,000) = 0.727X.$$

Where X is the intestinally absorbed PD and Y is the excretion of PD in mmol/d. Estimation of the microbial N

supply was based on daily excretion of allantoin only given that its quantity in urine is directly proportional to total PD (Khan et al., 2001). The urinary allantoin concentration was determined by a colorimetric method (Chen and Gomez, 1992), while the relationship first proposed by Balcells et al. (1991) was applied in the computation of MN production.

In sacco degradability

The rate and extent of degradation of a common substrate (barley straw) incubated in the rumen of sheep on the four dietary treatments was determined. Barley straw (2 g) ground to 2 mm particle size was weighed into nylon bags (size 150×80 mm, pore size 44 micron) together with a marble (5 g). The bags (in duplicate) were then suspended into the rumen of the four animals through the rumen fistula for 6, 12, 24, 48, 72 and 96 h using fishing line. The bags were removed from the rumen and washed thoroughly and then dried (65°C/72 h) in a forced draught oven, cooled and then weighed to determine the loss in weight.

The estimation of DM degradability of the barley straw was based on the model proposed by Ørskov and McDonald (1979), DM degradability (P) = a+b(1-e^{-ct}).

The effective extent of degradation (ED) was calculated on an hourly basis using the following equation: P = a+((bc/(c+k))(1-e^{-(c+k)t})), where k is the fractional outflow rate of solids from the rumen estimated at 0.03/h (Bonsi et al., 1994). The variables a, b, PD, c, ED and lag time (LT) were determined.

Protozoa count

Samples of rumen fluid (25 ml) were collected before the Cr-EDTA marker was injected and thereafter at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 21 and 24 h. About 15 ml was used for rumen kinetics (see Part II that follows this paper). Out of the remaining 10 ml of rumen fluid, 5 ml of the fresh sample was used for the determination of pH using a glass electrode pH meter, while the other 5 ml was reserved for protozoa count. The total protozoa were enumerated in the rumen fluid as described by Bird et al. (1979). In brief about 4 ml of the fresh rumen fluid was added to 16 ml of

4% (v/v) formal-saline solution in McCartney bottles, shaken thoroughly to mix and kept at 3-4°C for later determination of the protozoa numbers. During counting, the diluted rumen fluid was shaken well and pipetted into a counting chamber (C. A. Hanser & Sons, Maxley) of 0.0625 square mm and 0.2 mm depth. Counting was done using a light microscope (magnification×40), and a minimum of 3 separate blocks each covering 12 out of the 16-cell sub-units which were counted. The protozoa were assessed as total count only without attempting to differentiate between the various species, although it was apparent that in most samples the entodiniomorphs were the dominant species followed by Holotrich.

Analytical methods

Dry matter, organic matter, ash, N, ammonia N and volatile fatty acids : The DM and OM content of feed, refusals and faeces were estimated on the dried samples (AOAC, 1990). The total N content in the feed ingredients, refusals, faeces and urine was determined using an automated semi-microkjeldahl system (AOAC, 1990). The concentration of ammonia N in the rumen fluid supernatant was estimated using an autoanalyser (Technicon), according to the method described by Crook and Simpson (1971) and modified by Beitz (1974). The proportion of un-ionised ammonia in the total ammonia concentration was calculated using the Henderson-Hasselbach equation (Siddons et al., 1984).

The total molar concentration (mmol/L) of all VFAs, and molar percentages of the major VFA, were estimated in the rumen fluid supernatant using gas liquid chromatography (GLC) (Model CP 3800GC), with isocaproic acid as an internal standard (Erwin et al., 1961). The ratio of glucogenic to acetogenic substrates in the rumen was calculated from the molar percentage of propionic to acetic acid. The percentage of propionic acid in total VFA was used to calculate the glucogenic ratio (G/E)

according to the method proposed by Blaxter (1967). The ratio was expressed as follows:

$G/E = \text{Propionate}/(\text{Propionate}+0.6 \text{ Acetate}+1.4 \text{ Butyrate})$, where the VFAs were expressed as molar percentages. The ratio of energy supplied by propionate to that of total VFA was used as an index for expressing the glucogenic potential of the nutrients absorbed from the gut (Preston and Leng, 1987).

Statistical analysis

The data were analyzed by ANOVA for a 4×4 Latin square design using Minitab computer statistical software (Ryan et al., 1985), and separation of means done using the Tukey test at 5%.

RESULTS

Chemical composition of the basal roughage and the dietary ingredients

The composition of the basal roughage and the ingredients used in the basal diet and dietary treatments are shown in Table 3. The main ingredients used in the basal roughage (i.e. wheaten chaff and barley straw) were generally low in N. However, the basal roughage whose ingredients included urea was quite high in N content.

Feed intake and digestibility

The intake of dry matter (DM) and organic matter (OM) for total diet and basal roughage were significantly higher in animals supplemented with sucrose intra- ruminally (E_R) compared to those on the control diet or supplemented with sucrose through both the rumen and abomasum (E_{RA}) (Table 4). However, the DM and OM intake of total diet and basal roughage were not significantly different ($p>0.05$) from those of animals supplemented with sucrose abomasally. The intakes of DM and OM of diet and basal roughage of animals on the control diet (E_0) and those supplemented

Table 4. Dietary intake and digestibility of DM and OM in sheep fed urea-treated roughage (E_0) and supplemented with sucrose intra- ruminally (E_R), abomasally (E_A) or via both routes (50:50) (E_{RA})

Constituent	Dietary treatments				
	E_0	E_R	E_A	E_{RA}	SEM
Daily intake					
Diet DM (g/kg ^{0.75})	61.4 ^a	72.3 ^b	67.9 ^{ab}	61.4 ^a	2.06 (***)
Diet OM (g/kg ^{0.75})	57.0 ^a	67.6 ^b	63.5 ^{ab}	57.3 ^a	1.91 (***)
Basal DM (g/kg ^{0.75})	58.4 ^a	63.3 ^b	58.8 ^{ab}	52.2 ^a	2.08 (**)
Basal OM (g/kg ^{0.75})	54.4 ^a	59.0 ^b	54.8 ^{ab}	48.6 ^a	1.94 (**)
Nitrogen (g/d)	23.0 ^a	26.6 ^b	23.9 ^{ab}	22.4 ^a	0.89 (**)
Total apparent digestibility					
DM (×10 ³)(g/kg)	5.95 ^a	6.27 ^{ab}	6.39 ^b	6.05 ^a	27.83 (***)
OM (×10 ²)(g/kgDM)	6.12 ^a	6.40 ^a	6.53 ^b	6.20 ^a	7.74 (**)
Nitrogen (×10 ²)(g/kg)	7.37 ^c	7.21 ^{bc}	7.02 ^b	6.70 ^a	8.48 (***)

E_0 , no sucrose supplementation (control), or sucrose supplement administered through the rumen (E_R), abomasum (E_A) or both routes in equal amounts (E_{RA}); SEM, standard error of mean; ns, not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$; means within row with different superscripts differ at $p<0.05$.

Table 5. The degradation characteristics of barley straw incubated in the rumen of sheep fed urea-treated low quality basal roughage and supplemented with sucrose through the rumen or abomasum

Parameter	Dietary treatments				SEM
	E ₀	E _R	E _A	E _{RA}	
Soluble fraction (a)(g/kg DM)	91.0	87.6	92.2	97.6	5.8 (ns)
Degradable fraction (b)(×10 ²)(g/kg DM)	5.40	5.30	5.20	5.50	20.7 (ns)
PD (a+b)(×10 ²)(g/kg DM)	6.30	6.21	6.15	6.46	19.6 (ns)
Effective degradability (×10 ²)(g/kg DM)	4.20	3.80	3.70	3.70	29.6 (ns)
Rate of degradation (c)(×10 ⁻²)(/h)	5.0	4.0	3.0	3.0	0.006 (ns)
Lag time (h)	1.67	1.90	2.58	1.93	0.76 (ns)

E₀, no sucrose supplementation (control), or sucrose supplement administered through the rumen (E_R), abomasum (E_A) or both routes in equal amounts (E_{RA}); PD, potential degradability.

abomasally (E_A) were not different ($p>0.05$). The total apparent digestibility of DM and OM also showed a similar pattern, with significantly higher values being noted in animals on dietary treatments E_R and E_A. Nitrogen digestibility was highest in animals on the control diet and those supplemented with sucrose through the rumen. However, it was lowest in animals that were supplemented sucrose through both intra-ruminal and abomasal routes (i.e. E_{RA}) (Table 4).

In sacco degradation

There was no difference ($p>0.05$) in the soluble fraction (a), slowly degradable fraction (b), potential degradability (PD), effective degradability (ED) and rate of degradation (c) between the four treatments (Table 5).

Nitrogen retention, excretion of purine derivatives and microbial protein supply

N intake of animals supplemented with sucrose entirely through the rumen (E_R) or abomasum (E_A) was higher ($p<0.05$) than that of control animals (E₀) or those that received the sucrose supplement through both rumen and abomasum (E_{RA}) (Table 6). Faecal N excretion was higher ($p<0.05$) for the sucrose supplemented animals than for the

control group, but there was no difference ($p>0.05$) in faecal N excretion between the three supplemented treatments (i.e. E_R, E_A and E_{RA}). The average daily N balance of the sucrose-supplemented animals tended to be higher than the control, but the difference was not significant ($p>0.05$). There was no difference ($p>0.05$) in the daily urinary allantoin excretion and microbial N production (g/d) in animals on the dietary treatments, even on the basis of OM apparently digested in the rumen (OMADR).

Rumen pH, concentration of VFA, ammonia, and protozoa count

Results on the variation in pH, VFA and ammonia concentration, and total protozoa count in the rumen of the sheep on the four dietary treatments are presented in Table 7, Figure 1 and 2.

Rumen fluid pH of the animals on the four dietary treatments differed significantly ($p<0.001$), with animals receiving the sucrose supplement wholly through the rumen (E_R) having a lower ($p<0.05$) mean rumen fluid pH than on the other treatments. Although the rumen fluid pH of animals receiving dietary treatment E_{RA} (6.32) was lower compared to that of animals on the control diet (6.40) or

Table 6. The N retention and microbial protein production in sheep fed urea-treated basal roughage (E₀) and supplemented with sucrose intraruminally (E_R), abomasally (E_A) or by both routes (E_{RA})

N-value	Dietary treatments				SEM
	E ₀	E _R	E _A	E _{RA}	
Intake (g/d)	23.0 ^a	26.6 ^b	23.9 ^{ab}	22.4 ^a	0.89 (**)
Excretion (g/d)					
In urine	15.1	15.6	14.3	13.0	1.53 (ns)
In faeces	6.20 ^a	7.36 ^b	7.04 ^{ab}	7.38 ^b	0.30 (*)
Balance (g/d)	1.78	3.68	2.56	2.07	1.64 (ns)
Urinary allantoin (mmol/kg ^{0.75} d)	0.38	0.43	0.44	0.43	0.103 (ns)
Microbial N (g/d)	6.39	7.67	7.75	7.58	1.67 (ns)
Microbial N (g/kg OMADR)	18.8	18.1	18.5	18.9	4.34 (ns)

N = Nitrogen; OMADR = Organic matter apparently digested in the rumen (0.65 DOM); ns = Not significant, * $p<0.05$, ** $p<0.01$; means within row with different superscripts differ at $p<0.05$.

Table 7. The variation with diet in rumen pH, concentration of VFA and ammonia, and protozoa in the rumen of sheep fed urea-treated low quality basal roughage (E_0) and supplemented with sucrose intraruminally (E_R) abomasally (E_A) or by both routes (50:50) (E_{RA})

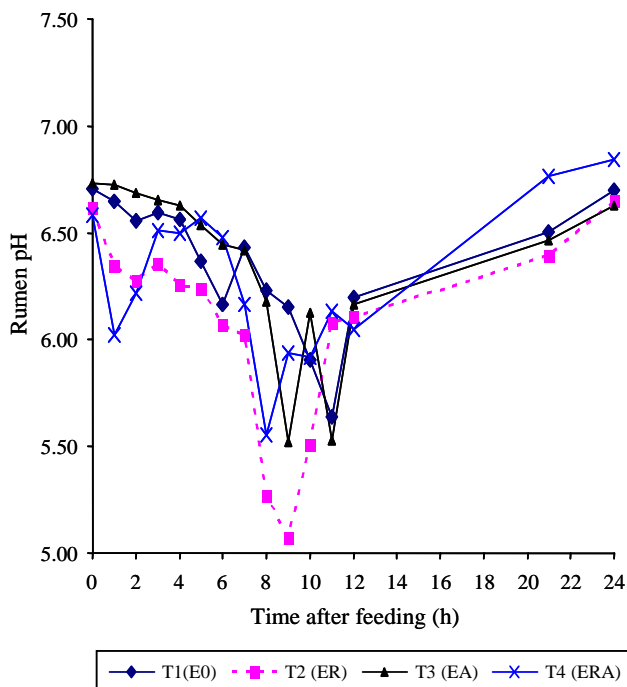
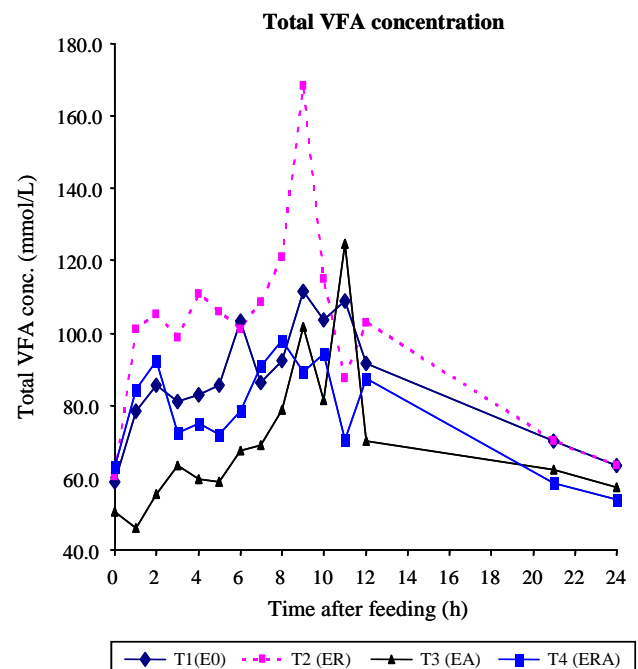
Constituent	Dietary treatments				SEM
	E_0	E_R	E_A	E_{RA}	
Rumen pH	6.40 ^b	6.13 ^a	6.44 ^b	6.32 ^b	0.06 (***)
Total VFA (mmol/L)	84.3 ^b	98.2 ^c	65.4 ^a	78.2 ^{ab}	3.60 (***)
Acetate (%)	68.8 ^c	61.3 ^a	70.9 ^d	63.5 ^b	0.51 (***)
Propionate (%)	20.7 ^a	27.8 ^b	19.7 ^a	26.8 ^b	0.60 (***)
Butyrate (%)	8.3 ^c	9.1 ^c	6.4 ^a	7.4 ^b	0.24 (***)
Other VFA (%)	2.25 ^a	1.82 ^a	3.07 ^b	2.31 ^a	0.18 (***)
Propionate/acetate	0.30 ^a	0.46 ^b	0.28 ^a	0.43 ^b	0.01 (***)
G/E	0.28 ^a	0.39 ^b	0.28 ^a	0.36 ^b	0.01 (***)
Total ammonia ($\times 10^2$)(mg/L)	2.35	2.44	2.25	2.08	18.1 (ns)
Un-ionised ammonia (mg/L)	0.72 ^b	0.40 ^a	0.74 ^b	0.55 ^{ab}	0.08 (**)
Protozoa ($\times 10^5$)/ml	5.0 ^b	7.5 ^c	2.8 ^a	3.8 ^{ab}	0.44 (***)

E_0 , no sucrose supplementation (control), or sucrose supplemented through the rumen (E_R), abomasum (E_A) or both routes in equal amounts (E_{RA}); G/E refers Glucogenic potential index; SEM, standard error of mean; ns, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; means within row with different superscripts differ ($p < 0.05$).

those supplemented sucrose through the abomasum (6.44), the difference was not significant ($p > 0.05$) (Table 7). The dietary and temporal variation in rumen pH showed that following introduction of feed the rumen fluid pH on all four dietary treatments decreased, reaching the lowest levels at about 8-12 h post-feeding, and remained below 6.0 for the longest period of time (3-4 h) in animals receiving dietary treatment E_R , followed by those on E_{RA} and shortest

in those on the control diet (Figure 1).

There was a very highly significant ($p < 0.001$) difference in total VFA concentration and also the molar proportions of the various VFA in the rumen between animals receiving the four dietary treatments. The total VFA concentration in the rumen was highest in those animals on dietary treatment E_R (98.2 mmol/L) and lowest in those on E_A (65.4 mmol/L). The unsupplemented animals (control) and those that received the sucrose supplement through both ruminal and

**Figure 1.** The temporal variation and variation with diet in pH in the rumen of sheep fed urea-treated basal roughage (E_0) and supplemented with sucrose intra-ruminally (E_R), abomasally (E_A) or by both routes (E_{RA}).**Figure 2.** Total VFA concentration in the rumen of sheep fed urea-treated basal roughage (E_0) and supplemented with sucrose intraruminally (E_R), abomasally (E_A) or by both routes (E_{RA}).

abomasal routes (E_{RA}) had intermediate total VFA concentration (i.e. 84.3 and 78.2 mmol/L) but were not significantly different ($p>0.05$) (Table 7). The molar proportion of acetate was highest in those animals that received the sucrose supplement through the abomasal route and lowest in the intra-ruminally supplemented group, while the control group and those that received the supplement through both routes were intermediate. The temporal variation in total VFA in the rumen showed a pattern that was inversely related to pH (Figure 2).

The molar percentage of propionate in rumen fluid of animals receiving the four dietary treatments differed significantly ($p<0.001$). Animals that received the sucrose supplement wholly (E_R) or partly (E_{RA}) into the rumen generally had a higher ($p<0.05$) molar percentage of propionate than those that received no supplement (E_0) or received the supplement through the abomasal route (E_A). The molar percentage of butyrate in the rumen of animals on the four dietary treatments ranged from 6.4-9.1% and the difference between dietary treatments was significant ($p<0.001$). Animals receiving the sucrose supplement entirely through the rumen or on the control diet had the highest molar % of butyrate though the two were not significantly different ($p>0.05$). The molar percentage of the other VFA in the rumen of animals on all four treatments was generally low (1.8-3.1%) but significantly different

($p<0.001$), with abomasally-supplemented animals having a higher ($p<0.05$) butyrate % than the other three treatments. There was a significant ($p<0.001$) difference in both propionate/acetate ratio and glucogenic potential index (G/E ratio) between animals receiving sucrose supplement entirely or partly through the rumen (i.e. E_R and E_{RA}) and the control or abomasally-supplemented animals (i.e. E_0 and E_A). However, there was no difference ($p>0.05$) in both propionate/acetate ratio and G/E ratio between the animals on dietary treatments E_R and E_{RA} or between those on the control diet (E_0) and the abomasally-supplemented animals (E_A) (Table 7). The temporal variation of propionate/acetate and G/E ratio also showed clearly that ratios for animals on dietary treatments E_R and E_{RA} were consistently higher than those on the control diet (E_0) or the abomasally-supplemented animals (E_A). The differences between the two sets of treatments (i.e. E_R and E_{RA} vs. E_0 and E_A) were even more apparent 1-2 h after the administration of each of the two doses of sucrose supplement (Figures 3 and 4).

The total ammonia concentration in the rumen of animals on the four dietary treatments ranged from 208 mg/L in E_{RA} to 244 mg/L in E_R , but were not significantly different ($p>0.05$) between the treatments. However, the concentration of un-ionized ammonia (NH_3) was lower ($p<0.05$) in those animals that received the sucrose supplement wholly or partly through the rumen (i.e. E_R and

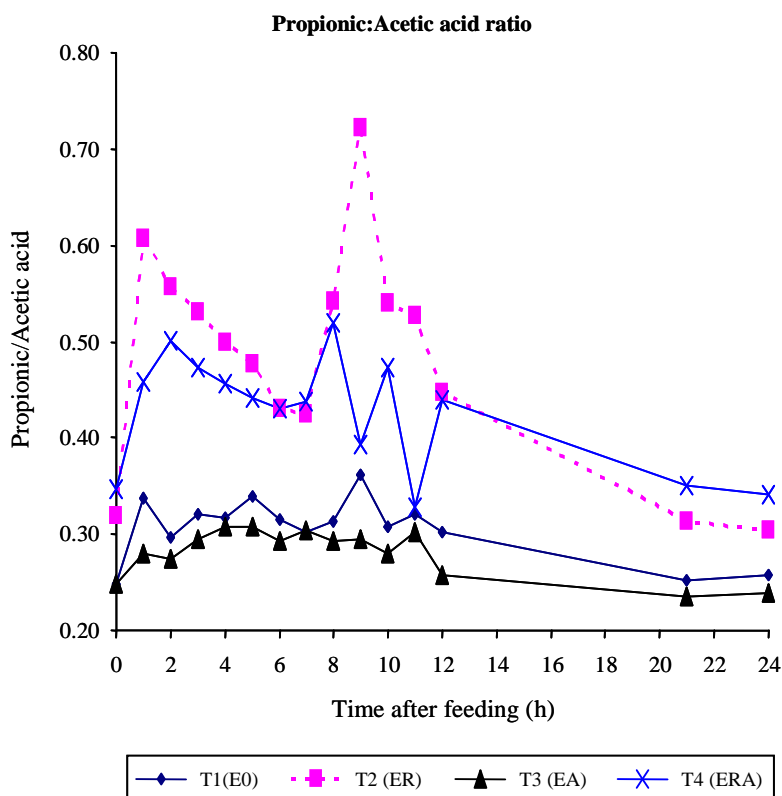


Figure 3. The temporal variation and variation with diet in the molar proportion of propionic to acetic acid ratio in the rumen of sheep fed urea-treated basal roughage supplemented with sucrose intra-ruminally or abomasally.

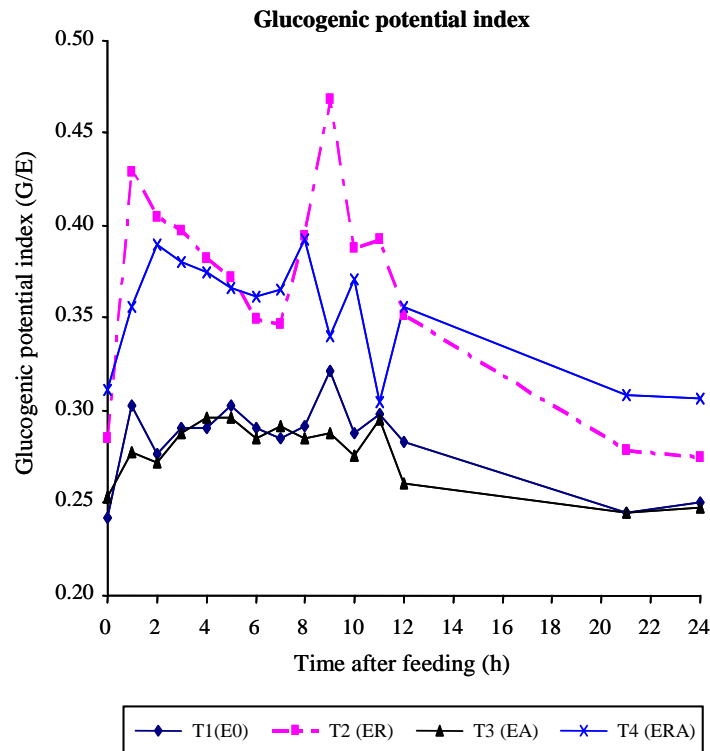


Figure 4. The glucogenic potential index (G/E) in the rumen of sheep fed urea-treated basal roughage supplemented with sucrose intraruminally or abomasally.

E_{RA}) (Table 7). The difference in the total protozoa count in rumen fluid of animals receiving the four dietary treatments was significant ($p < 0.001$), with the intraruminally-supplemented animals (E_R) having a significantly ($p < 0.05$) higher count than the other dietary treatments (Table 7). The

temporal variation of protozoa in the rumen also showed that animals supplemented with sucrose wholly through the rumen (E_R) generally had consistently higher numbers of protozoa on an hourly basis compared to the other three dietary treatments (Figure 5).

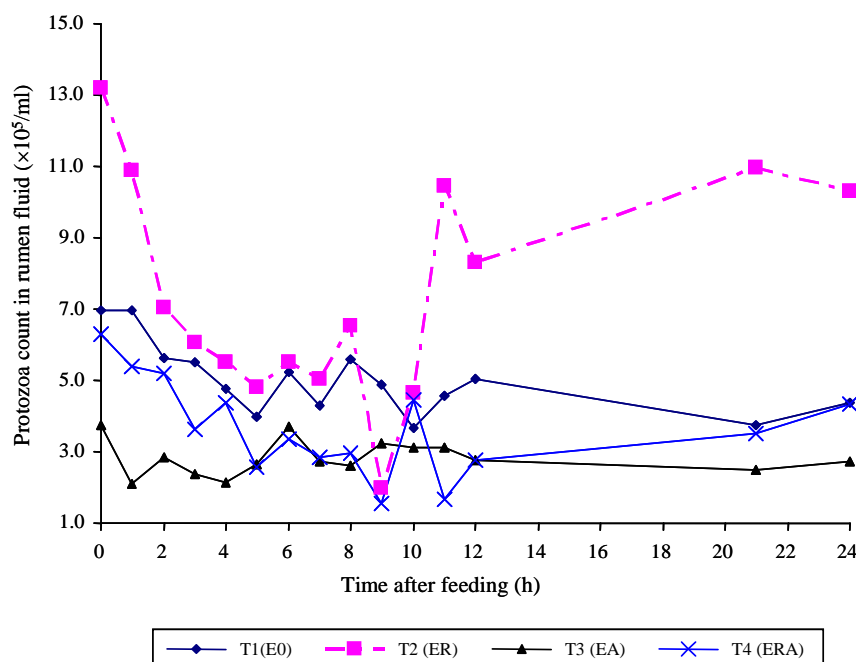


Figure 5. The temporal variation in total protozoa numbers with diet in the rumen fluid of sheep fed urea-treated low quality basal roughage (E_0) and supplemented with sucrose via the rumen (E_R), abomasum (E_A) or both routes (E_{RA}).

DISCUSSION

Feed intake and digestibility

The N content of the basal roughage (22.2 g/kg DM or 13.9% CP) was quite high due to the addition of urea. Given that wheaten chaff and barley straw contained 7.1 and 5.5 g N/kg DM respectively, mixing the two in the ratio of 3:1 (DM basis) to constitute the basal roughage without incorporating any urea would have produced a basal roughage containing 6.7 g N/kg DM (4.2% CP) which is low compared to the 22.2 g N/kg DM (13.9% CP) that was attained after treatment with 2.5% urea. All animals were therefore considered to be adequately supplied with more than the minimum requirements of N for optimal microbial growth in the rumen (Satter and Slyter, 1974), as confirmed by the apparently high rumen ammonia concentration in animals on all four dietary treatments (i.e. 208-244 mg/L). The increase in N content following urea-treatment is in agreement with results reported by other workers (Cloete et al., 1983; Cloete and Kritzler, 1984; Djajanegara and Doyle, 1989).

The high intake of basal roughage DM and OM in animals supplemented with sucrose entirely through the rumen (E_R) or abomasum (E_A) may be attributed to favourable conditions for microbial growth in the rumen and/or hindgut, and possibly the enhanced nutrient balance, especially the protein: energy ratio (P/E), at the tissue metabolism level (Egan, 1977). Voluntary intake in ruminants, especially of roughage is highly complex and is determined by, among other factors, the rate at which ingested feed material is broken down to particle sizes that are small and dense enough to pass from the rumen to the lower gut through the reticulo-omasal orifice (Lechner-Doll et al., 1991). Fibre breakdown in the rumen is mainly accomplished through rumination and complemented by microbial degradation (Ulyatt et al., 1986).

In this study, the *in sacco* degradation of barley straw was not negatively affected by sucrose supplementation and the 96-h potential degradability (PD) level was fairly high for roughage. This suggests that the basal roughage diet contained adequate amounts of nutrients to meet microbial growth requirements, and that the presence of sucrose in the rumen did not adversely affect cellulolytic activity.

Poor microbial fermentation environment as influenced by the diet can hinder achievement of 100% potential degradability. Such an environment may be manifested in the form of insufficient NH_3 , low rumen pH, and the fermentation of large amounts of soluble sugars and starch in the rumen following dietary intake (Chen and Hovell, 1996). These factors are likely to affect microbial growth in the rumen by increasing lag time and in the process lead to a reduction in rate of fibre degradation in the rumen, even without affecting the extent of degradation (Chen and

Hovell, 1996). Soluble sugars and starch are generally fermented very rapidly in the rumen, depressing pH and this can adversely affect microbial growth and cellulolytic activity (Mould et al., 1983; Mould and Ørskov, 1983; Russell and Wilson, 1996). However, the magnitude of the depression in fibre digestion depends on the period of time when the pH is depressed to a level below 6.0 (Hoover, 1986; Royes et al., 2001). In this study, it was only in animals that were supplemented with sucrose intraruminally (E_R) that a mean rumen fluid pH as low as 6.13 was attained, and pH was below 6.00 for less than 3h per day which did not adversely affect cellulolytic activity in the rumen. Any difference in apparent digestibility between the dietary treatments cannot therefore be attributed to the rumen fermentation environment, but to other parts of the gut and certainly in the hind gut.

Nitrogen digestibility, retention and microbial protein supply

The higher N intake in animals supplemented with sucrose entirely through the rumen or abomasum was attributed to their higher total intake of DM. The basal roughage had a relatively high N content (22.2 g/kg DM) with a large proportion of the N being in NPN. The total apparent digestibility of N was also fairly high mainly because of the large proportion of NPN from urea. The relatively high faecal N excretion in animals supplemented with sucrose abomasally, and in those supplemented through both rumen and abomasum, was attributed to the extensive hindgut microbial fermentation activity (Siddons, 1968; Ørskov et al., 1972). Although the intake and digestibility of DM and therefore N intake of the animals on the control (E_0) or dietary treatment E_{RA} were not significantly different, animals on the latter had a significantly higher faecal N excretion mainly due to higher microbial fermentation activity in their hindgut. Faecal N that includes N of metabolic origin varies with DM intake (AFRC, 1993) and is estimated to be 21-40 g N per kg DM intake (NRC, 1988).

Control animals had the highest N digestibility (737 g/kg), while those supplemented with sucrose through both ruminal and abomasal routes had the lowest (670 g/kg), and this was in spite of the fact that the N intake (23 and 22.4 g/d) in the two dietary treatments were not significantly different, though lower compared to treatments E_R and E_A . However, animals on the control diet had a significantly lower faecal N excretion (6.20) than those on E_{RA} (7.38 g/d) and this resulted in a large difference in the N digestibility values between these two treatments (i.e. E_0 and E_{RA}). A high faecal N can depress the apparent digestibility of N as was evident in dietary treatments E_0 and E_{RA} . The amount of fermentable substrate reaching the hindgut can increase fecal N and therefore depress apparent N digestibility (Kay,

1969). The relatively high N digestibility of the urea-treated basal roughage that was observed in this study is in agreement with reports from other workers (Ferrell et al., 1999).

There was no evidence of reduction in total ammonia concentration in the rumen fluid, or increase in microbial protein synthesis between the unsupplemented animals and those supplemented with sucrose through the rumen, abomasum or by both routes. This was rather surprising as the presence of sucrose in the rumen was expected to stimulate higher microbial growth and therefore higher microbial synthesis, thereby depressing rumen ammonia level and enhancing microbial protein synthesis. This suggests that the basal diet was well supplied with fermentable (energy) substrates and that fermentable metabolisable energy (FME) was not limiting microbial synthesis on the control diet. This is partly supported by the observation that microbial N production on all four dietary treatments averaged 18.1-18.9 g per kg organic matter apparently digested in the rumen (OMADR), and this level is within the 14-49 g normally achieved with most diets (ARC, 1984).

Rumen fluid pH and VFA production

Sucrose is highly soluble and rapidly fermented by rumen microbes leading to a rapid build up in the concentration of short-chain fatty acids. This may depress rumen fluid pH and reduce cellulolytic activity in the rumen, unless the acidity is neutralized by the various processes in the rumen such as saliva output from rumination (Wolin, 1981), ruminal ammonia, and absorption of VFAs across the rumen epithelium or outflow to the lower parts of the gut (Sutherland, 1976). Although the rumen pH of animals on the four dietary treatments was at times lower than 6.0, especially those on E_R and E_{RA} , this was relatively short-lived (<3 h) and this may explain why microbial degradation of fibre in the rumen was not adversely affected. The temporal variation of pH was closely but inversely related to that of total VFA, thus indicating that it was the production of VFA that was responsible for the observed trend in pH.

The high total VFA concentration in the rumen of animals supplemented with sucrose wholly through the rumen (E_R) was attributed to the high intake of DM and the presence of extra fermentable substrates from the sucrose supplement. The relatively high total VFA concentration in the rumen of control animals that received no sucrose supplement (84.3 mmol/L) suggests that the basal roughage was well fermented in the rumen. This may be partly attributed to the high ruminal ammonia concentration. Perdok and Leng (1989) suggested that for optimal microbial function, the rumen ammonia concentration of sheep fed roughage has to be maintained at about 100 to

200 mg/L. Although the total VFA concentration in the rumen of animals receiving the sucrose supplement through both rumen and abomasum was lower than for the control, it was not significantly different, and this can be explained by the fact that animals on the two dietary treatments had similar intakes of dietary and basal DM (also OM), and therefore almost similar amount of fermentable OM in the rumen.

A supplement can boost the total quantity of fermentable substrates and/or have a stimulatory effect on microbial fermentation in the rumen, thus contributing to higher total VFA production. The concentration of total VFA in the rumen is positively correlated to rate of fermentation and therefore production of VFA (Leng, 1970). In the present study, *in sacco* degradation results showed that the sucrose supplement neither enhanced nor impaired the degradation of barley straw in the rumen. Therefore, it can be presumed that the sucrose supplement only played the role of boosting the quantity of fermentable substrates in the rumen over and above those already supplied by the basal roughage. As a result, animals with higher total diet and roughage intake were expected to have higher rates of total VFA production, and therefore higher VFA concentrations (Leng, 1970; Obara et al., 1991). This fact is supported by the observation that the total VFA concentration in the rumen of animals on dietary treatments E_0 , E_R and E_{RA} were proportional to the level of dietary intake. However, the same argument does not explain why animals supplemented with sucrose entirely through the abomasum (E_A) had a lower total VFA concentration than those on dietary treatment E_R , in spite of the two treatments having similar dietary and basal DM (and OM) intakes. This indicates that factors other than intake *per se* may also have influenced the total VFA concentration in the rumen of animals supplemented with sucrose wholly through the abomasum. This was attributed to a possible dilution effect in the rumen from the extra water intake occasioned by osmotic changes brought about by extensive hindgut fermentation (see rumen kinetics section in Part II).

Besides increasing the total VFA concentration in the rumen, sucrose supplementation wholly or partially intraruminal also favoured a higher percentage of propionate and butyrate as evident from the results of molar proportions of various VFA which showed acetate: propionate: butyrate ratios of 69:21:8 (E_0), 61:29:9 (E_R), 71:20:6.5 (E_A) and 64:27:7.4 (E_{RA}). The propionate/acetate and G/E ratio also appeared to be increased significantly by intraruminal sucrose supplementation, and these results are in agreement with reports from other workers (Syrjala, 1972; Rook et al., 1987). Dietary composition is a major factor influencing both total VFA concentration, and also the ratio of various VFAs in the rumen, and especially propionate/acetate (Wolin, 1981; Brockman, 1993; Russell and Strobel, 1993).

In particular, the supplementation of roughage with readily fermentable carbohydrate energy sources (soluble sugars and starch) normally results in a higher propionate/acetate ratio (Obara et al., 1991; France and Siddons, 1993). Propionate is considered to be highly glucogenic and when its proportion in total VFA is high it may increase the glucogenic potential index (G/E) of the energy absorbed from the gut. This is likely to increase gluconeogenesis in the liver (Wolin, 1981; Leng, 1982; Preston and Leng, 1987), and therefore have a beneficial implication for tissue metabolism; especially of acetogenic substrates such as acetate (see the report on acetate clearance in Part II).

Protozoa count in the rumen

The high protozoa count in the rumen of animals supplemented with sucrose intra-ruminally has been reported before (Bird and Leng, 1984; Habib, 1988). As the diurnal pattern confirmed, their population in the rumen is greatly reduced when the rumen pH is lower than 6.0. This study also confirmed earlier observation by other workers that a high protozoa count in the rumen is associated with a high proportion of butyrate (Luther et al., 1966; Habib, 1988). A large population of protozoa can be expected in the rumen of animals supplemented with sugars and starch (Bird et al., 1979; Newbold et al., 1986), and though they are credited for ameliorating rumen pH (Mackie et al., 1978; Kariya et al., 1989), they tend to reduce the microbial N flow to the small intestines. A reduction in microbial protein flow to the small intestines is likely to reduce productivity, especially in those animals subsisting on low quality basal roughage in tropical and sub-tropical areas (Bird and Leng, 1984).

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