The importance of the form of nitrogen on microbial protein synthesis in the rumen of cattle receiving grass silage and continuous intrarumen infusions of sucrose

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1. In a 4 × 4 Latin square design experiment, four cattle were given grass silage in two meals per d to satisfy maintenance energy requirements. In addition, sucrose (170 g/kg silage dry matter (DM)) was infused intraruminally at a constant rate with no nitrogen supplementation; with the infusion intraruminally of either casein (23 g/kg silage DM) or urea (8 g/kg silage DM); or with soya-bean meal (64 g/kg silage DM) fed in two equal portions.

2. Samples of duodenal digesta representative of a 24 h period were obtained using chromium-EDTA and ytterbium acetate for flow estimation and ^{35}S as a marker of microbial N entering the small intestine. Samples of rumen fluid were also taken for estimation of rumen pH and concentrations of ammonia-N and volatile fatty acids. Estimates of apparent organic matter (OM) and N digestibility and of the rates of silage DM and N disappearance from porous synthetic-fibre bags incubated in the rumen were also made.

3. The N supplements had no significant effects on rumen pH, concentrations of volatile fatty acids, their molar proportions or the disappearance of DM or N from porous synthetic-fibre bags. N supplementation increased rumen ammonia-N concentrations (urea, $P < 0.05$; casein, soya-bean meal, not significant).

4. N supplementation had no significant effects on the digestion of OM, acid-detergent fibre or soluble carbohydrate.

5. Infusion of casein increased the quantities of total non-ammonia-N (not significant) and microbial N ($P < 0.05$) entering the small intestine daily and the efficiency of rumen microbial N synthesis (not significant). Giving soya-bean meal twice daily resulted in marginal increases in the quantities of non-ammonia-N and microbial N entering the small intestine, while infusing urea intraruminally had no effect.

The efficiency of rumen microbial nitrogen synthesis in animals given diets of grass silage (e.g. Thomas *et al.* 1980; Thomson *et al.* 1981) is often lower than the mean value of 32 g N/kg organic matter (OM) apparently digested in the rumen reported for all diets (Agricultural Research Council, 1984). Continuous intrarumen infusion of soluble carbohydrates (CHO), such as sucrose, has been shown to stimulate rumen microbial N synthesis in cattle (Rooke *et al.* 1987) and sheep (Huhtanen & Ala-Seppala, 1987) fed on silage. Infusion of casein with soluble CHO (Rooke *et al.* 1987) resulted in an even more marked stimulation of the amounts of microbial N synthesized in the rumen of cattle.

Since Rooke *et al.* (1987) only infused a protein-N source (casein) with soluble CHO, it was decided to investigate whether the form of the N supplement (non-protein or protein-N) was an important factor in the observed stimulation of rumen microbial N synthesis. This was achieved by the infusion of casein or urea intraruminally with sucrose and compared with giving soya-bean meal to ascertain whether feeding a rumen-degradable protein supplement twice daily would produce a similar stimulation of microbial N synthesis to that expected when N was continuously infused.

A preliminary report of some of this work has been published (Rooke & Armstrong, 1987*a*).

EXPERIMENTAL

Animals

Two Jersey cattle (approximate live weights, 400 and 475 kg) and two Friesian cattle (approximate live weights, 525 and 630 kg) were used in the experiment. All were adult

females and non-pregnant and non-lactating. Each was equipped with a rumen cannula and either a simple (Friesians) or re-entrant (Jerseys) cannula in the proximal duodenum.

Diets and experimental procedure

The cattle were fed on grass silage and sucrose was infused into the rumen at a continuous rate throughout the experiment. The animals were given, according to a 4×4 Latin square experimental design, either no N supplement or supplements of either urea or casein infused intraruminally, or a supplement of soya-bean meal which was fed.

The grass silage was prepared from a second cut of a mixture of perennial (*Lolium perenne*) and hybrid ryegrasses (*L. perenne* × *L. multiflorum*) (first cut, 28 May 1985), harvested with a precision-chop forage harvester on 17 July 1985. The grass was wilted for 24 h and ensiled in a 100 t bunker silo with the application of an additive (Pioneer 1177; Pioneer Overseas Corp., Johnston, Iowa, USA) such that 2×10^4 lactic acid bacteria were applied/g grass ensiled.

Feed was offered to each animal twice daily in equal amounts at 08.00 and 16.00 hours throughout the experiment. The amounts of silage offered supplied sufficient metabolizable energy, estimated from its modified acid-detergent-fibre content (Givens, 1986) to provide the maintenance energy requirements of each animal (Ministry of Agriculture, Fisheries and Food, 1975). Water and mineralized salt licks were freely available.

Each experimental period was of 21 d duration and consisted of a 14 d infusion period followed by a 7 d rest period. During each experimental period a solution of sucrose was infused intraruminally at a rate of 125 ml/h. The quantities of sucrose infused daily were such that 170 g sucrose were infused/kg silage dry matter (DM) offered to each animal. The composition of the soya-bean meal fed and the amounts of casein and urea infused intraruminally and soya-bean meal fed daily are given in Table 1. Throughout the infusion period chromium-EDTA (120 mg Cr/kg silage DM intake) was included in the nutrient infusion and ytterbium acetate (100 mg Yb/kg silage DM intake) was infused separately; finally 2·5 mCi $^{35}SO_4$ were added to the nutrient infusion on day 9 of each experimental period.

Sampling procedures

Representative samples of the silage, soya-bean meal and infusates were obtained from each period. Spot samples of duodenal digesta (500 g), rumen fluid (50 ml) and of faeces (by 'grab' sampling or as freshly voided faeces) were obtained from days 12 to 14 of each period using a sampling schedule (Faichney, 1980) to give twelve samples at 2-h intervals representative of a 24 h feeding period. From each sample 250 g digesta were composited to give a representative 24 h digesta sample. From this composite sample a duodenal microbial fraction was prepared by differential centrifugation according to Rooke *et al.* (1985); briefly duodenal digesta were filtered through muslin to remove fibre, centrifuged at 500 ***g*** for 5 min to remove feed particles and the duodenal microbial fraction recovered from the supernatant fraction by centrifugation at 10000 ***g*** for 30 min. A centrifuged duodenal digesta sample was also prepared by centrifuging whole digesta at 10000 ***g*** for 15 min. Faecal samples were dried at 65° and then composited on a DM basis to give one sample/animal per period. Finally, four porous synthetic-fibre (psf) bags (45 μm, 150×60 mm) containing fresh silage equivalent to 5 g silage DM were inserted into the rumen of each animal at 11.00 hours on day 12, and removed at intervals of 6, 12, 26 and 52 h thereafter. On removal from the rumen the psf bags were thoroughly washed in cold water and then dried at 65° in a forced-draught oven.

Table 1. *The chemical composition (g/kg dry matter) of the silage and soya-bean meal. The quantities of casein and urea infused daily intraruminally and of soya-bean meal fed twice daily (g/d) are also given*

	Silage*	Soya-bean meal	Casein	Urea
Dry matter (g/kg)	232	865		
Organic matter	917	924		
Acid-detergent fibre	308			
Water-soluble carbohydrate	15			
Total nitrogen	20·2	75·8		
Ammonia-N (g/kg N)	89			
Acetic acid	17			
Butyric acid	1			
Lactic acid	92			
Ethanol	6			
pH	3·9			
Daily intake (g/kg silage DM)	—	64	23	8

* Dry matter determined by toluene distillation.

Analytical procedures

Total and centrifuged duodenal digesta samples and duodenal microbial fractions were freeze-dried. All samples were milled before analysis by the methods described by Rooke *et al.* (1987). Briefly, OM was determined by ashing dried samples at 550°, N by the Kjeldahl procedure, soluble CHO as the sum of free glucose plus α-linked glucose polymers (MacRae & Armstrong, 1969) and acid-detergent fibre according to Van Soest & Wine (1967). The ^{35}S:non-ammonia-N (NAN) contents of the total duodenal, centrifuged duodenal and duodenal microbial fractions were analysed according to Mathers & Miller (1980). The Cr contents of infusates and digesta samples were determined as described by Rooke *et al.* (1985) and the Yb contents of infusates and digesta samples according to Siddons *et al.* (1985*b*) except that Yb concentrations were measured by the known-addition method. Rumen volatile fatty acids were determined by gas–liquid chromatography (Cottyn & Boucque, 1968) and silage analyses were performed as described by Rooke *et al.* (1988).

Calculation of results

Faecal excretion was estimated using Yb as a non-absorbable marker (Siddons *et al.* 1985*b*; Peyraud, 1987). Nutrient flows to the small intestine were estimated according to the dual-phase-marker method of Faichney (1986) from the concentrations of Cr and Yb in duodenal digesta DM and centrifuged duodenal digesta DM for animals with both types of duodenal cannula. An effective infusion rate for Cr was calculated from the faecal ratio Cr:Yb (Faichney, 1986) since the ratio Cr:Yb (J. A. Rooke and C. Rymer, unpublished results and values from this experiment) in faecal DM was significantly ($P < 0·01$) less (0·89-fold) than the ratio Cr:Yb infused daily, suggesting a net loss of Cr from the gastrointestinal tract. Additionally the Cr:Yb ratio in duodenal digesta DM obtained from cattle with re-entrant cannulas was also significantly ($P < 0·01$) less than the infused Cr:Yb ratio but not significantly different from the faecal Cr:Yb ratio. Thus all Cr absorption was assumed to have taken place before the proximal duodenum, possibly as a result of changes of rumen osmolality (Dobson *et al.* 1976) associated with feeding silage twice daily. The

Table 2. *Mean values for pH and for the concentrations of ammonia-nitrogen (mg/l) and volatile fatty acids (mmol/l) in the rumen fluid of cattle given diets of grass silage and intrarumen infusions of sucrose unsupplemented or supplemented with three N supplements*†. *The molar proportions of individual fatty acids (mmol acid/mol total volatile fatty acids) are also given*

N supplement...	None	Casein	Urea	Soya bean	SE‡
pH	6·75	6·72	6·71	6·71	0·039
Ammonia-N	49	61	72*	58	4·5
Volatile fatty acids					
Total	96	93	100	99	3·6
Acetic	657	656	653	644	18·7
Propionic	197	203	194	199	3·4
*Iso*butyric	5	4	7	7	0·7
n-Butyric	112	108	113	118	15·0
*iso*valeric	12	14	14	14	1·9
n-Valeric	20	19	20	19	3·3

* Mean value was significantly different from that for diet with no N supplement $P < 0·05$.
† For details, see p. 114.
‡ SE of mean with 5 df for four observations; for casein three observations only.

Table 3. *The disappearance of silage dry matter and nitrogen from porous synthetic-fibre bags incubated in the rumen of the cattle*

(Mean values with their standard errors are given for constants a', b and c relating to the equation $p = a' - be^{-ct}$ for each of the four diets where p is the proportion of dry matter or N which had disappeared from bags after time t (h))

Constant...	a'		b		c			
	Mean	SE	Mean	SE	Mean	SE	r^2	df
Dry matter								
N supplement†								
None	0·88	0·073	0·52	0·067	0·035	0·0106	0·91	17
Casein	1·00	0·180	0·64	0·167	0·024	0·0117	0·90	12
Urea	0·98	0·088	0·55	0·081	0·033	0·0110	0·95	17
Soya-bean	0·86	0·076	0·51	0·070	0·039	0·0131	0·93	17
Nitrogen								
N supplement†								
None	0·94	0·017	0·27	0·021	0·082	0·0162	0·92	17
Casein	0·94	0·023	0·26	0·027	0·069	0·0178	0·92	12
Urea	0·93	0·012	0·25	0·015	0·088	0·0128	0·91	17
Soya-bean	0·93	0·013	0·26	0·017	0·096	0·0160	0·89	17

No significant differences between diets.
† For details, see p. 114.

proportion of microbial NAN in duodenal NAN was calculated according to Mathers & Miller (1980). The proportionate disappearances of DM and N from psf bags in the rumen were fitted using a Maximum Likelihood Program (Ross, 1980) to an exponential function of the form

$$p = a' - be^{-ct},$$

Table 4. *The mean quantities (kg/24 h) of organic matter (OM), soluble carbohydrate (CHO) and acid-detergent fibre (ADF) consumed and entering the small intestine of cattle infused intraruminally. The quantities of OM excreted in the faeces (kg/24 h), the apparent digestibility of OM and the proportion of digestible OM digested in the rumen (DOMDR) are also given*

Nitrogen supplement* ...	None	Casein	Urea	Soya bean	SE†
		OM			
Intake from:					
Silage	5·23	5·46	5·26	5·25	—
Soya-bean meal	—	—	—	0·30	—
Infusion	0·80	0·86	0·97	0·84	—
Total	6·03	6·32	6·23	6·39	—
Entering small intestine	2·37	2·63	2·59	2·54	0·096
In faeces	1·33	1·46	1·54	1·36	0·065
Apparent digestibility	0·78	0·77	0·76	0·76	0·011
DOMDR	0·78	0·77	0·78	0·76	0·016
		Soluble CHO			
Intake from:					
Silage	0·09	0·09	0·08	0·08	—
Infusion	0·80	0·70	0·92	0·84	—
Total	0·89	0·79	1·00	0·92	—
Entering small intestine	0·03	0·03	0·02	0·03	0·002
		ADF			
Intake from:					
Silage	1·70	1·87	1·70	1·68	—
Soya-bean meal	—	—	—	0·05	—
Total	1·70	1·87	1·70	1·73	—
Entering small intestine	0·49	0·52	0·51	0·53	0·029

No significant differences between diets.
* For details, see p. 114.
† SE of mean with 5 df for four observations; for casein three observations only.

where p is the proportion of DM or N disappearing after time t (h) and a', b and c are constants.

Statistical analysis

Results were analysed by analysis of variance for Latin square design experiments using a least squares solution as one animal was removed from one period for reasons not connected with the experimental diets fed. Differences between each N supplemented diet and the diet containing no N supplement were determined according to Dunnett (1955). Differences between fitted curves for the psf-bag values were analysed by parallel curve analysis (Ross, 1980).

RESULTS

The silage given in the experiment was of moderate N content (Table 1) and was well-fermented, having a low pH, high concentration of lactic acid and low concentrations of acetic and butyric acids.

The addition of different N sources to diets of grass silage supplemented with intrarumen infusions of sucrose increased rumen ammonia-N concentrations (Table 2); however, these

Table 5. *The mean quantities of total nitrogen consumed by the cattle (g/24 h), infused intraruminally and the quantities of non-ammonia-N (NAN), microbial N and feed NAN entering the small intestine, the apparent efficiency of microbial N synthesis (g N/kg organic matter apparently digested in the rumen) and the apparent degradability of feed N in the rumen*

N supplement†...	None	Casein	Urea	Soya bean	SE‡
N intake from	114	120	116	117	—
Silage					
Soya-bean meal	—	—	—	23	—
Infusion	—	22	24	—	—
Total	114	142	140	140	—
N entering small intestine					
Total NAN	120	142	128	127	5·4
Microbial N	105	126*	108	112	3·4
Feed NAN§	15	16	20	15	3·2
Efficiency of microbial N synthesis	—	34	29	30	1·7
Apparent feed N degradability§	0·88	0·89	0·87	0·90	0·020

* Mean value was significantly different from that for the diet with no N supplement ($P < 0·05$).
† For details, see p. 114.
‡ SE of mean with 5 df for four observations; for casein, three observations only.
§ Includes endogenous N secretions. Values for degradability of feed N calculated from the difference between N intake and duodenal (NAN − microbial N).

increases in ammonia-N concentrations were significant ($P < 0·05$) only when urea was the N source. No N supplement had any significant effect on rumen pH, volatile fatty acid concentrations or molar proportions (Table 2). Similarly, neither the rate (c) nor extent (total, a'; not instantly solubilized, b) of silage DM or N disappearance from psf bags (Table 3) incubated in the rumen was affected by N supplementation.

Neither the quantities of OM, soluble CHO or acid-detergent fibre entering the small intestine nor the faecal excretion of OM (Table 4) were changed by N supplementation.

Table 5 shows that only the infusion of casein increased the quantities of NAN entering the small intestine daily, but this increase was not significant ($P > 0·05$). Supplying N in the form of urea or soya-bean meal had small and non-significant effects on NAN flow to the small intestine. From Table 5 it can be seen that the increase in the quantities of NAN entering the small intestine when casein was infused arose from a significant ($P < 0·05$) increase in the quantities of microbial N synthesized in the rumen. Neither urea nor soya-bean meal had any effect on microbial N flow to the small intestine. The efficiency of rumen microbial N synthesis was not significantly increased by N supplementation although higher values were observed when casein was infused. Neither the quantities of undegraded feed NAN entering the small intestine nor apparent feed N degradability (Table 5) were influenced by the diets fed.

DISCUSSION

Infusion of casein or urea intraruminally

A major aim of the present experiment was to establish whether the increases in rumen microbial N synthesis observed previously (Rooke *et al.* 1987) when casein was added to a continuous intrarumen infusion of glucose syrup were related to the form of the N

supplied. Clearly infusion of protein-N was an important factor, as in the present experiment casein, but not urea, increased the quantities of microbial N synthesized within the rumen when co-infused with sucrose. Rumen bacterial protein synthesis has been shown to be stimulated by the supply of peptide and amino acid-N in the presence of simple N sources both in vitro (Maeng *et al.* 1976; Cotta & Russell, 1982) and in vivo (e.g., Hume, 1970; McAllan & Smith, 1984).

Energetically the advantage to rumen bacteria of using preformed amino acids and peptides may arise from the reduced costs of amino acid and peptide transport rather than from reduced biosynthetic costs (Demeyer & Van Nevel, 1986). In maize-silage-fed cattle Cottrill *et al.* (1982) observed increases in microbial N synthesis when fish meal replaced urea in the diet. In grass-silage-fed animals, urea did not influence the quantities of microbial N synthesized in the rumen of sheep (Siddons *et al.* 1979) whereas in cattle, protein-N in the form of soya-bean meal has been shown to stimulate microbial N synthesis (Rooke *et al.* 1986). Since silage contains large quantities of non-protein-N in the form of ammonia and amino acid-N, and Chen *et al.* (1987) have shown that rumen bacteria have a preference for peptide-N, then it is possible that in the present experiment casein-N supplied peptide-N rather than amino acid-N.

Infusion of casein and feeding soya-bean meal

When soya-bean meal was fed twice daily in the present experiment there was only a marginal increase in the quantities of microbial N synthesized in the rumen in contrast to the response obtained when casein was infused. Apart from the fact that different protein sources will supply different quantities of each amino acid to the rumen microflora, the most likely explanation for the limited response obtained with soya-bean meal is that the rates of release of N into the rumen from the soya-bean meal and of energy from sucrose were less well synchronized than when casein was infused. Infusing casein supplied approximately 1 g casein-N/h to the rumen microflora. Using the values of Rooke *et al.* (1985) and eqn (2) of Ørskov & McDonald (1979), it can be calculated that only in the hour immediately after each of the daily feeds would soya-bean meal have supplied more than 1 g N. In contrast, for 14 h of 24 h, soya-bean meal would have supplied less than 0·5 g N/h. Further evidence for the importance of synchronizing the supply of supplementary energy and protein-N in silage-based diets can be seen from the findings of Newbold *et al.* (1987). These authors gave a protein supplement consisting predominantly of soya-bean meal to sheep receiving diets of silage and molasses, the whole diet being fed in twenty-four hourly meals/d and thus supplies of energy and protein were synchronized; significant increases in the quantities of microbial N synthesized in the rumen were observed when the protein supplement was fed. Similarly when protein supplements were fed twice daily, accompanied by cereals, stimulation of the quantities of microbial N synthesized have been often (Cottrill *et al.* 1982; Rooke *et al.* 1986) but not always (Siddons *et al.* 1979; Rooke & Armstrong, 1987*b*) observed. The need for synchronization of the supplies of protein and energy is probably more acute when silage is fed because of the relatively high rumen pH (e.g. 6·7–6·8 in the present experiment) observed with silage which thus encourages extensive absorption of ammonia-N from the rumen as ammonia is largely in the unionized form (Siddons *et al.* 1985*a*).

Although the present experiment confirmed the stimulation of microbial N synthesis by casein which had been previously observed (Rooke *et al.* 1987) the extent of the stimulation was much less marked (0·7 (present experiment) *v.* 1·9 (Rooke *et al.* 1987) g microbial N/g casein-N infused). Since the silage:infused CHO ratio was similar in the two experiments (6·4:1 present experiment; 7·3:1 Rooke *et al.* (1987)) and responses to the infusion of sucrose have been obtained in other studies (Gill & Ulyatt, 1977; Huhtanen & Ala-Seppala,

1987), the differing silages fed in each experiment were probably responsible for the between-experiment differences. The silage used by Rooke *et al.* (1987) had lower N and higher acid-detergent fibre contents than that used in the present experiment, and was associated with a lower mean rumen ammonia-N concentration (28 mg N/l) than in the current experiment (49 mg N/l). It is possible that the marked stimulation of microbial N synthesis observed by Rooke *et al.* (1987) when casein was infused was caused by two factors; first, by meeting an absolute requirement for N by the rumen biomass and second, by meeting a requirement for protein-N. Since urea gave no response in the present experiment, there was possibly only a requirement for protein-N to be met with the higher-N-containing silage.

The results for the present experiment have confirmed the importance for rumen microbial N synthesis of including a supply of rumen degradable-protein-N in silage-based diets, although the extent of stimulation achieved is clearly dependent both on silage composition and the synchronization of supply of protein and energy.

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