The digestion by cattle of silage-containing diets fed at two dry matter intakes

1. Digestion of organic matter and nitrogen

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(*Received 27 June 1984 – Accepted 26 November 1984*)

1. In a 4 × 4 Latin square experiment four cattle were given in two meals per d diets consisting of (g/kg dry matter (DM)) 500 barley, 400 grass silage and 100 soya-bean meal. The diets were given at either 1·15 (L) or 2·3 times (H) maintenance energy requirements and the soya-bean meal was either untreated (U) or formaldehyde-treated (T).

2. A 24 h collection of duodenal digesta and a 7 d collection of faeces were made using chromium sesquioxide for flow estimation and ^{35}S as a marker of microbial nitrogen entering the small intestine. Samples of rumen fluid were also taken for estimation of rumen pH, ammonia and volatile fatty acid concentrations.

3. Spot samples of duodenal digesta were obtained after administration of Cr_2O_3-mordanted silage-fibre and soya-bean meal, to determine the rates of outflow of these markers from the rumen. Similar samples were also obtained after cessation of a continuous intraruminal infusion of ruthenium phenanthroline, ^{35}S and CoEDTA.

4. Incubations of each feedingstuff in porous synthetic fibre (psf) bags were carried out in the rumen and the rates of N disappearance from the bags determined.

5. Increasing DM intake significantly ($P < 0·001$) increased the quantities of organic matter (OM), total N and amino acid-N entering the small intestine and amounts subsequently voided in the faeces. Apparent digestibilities of OM and N were unaffected by DM intake; the proportions of total digestible OM digested in the rumen were significantly lower ($P < 0·01$) at the higher level of DM intake.

6. Formaldehyde treatment of the soya-bean meal increased the quantities of N entering the small intestine; these increases were not significant.

7. Increased DM intake increased the quantities of both microbial N ($P < 0·001$) and undegraded feed N ($P < 0·01$) entering the small intestine; HCHO-treatment also significantly ($P < 0·05$) increased the quantities of undegraded feed N entering the small intestine. The efficiency of microbial N synthesis within the rumen was not significantly affected by dietary treatments whereas apparent feed N degradability was reduced significantly ($P < 0·05$) both by increasing DM intake and by HCHO-treatment of the soya-bean meal.

8. Rates of disappearance of N from psf bags in the rumen were different for different feedingstuffs. However, for a given feedingstuff, the rate of N disappearance was not affected by the diets fed.

9. The rates of decline in marker concentrations measured in duodenal digesta were significantly increased as DM intake increased with the exception of Cr_2O_3-soya-bean meal. The markers could be ranked ($P < 0·05$) in the following order of increasing outflow rate: ruthenium phenanthroline, ^{35}S-labelled amino acids and Cr_2O_3-silage fibre < Cr_2O_3-soya-bean meal < CoEDTA.

10. Estimates of the degradabilities of feedingstuffs were calculated from N disappearance rates from psf bags and either experimentally determined outflow rates or those proposed by the Agricultural Research Council (1984). Such estimates for the degradability of the whole diet were then compared with those determined in vivo using ^{35}S as a marker.

In France and the UK the digestible crude protein system for calculating the allowances of dietary protein needed to meet the requirements of ruminant livestock has been largely replaced by systems in which the supply of amino acids to the tissues is calculated from the amount of protein entering the small intestine (Verite *et al.* 1979; Agricultural Research Council (ARC), 1980, 1984). This protein is recognized to be derived from two sources: (1) microbial protein which is synthesized by rumen micro-organisms from dietary nitrogen degraded within the rumen, using energy released during rumen fermentation of feed

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organic matter (OM), and (2) feed protein which escapes rumen fermentation. In the ARC (1980) proposals, the amount of microbial protein entering the small intestine is calculated using factors describing the extent of OM fermentation within the rumen and the efficiency with which N is incorporated into microbial protein per unit OM fermented within the rumen. The extent to which a feed protein is fermented within the rumen (its degradability) is recognized to be a characteristic of that protein source. Continuing research has led to the original ARC (1980) proposals being modified (ARC, 1984). In particular, feed protein degradability is recognized to be dependent not only on the protein source but also on the rate at which undegraded feed particles leave the rumen; the latter is partially dependent on the amounts of feed ingested. The N digestion of silage diets is also recognized to differ from that of diets based on dry forages in that the efficiency with which N is incorporated into microbial protein within the rumen is lower than normal (Miller, 1982).

In the experiment described in the present paper, the effect of increasing dry matter (DM) intake on the digestion of silage-based diets was studied with particular regard to those factors used by the ARC (1980) for the calculation of the quantities of microbial protein entering the small intestines of cattle. In addition, a comparison was made at different DM intakes between feed-protein degradability measured in vivo or estimated from the disappearance rates of N from porous synthetic fibre (psf) bags suspended in the rumen; the latter estimates were calculated using either experimentally determined rumen outflow rates or those outflow rates proposed for use in the revised ARC (1984) proposals. A preliminary account of some of the results presented in the present paper has been given (Rooke & Armstrong, 1983).

EXPERIMENTAL

Animals

The four female Jersey cattle, aged between 3 and 4 years, used during the experiment had mean (with SE) weights of 309 (10·1) kg at the beginning and 349 (9·1) kg at the end of the experiment. Each heifer was equipped with a rumen cannula and a re-entrant cannula in the proximal duodenum (McMeniman & Armstrong, 1979).

Diets

Grass silage was prepared from primary growth perennial ryegrass (*Lolium perenne*) which was harvested on 3 June 1981 after wilting for 49 h under poor climatic conditions (15·9 mm rain, 2 June 1981). The herbage was harvested with a precision chop forage-harvester and ensiled by means of an Eberhard Silopresse (Benedict Agricultural Ltd, London) with the application of 2·5 litres/tonne of an additive containing 850 g formic acid/kg (Add-F, BP Nutrition UK Ltd). The silo was opened after 180 d and from then on silage was removed at weekly intervals, weighed and stored in tightly closed plastic bags. The barley was grown at Cockle Park Experimental Farm, University of Newcastle upon Tyne and cold-rolled before use. The soya-bean meals given to the cattle were provided by BP Nutrition UK Ltd and were identical except that one portion had been subjected to a pretreatment with 2·8 g formaldehyde/kg meal. The daily quantities of feed were offered in two equal portions at 08.00 and 16.00 hours. Water and mineralized salt licks were freely available. The chemical analysis of the dietary ingredients is shown in Table 1.

Experimental procedure

The experiment was designed as a 4×4 Latin square. The cattle were given four diets, the composition of each of which on a DM basis (g/kg) was 500 barley, 400 silage and 100 soya-bean meal. The soya-bean meal was given either untreated (U) or HCHO-treated (T) and the whole diet was given at two levels of DM intake. The lower level of DM intake

Table 1. *The dry matter (DM) contents (g/kg) and the chemical compositions (g/kg DM) of the feedingstuffs*

	Silage*	Barley	Soya-bean	
			Untreated	Formaldehyde-treated
Dry matter	226	854	870	854
Organic matter	916	974	928	929
Nitrogen	20·8	15·7	83·7	84·0
Amino acid-N	15·1	13·5	76·0	76·3
Soluble N	12·8	—	—	—
Ammonia-N	2·0	—	—	—
Water-soluble carbohydrate	34·6	—	—	—
Acid-detergent fibre	346	—	—	—
Acetic acid	18·3	—	—	—
Butyric acid	2·2	—	—	—
Formic acid	10·9	—	—	—
Lactic acid	96·7	—	—	—
pH	3·8	—	—	—

* Dry matter determined by toluene distillation.

(L) was formulated to provide sufficient metabolizable energy (ME) to provide 1·15 times the maintenance energy requirement of each animal, calculated from the live weight of the animal at the start of the experiment (Ministry of Agriculture, Fisheries and Food, 1975). The higher level of DM intake (H) was formulated to provide 2·3 times maintenance energy requirement. In the event, although all animals consumed this higher level of DM intake satisfactorily immediately before the beginning of the experiment, it subsequently proved necessary to reduce the intake of two of the animals to 2·0 times maintenance in order to avoid feed refusals.

Each experimental period was 35 d long. After a period of 14 d for adaptation to the diets, the following collections were made from each animal during the 21 d dietary period. On day 1 the two animals receiving the diets containing untreated soya-bean meal were given 200 g (LU) or 300 g (HU) Cr_2O_3-mordanted soya-bean meal, prepared as described by Ganev *et al.* (1979) and mixed with the concentrate portion of the 08.00 hours feed. Similarly, the two animals receiving the HCHO-treated soya-bean meal in the diet were given either 200 g (LT) or 300 g (HT) Cr_2O_3-mordanted, washed silage-fibre, prepared as described by Udén *et al.* (1980) and mixed with the silage portion of the 08.00 hours feed. Subsequently, on days 2, 3 and 4, spot samples of duodenal digesta (approximately 500 g/sample) were obtained from each animal three times daily, i.e. between 07.30 and 08.00 hours, 13.00 and 13.30 hours and 19.00 and 19.30 hours. These digesta samples were oven-dried at 65°, milled and stored at −20° before analysis for Cr_2O_3.

Samples of rumen contents, free from large particulate material, were obtained on day 2 of each period at 1·5 h intervals from 09.00 to 24.00 hours and then at 06.00 and 07.30 hours on day 3 by means of an in-dwelling probe covered with a nylon-gauze filter. Rumen pH was recorded immediately, 10 ml rumen fluid were added to 10 ml 0·2 M-hydrochloric acid for analysis for ammonia-N concentrations and 4 ml rumen fluid were added to 1 ml 2·5 M-metaphosphoric acid containing 0·2 M-crotonic acid for determination of volatile fatty acid concentrations. Samples were stored at −20° before analysis.

The rate of disappearance of feed N from psf bags was determined for each feedingstuff

when each diet was given to each animal. The bags and the methods used have been described by Rooke *et al.* (1982) with the exception that the bags were made from nylon filter cloth HS013, pore size 36 μm (Henry Simon Ltd, Stockport, Cheshire). On day 3 of each period, bags containing 5 g DM of either untreated soya-bean meal or barley were incubated for periods of 2, 4, 6, 9, 12 and 24 h in the rumen of each of the four animals (six bags/feedingstuff, twelve bags/animal). Similarly, beginning on day 4, bags containing either HCHO-treated soya-bean meal or silage were incubated for periods of 2, 6, 9, 12, 24 and 48 h in the rumen of each of the four animals.

A 7 d collection of urine-free faeces was made from days 11 to 18 of each period. Before this, from day 5 onwards, Cr_2O_3-impregnated paper (2 × 15 g/d) was administered to each animal via the rumen cannula after each feed until after the feed at 16.00 hours on day 19. On day 19, beginning at 08.00 hours, a 24 h total collection of duodenal digesta was made from each animal. Before this, beginning on day 17 (48 h before the duodenal digesta collection), a continuous aqueous infusion (30 ml/h) into the rumen of each animal was started. The infusion supplied: $Na_2{}^{35}SO_4$ (450 μC_i/d, low DM intake (L); 900 μC_i/d, high DM intake (H)), ruthenium phenanthroline (RuP; Tan *et al.* 1971; Beever *et al.* 1978; 38 mg/d (L), 76 mg/d (H)) and CoEDTA (Udén *et al.* 1980; 80 g/d (L), 120 g/d (H)). The infusion was maintained until 08.00 hours on day 20. From the bulk sample of duodenal digesta representing the 24 h collection of duodenal digesta held at 4°, a sample of duodenal microbial material was prepared by differential centrifugation (Elliott & Armstrong, 1982) immediately after the completion of the collection. Spot samples of duodenal digesta (500 g) were also obtained at the end of the 24 h collection of duodenal digesta and on each of days 20 and 21 between the following times: 07.30–07.45 hours, 10.30–10.45 hours, 13.30–13.45 hours and 15.30–15.45 hours. Representative samples of the faecal output, the 24 h collection of duodenal digesta and the previously mentioned spot samples of duodenal digesta were freeze-dried, milled and stored at −20°.

Analytical methods

Silage DM was determined by toluene distillation, water-soluble carbohydrate by the anthrone method, volatile fatty acids and lactic acid in silage as their benzyl esters and fibre as acid-detergent fibre; details of all the methods are to be found in Ministry of Agriculture, Fisheries and Food (1982). The soluble N content of the silage was determined according to Siddons *et al.* (1982). Total N content of all samples including those from the psf bags was determined by Kjeldahl digestion followed by titrimetric determination of the ammonia released by steam distillation; the last-mentioned part of the procedure was also used to determine rumen ammonia-N concentrations and to determine ammonia-N concentrations in silage and duodenal digesta after extraction of the ammonia from the samples with 0·2 M-HCl. The amino acid compositions of the feedingstuffs, duodenal digesta and duodenal microbial preparations were measured by automated ion-exchange chromatography after hydrolysis with 6 M-HCl. The specific radioactivities of duodenal digesta and microbial samples containing ^{35}S were determined according to Mathers & Miller (1980). Volatile fatty acids in rumen fluid were determined by gas–liquid chromatography according to Cottyn & Boucque (1968). The concentrations of Cr_2O_3 and Co in digesta samples were determined by atomic absorption spectrophotometry after ashing of freeze-dried samples and digestion with a potassium bromate–acid mixture (Williams *et al.* 1962). The Ru concentration of digesta samples was measured by X-ray fluorescence spectrometry (Evans *et al.* 1977).

Calculation of results

Flows of digesta DM entering the small intestine and voided in the faeces were corrected for complete recovery of Cr_2O_3. The faecal correction was necessary because some faecal

material was discarded on account of urine contamination. The amounts of microbial amino acid N (AAN) entering the small intestine daily were calculated from the amounts of microbial total N entering the small intestine and the measured proportion of the microbial total N accounted for by AAN. Feed AAN entering the small intestine was calculated as the difference between total AAN and microbial AAN entering the small intestine.

The disappearance of N from psf bags was fitted by a maximum likelihood procedure (Ross, 1980) to an equation:

$$p = a' - b\,e^{-ct}, \tag{1}$$

where p is the proportionate amount of N which had disappeared from the bags after time t (h), and a', b and c are constants; the equation is identical to that described by Ørskov & McDonald (1979) with the exception that constant a given by Ørskov & McDonald (1979) is equal to $(a'-b)$ in eqn (1). For the HCHO-treated soya-bean meal it was necessary to amend the equation according to McDonald (1981) to allow for the introduction of a lag phase into the equation. The rates of decrease (k) in the concentrations of Cr_2O_3-mordanted silage fibre and those of soya-bean meal, and of Cr_2O_3 (from impregnated paper), RuP, CoEDTA and of ^{35}S-labelled amino acids (^{35}S-AA) in spot samples of duodenal digesta, were calculated from the slopes of the equation relating the natural logarithm of marker concentration (Y) to time (h; X). These rates of decline in marker concentration were then used to calculate values for degradability (P) of feedingstuff N according to the equation given by Ørskov & McDonald (1979):

$$P = a + bc/c + k, \tag{2}$$

where, in addition to the constants a, b and c given previously, k is the exponential rate constant relating to the outflow of material from the rumen. The equation for HCHO-treated soya-bean meal was amended according to McDonald (1981) to allow for the lag phase as follows:

$$P = a + (bc/(c+k))(\exp(-(c+k)\,t_o)), \tag{3}$$

where t_o equals 6 h rumen incubation, the length of the lag phase.

Statistical analysis

Analysis of variance techniques for Latin square experiments were used where measurements were completely replicated throughout the experiment. Significant differences relating to diet effects were further partitioned into effects due to differences in DM intake (L *v.* H) or to differences resulting from the HCHO-treatment of the soya-bean meal (U *v.* T). It was necessary to calculate a missing value for one estimate of the rate of decline in the specific radioactivity of ^{35}S-labelled amino acids (^{35}S-AA) in duodenal digesta spot-samples as a result of the poorness of fit observed for this measure ($r^2 < 0{\cdot}80$) when fitted to the model described above. In cases where the experimental design was not fully replicated (i.e. the decline in Cr_2O_3-mordanted soya-bean meal and silage fibre concentrations in spot samples of duodenal digesta, paired t tests were carried out to investigate the effects of level of DM intake. Significant differences between fitted curves for the disappearance of N from psf bags were determined by parallel curve analysis (Ross, 1980).

RESULTS

The mean recoveries (with SE) of Cr_2O_3 (g/g administered) during the experiment were 0·99 (0·029) during the 24 h collections of duodenal digesta and 0·91 (0·024) during the 7 d collections of faeces.

The daily intakes of DM and organic matter (OM) by the cattle given each of the four diets and the digestion of OM are shown in Table 2. It can be seen that whilst increasing

Table 2. *The mean quantities (kg/24 h) of dry matter (DM) and organic matter (OM) consumed by the cattle given the four diets*

(The quantities of OM entering the small intestine and voided in the faeces daily are also given as are values for the apparent digestibility of OM, both in the whole digestive tract and before the small intestine (expressed as a proportion of the digestible OM intake, DOMDR))

	Diet†					Statistical significance of effect	
	LU	LT	HU	HT	SE	L *v.* H	U *v.* T
DM intake	3·27	3·27	6·16	6·16	0·121	***	NS
OM intake	3·09	3·09	5·82	5·79	0·118	***	NS
OM entering small intestine	1·12	1·16	2·53	2·54	0·108	***	NS
OM excreted in faeces	0·69	0·71	1·29	1·20	0·029	***	NS
OM digestibility	0·78	0·77	0·78	0·80	0·011	NS	NS
DOMDR	0·82	0·81	0·73	0·70	0·025	**	NS

LU, low-DM intake, untreated soya-bean meal; LT; low-DM intake, HCHO-treated soya-bean meal; HU, high-DM intake, untreated soya-bean meal; HT, high-DM intake, HCHO-treated soya-bean meal; NS, not significant.

** $P < 0·01$, *** $P < 0·001$.

† For details, see Table 1 and p. 692.

Table 3. *The mean quantities of total (TN) and amino acid nitrogen (AAN) consumed (g/24 h) by the cattle given the four diets*

(The quantities of non-ammonia-N (NAN) and AAN entering the small intestine and of TN voided in the faeces daily are also given. In addition, values are given for the quantities of NAN entering the small intestine daily expressed as a proportion of the quantity of feed N ingested (NAN/N intake) and for N digestibility in the whole gut)

	Diet†					Statistical significance of effect	
	LU	LT	HU	HT	SE	L *v.* H	U *v.* T
TN intake	80·1	80·4	151·3	150·7	2·97	***	NS
AAN intake	66·6	66·8	125·5	125·0	2·42	***	NS
Entering small intestine							
NAN	60·6	69·6	136·0	149·8	9·19	***	NS
AAN	47·8	55·5	103·1	116·2	6·40	***	NS
NAN/N intake	0·76	0·87	0·90	0·99	0·068	NS	NS
N excreted in faeces	19·4	22·3	46·9	49·8	1·49	***	NS
Apparent digestibility of N	0·76	0·72	0·69	0·69	0·025	NS	NS

DM, dry matter; LU, low-DM intake, untreated soya-bean meal; LT, low-DM intake, HCHO-treated soya-bean meal; HU, high-DM intake, untreated soya-bean meal; HT, high-DM intake, HCHO-treated soya-bean meal; NS, not significant.

*** $P < 0·001$.

† For details, see Table 1 and p. 692.

Table 4. *The quantities (g/24 h) of microbial total (TN) and amino acid-nitrogen (AAN) entering the small intestine daily and values for the apparent efficiency of microbial N synthesis within the rumen (expressed as g TN or AAN/kg OM apparently digested in the rumen)*

(Also shown are values for the apparent quantities (g/24 h) of feed non-ammonia-N (NAN) and AAN entering the small intestine daily, together with values for the apparent degradability of feed total and AAN within the rumen)

	Diet†					Statistical significance of effect	
	LU	LT	HU	HT	SE	L *v.* H	U *v.* T
Microbial N entering small intestine							
TN	45·0	46·8	97·3	89·8	4·92	***	NS
AAN	35·4	36·8	73·9	69·7	3·69	***	NS
Efficiency of microbial N synthesis							
TN	22·9	24·0	30·6	29·0	3·12	NS	NS
AAN	18·0	19·1	23·4	22·4	2·47	NS	NS
Feed N entering small intestine‡							
NAN	15·6	23·2	38·7	60·0	5·40	**	*
AAN	12·3	18·6	29·1	46·5	3·89	**	*
Apparent feed N degradability‡							
TN	0·81	0·71	0·75	0·60	0·042	NS	*
AAN	0·82	0·72	0·77	0·63	0·038	*	*

DM, dry matter; LU, low-DM intake, untreated soya-bean meal; LT, low-DM intake, HCHO-treated soya-bean meal; HU, high-DM intake, untreated soya-bean meal; HT, high-DM intake, HCHO-treated soya-bean meal; NS, not significant.

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details, see Table 1 and p. 692.

‡ No allowance made for endogenous N secretions.

the DM intake significantly ($P < 0·001$) increased the quantities of OM which entered the small intestine and those which were subsequently voided in the faeces, the HCHO-treatment of the soya-bean did not influence overall OM digestion significantly. Increasing intake of OM significantly ($P < 0·01$) reduced the proportion of the total apparently digested OM which disappeared before the small intestine.

Similarly, increasing the intake of total N and AAN by increasing DM intake (Table 3) increased ($P < 0·001$) the quantities of non-ammonia-N and AAN entering the small intestine and the quantities of total N voided in the faeces. In comparison with the quantities of N entering the small intestine when the untreated soya-bean meal was given, HCHO-treatment of the soya-bean meal increased the quantities of both non-ammonia-N and AAN entering the small intestine by 12 and 14% respectively; however, these increases were not significant. The values relating to the digestion of individual amino acids will be presented in a following paper.

Table 4 shows the partition of both total N and of AAN entering the small intestine daily into microbial N and that arising from rumen-undegraded feed N. It should be noted that the latter has been calculated as the difference between the quantities of total non-ammonia-N (or AAN) and microbial total N (or AAN) entering the small intestine daily. No allowance has been made for endogenous N secretions in the digesta entering the small intestine as there is no reliable information for endogenous secretion for cattle cannulated between the pyloric sphincter and the bile duct; this point has been previously discussed (Rooke *et al.*

Table 5. *The mean values for rumen pH and for rumen ammonia-nitrogen (mg N/l) and volatile fatty acid (mmol/l) concentrations for the cattle given the four diets*

(The molar proportions of individual fatty acids (mmol acid/mol total volatile fatty acids) are also given)

	Diet†				Statistical significance of effect		
	LU	LT	HU	HT	SE	L *v.* H	U *v.* T
pH	6·81	6·82	6·60	6·52	0·103	*	NS
Ammonia-N	77·9	57·8	68·2	45·8	4·33	*	**
Volatile fatty acids							
Total	70·8	66·1	87·2	88·1	9·30	*	NS
Acetate	660	676	649	656	9·1	NS	NS
Propionate	207	192	206	203	8·7	NS	NS
n-Butyrate + isobutyrate	111	101	121	119	4·1	NS	NS
n-Valerate + isovalerate	23	32	24	23	3·1	NS	NS

DM, dry matter; LU, low-DM intake, untreated soya-bean meal; LT, low-DM intake, HCHO-treated soya-bean meal; HU, high-DM intake, untreated soya-bean meal; HT, high-DM intake, HCHO-treated soya-bean meal; NS; not significant.

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

† For details, see Table 1 and p. 692.

1982). Increasing DM intake significantly increased the quantities of microbial and feed N (both total and AAN) entering the small intestine. In addition, HCHO-treatment of the soya-bean meal significantly increased the quantities of feed total N and AAN entering the small intestine. The apparent efficiency of microbial total N or AAN synthesis (g N/kg OM apparently digested in the rumen (OMADR)) was not significantly affected by either DM intake or by HCHO-treatment. Apparent feed total N (and AAN) degradability in the rumen was, however, significantly reduced as a result of the HCHO-treatment of the soya-bean meal. Increasing DM intake also reduced feed N degradability although this effect was only significant in the case of AAN.

Mean values during the experiment for rumen pH and for rumen ammonia-N and volatile fatty acid concentrations calculated on a 24 h basis are shown in Table 5. Increasing DM intake significantly ($P < 0{\cdot}05$) decreased the mean values observed for rumen pH and ammonia-N concentrations but increased volatile fatty acid concentrations. Formaldehyde treatment of the soya-bean meal had no significant effect on pH or on volatile fatty acid concentrations; however, ammonia-N concentrations were significantly ($P < 0{\cdot}05$) reduced by the HCHO-treatment of the soya-bean meal. No dietary treatment had any significant effect on the molar proportions of the individual fatty acids in the total volatile fatty acids.

The changes in rumen pH, total volatile fatty acid concentrations and the molar proportions of individual volatile fatty acids observed throughout the day are shown in Fig. 1. It can be seen that the significant decrease in rumen pH and increase in volatile fatty acid concentration noted previously (Table 5) when DM intake was increased were associated with consistent differences in rumen pH and volatile fatty acid concentrations at each sampling time throughout the 24 h period. The molar proportions of individual volatile fatty acids in the total volatile fatty acids are also shown in Fig. 1. It can be seen that, irrespective of feeding level, there were increases in the molar proportions of propionic acid after both daily feeds at the expense of acetic acid.

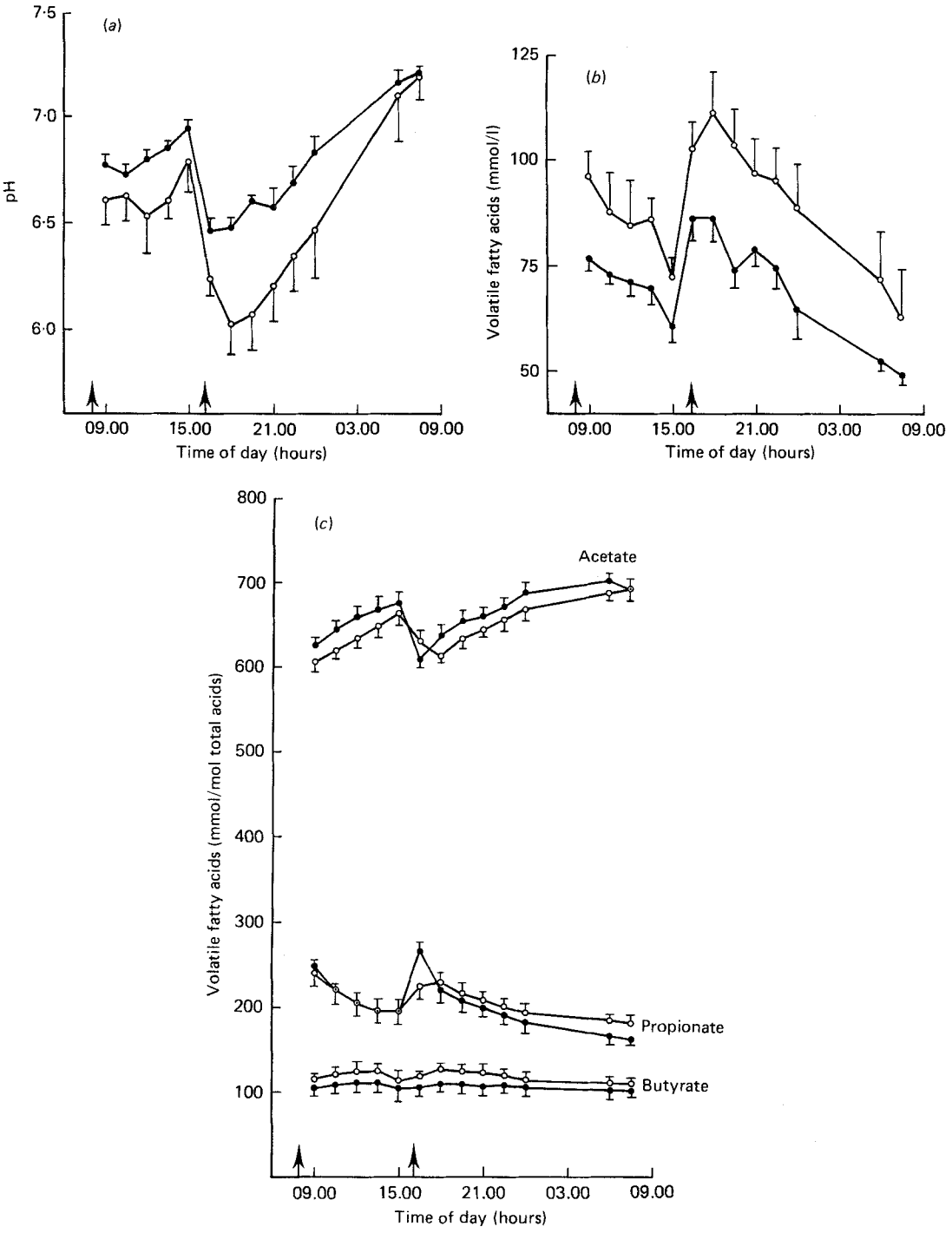

Fig. 1. Daily variations in (*a*) pH, (*b*) total volatile fatty acid concentrations (mmol/l) and (*c*) molar proportions of each volatile fatty acid (mmol/mol total acids) in the rumen of cattle given low (●) or high (○) dry matter intakes (for details see p. 693). Mean values, with their standard errors represented by vertical bars, are given for eight observations. ↑, Times of feeding.

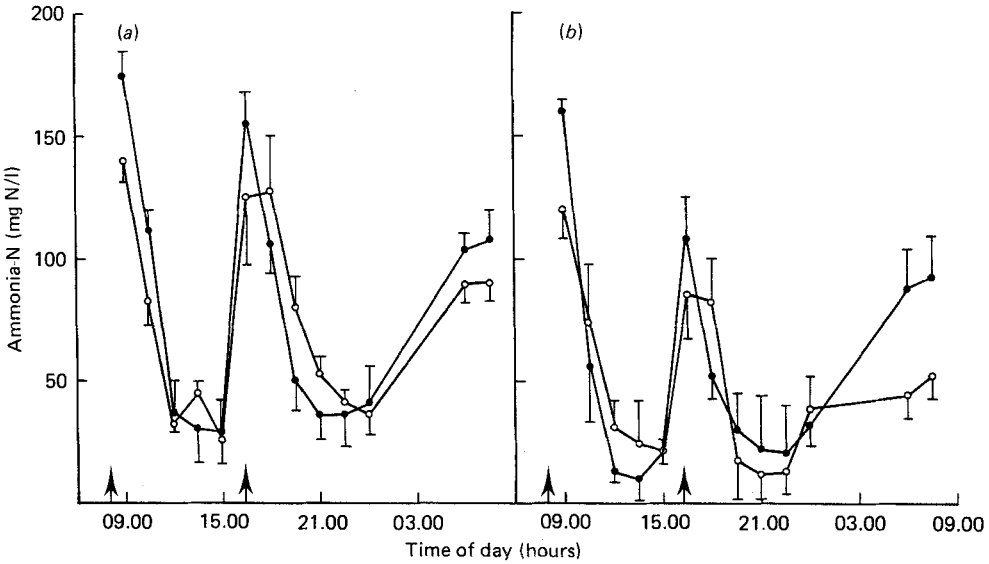

Fig. 2. Daily variations in rumen ammonia-nitrogen concentration of cattle given (*a*) untreated or (*b*) formaldehyde-treated soya-bean meal at low (●) or high (○) dry matter intakes (for details see p. 693). Mean values, with their standard errors represented by vertical bars, are given for four observations. ↑, Times of feeding.

The changes in rumen ammonia-N concentrations throughout the day are shown in Fig. 2. The significant decrease in ammonia-N concentrations (Table 5) resulting from the HCHO-treatment of the soya-bean meal was apparent at all sampling times. It should be noted that with all four diets ammonia-N concentrations fell to less than 50 mg N/l approximately 5 h after each meal.

Table 6 gives the mean proportions of N from each feedingstuff which remained in psf bags after varying times of rumen incubation. Statistical analyses demonstrated that while the rate of disappearance of N from bags differed significantly between feedingstuffs ($P < 0{\cdot}001$) for silage, barley and HCHO-treated soya-bean meal, not the diets given, the animals, nor the period of the Latin square experiment significantly influenced the disappearance of N from the bags for any of the three feedingstuffs. The constants for the curves fitted to the disappearance of N from the bags with time for the three feedingstuffs are given in Table 7. It should be noted that for the HCHO-treated soya-bean meal, the values required that a lag phase between 0 and 6 h rumen incubation during which no N disappeared from the bags be included in the model. In contrast to the foregoing, significant differences ($P < 0{\cdot}001$) between curves as a result of dietary effects were found for the untreated soya-bean meal. Further analysis established that there were no significant differences between curves when the different soya-bean meals were included in the diet. However, the fitted curve for N disappearance from the bags when DM intake was low was significantly different ($P < 0{\cdot}001$) in both intercept (*b*) and exponential (*c*) terms from that fitted when DM intake was high. In quantitative terms, however, these differences were small; after 24 h incubation, the differences in the proportionate amounts of N lost from the bags when calculated using the fitted parameters were 0·87 (L intake) and 0·83 (H intake).

A comparison was made in this experiment between different markers of the rate of outflow of digesta constituents from the rumen, as measured by the exponential rate of

Table 6. *The proportionate quantities of nitrogen from each feedingstuff lost from porous synthetic fibre bags after varying times of incubation in the rumens of cattle given each of four diets*

(Mean values with their standard errors are given for sixteen measurements for each feedingstuff at each incubation time)

	Time of rumen incubation (h)						
	2	4	6	9	12	24	48
Silage							
Mean	0·62	ND	0·65	0·68	0·65	0·78	0·83
SE	0·012		0·008	0·013	0·009	0·031	0·042
Barley							
Mean	0·35	0·51	0·66	0·77	0·82	0·92	ND
SE	0·005	0·006	0·009	0·013	0·009	0·008	
Soya-bean meal							
(i) Untreated							
Mean	0·34	0·40	0·45	0·54	0·62	0·91	ND
SE	0·018	0·017	0·024	0·024	0·036	0·019	
(ii) HCHO-treated							
Mean	0·00	ND	0·00	0·02	0·03	0·19	0·47
SE	0·031		0·027	0·018	0·015	0·011	0·007
SE	0·020	0·016	0·016	0·016	0·017	0·016	0·027

ND, not determined.

Table 7. *The disappearance of feedingstuff nitrogen from porous synthetic fibre bags incubated in the rumen of cattle*

(Mean values with their standard errors are given for constants a', b and c relating to the equation $p = a' - be^{-ct}$, where p is the proportion of N which had disappeared from bags at time t (h) for all values relating to silage, barley and formaldehyde-treated soya-bean meal and for values relating to the low (L) and high (H) levels of dry matter intake for the untreated soya-bean meal)

	Constant							
	a'		b		c			
	Mean	SE	Mean	SE	Mean	SE	Residual sum of squares	df
Barley	0·93	0·022	0·84	0·041	0·182	0·0200	0·601	93
Silage	0·89	0·043	0·29	0·036	0·035	0·0111	0·203	91
HCHO-treated soya-bean meal*	1†	—	1·03	0·014	0·015	0·0010	0·554	75
Untreated soya-bean meal								
L intake	1†	—	0·76	0·037	0·074	0·0076	0·252	45
H intake	1†	—	0·83	0·029	0·065	0·0065	0·383	46

* Fitted to a model which included a lag phase between 0 and 6 h rumen incubation where no N disappearance from the bags occurred.

† Model constrained such that a' was less than or equal to 1.

Table 8. *The exponential rates of decrease (/h) in the concentrations of four markers in the digesta entering the small intestines of cattle given each of four diets*

(Rates of decrease are also given for chromium-sesquioxide-mordanted soya-bean meal when diets LU and HU were given and for Cr_2O_3-mordanted silage-fibre when diets LT and HT were given)

Marker	Diet†				Statistical significance of effects		
	LU	LT	HU	HT	SE	L *v.* H	U *v.* T
Ruthenium phenanthroline	0·024	0·021	0·032	0·031	0·0030	*	*
^{35}S-labelled amino acids	0·024	0·018	0·036	0·035	0·0035	**	NS
Cr_2O_3 from impregnated paper	0·040	0·031	0·048	0·048	0·0073	**	NS
CoEDTA	0·057	0·051	0·062	0·067	0·0037	*	NS
Cr_2O_3-mordanted:							
Silage-fibre	—	0·022	—	0·036	0·0053	*	—
Soya-bean meal	0·048	—	0·051	—	0·0019	NS	—
SE‡	0·0029		0·0039				

DM, dry matter; LU, low-DM intake, untreated soya-bean meal; LT, low-DM intake, HCHO-treated soya-bean meal; HU, high-DM intake, untreated soya-bean meal; HT, high-DM intake, HCHO-treated soya-bean meal; NS, not significant.

* $P < 0·05$, ** $P < 0·01$.

† For details see Table 1 and p. 692.

‡ Standard errors for eight observations are given at each level of DM intake for between marker differences in outflow rates.

decrease in marker concentration in digesta entering the small intestine with time; these results are given in Table 8. It can be seen that for every marker examined, with the exception of Cr_2O_3-soya-bean meal, increasing DM intake significantly increased the rate of outflow of the marker from the rumen. When the outflow rates for different markers were compared using Neumann-Keuls test (Keuls, 1952), the markers could be ranked statistically ($P < 0·05$) in the following order of outflow rates: RuP, Cr_2O_3-mordanted silage-fibre and ^{35}S-AA < Cr_2O_3 (from impregnated paper) < Cr_2O_3-mordanted soya-bean meal < CoEDTA.

DISCUSSION

The results reported in the present paper represent the continuation of a long-term project concerning the digestion by cattle of the N constituents of silage-containing diets (Brett *et al.* 1979; Overend & Armstrong, 1982; Rooke *et al.* 1982, 1983*a*, *b*, 1984). The main aim of the present experiment was to determine the effect that increasing DM intake above 1·0 and 1·5 times maintenance energy requirements had on previously investigated aspects of the digestion of silage-containing diets.

The silage given to the cattle as part of their diet during the present experiment had been previously given to the same cattle as the only dietary ingredient at 1·3 times maintenance energy requirements (Rooke *et al.* 1983*b*). Under these conditions 813 g digestible OM/kg was digested before the small intestine; 0·93 g non-ammonia-N entered the small intestine/g silage N intake, the apparent degradability of the silage N was 0·82 and 27·2 g microbial N were synthesized/kg OMADR.

In the present experiment when concentrates were given with the silage at 1·15 times maintenance energy requirements, 820 g digestible OM/kg were digested before the small intestine, a value similar to that observed when the silage was given alone, and in agreement with previously published values from this laboratory for cattle (Brett *et al.* 1979; Overend & Armstrong, 1982; Rooke *et al.* 1982, 1983*a*, *b*, 1984). These values are substantially higher than the mean value for sheep and cattle of 0·65 adopted from the literature by the ARC (1980) and the value calculated by Thomas & Chamberlain (1982) of 0·70 for sheep given silage-based diets. The higher value of 820 g/kg obtained in this experiment may be partially explained by the longer retention times observed for digesta within the reticulo-rumen of cattle given forage diets when compared with sheep (Ternouth *et al.* 1979; Rees & Little, 1980). In addition, there is also evidence (Deswysen *et al.* 1978) that the retention times of digesta within the rumens of silage-fed ruminants are longer than for animals given dry forages.

In the present study when soya-bean meal was included with the silage in the diet, there was no stimulation of apparent efficiency of microbial N synthesis in the rumen; the relevant values, i.e. 22·9 (diet LU) and 24·0 (diet LT) g N/kg OMADR were less than that of 27·2 g N/kg OMADR when the silage was given as the only component of the diet (Rooke *et al.* 1983*b*). This is in contrast to previous results where the inclusion of soya-bean meal in silage-based diets has increased apparent efficiency of microbial N synthesis over silage-only diets (Brett *et al.* 1979; Rooke *et al.* 1983*a*). This difference might relate to the ammonia-N levels maintained in the rumen; in contrast to those in previous studies of Rooke *et al.* (1983*a*). In the present experiment rumen ammonia-N concentrations were not maintained above 50 mg N/l throughout the sampling period and thus the supply of ammonia-N for microbial N incorporation may have been limiting during some periods of the day.

The recent ARC (1980, 1984) proposals for calculating the N requirements of ruminant livestock are dependent on a number of factors. Thus the quantity of microbial N entering the small intestine is calculated from the amounts of energy made available, within the rumen, from fermentation of feed OM (expressed as 0·65 of the digestible OM intake) and from the efficiency of synthesis of microbial biomass (32 g N/kg OMADR; ARC, 1984). The degradability of a feed protein within the rumen is recognized to be a function of both the chemical structure of the feed and the residence time of the feed within the reticulo-rumen (ARC, 1980, 1984).

In the present study and others with sheep (Nicholson & Sutton, 1969), growing cattle (McAllan & Smith, 1983; Zinn & Owens, 1983) and dairy cows (Rohr *et al.* 1979; Tamminga *et al.* 1979), increasing DM intake has consistently decreased the proportion of the digestible OM intake apparently digested within the rumen. However, only in the present study and in those of McAllan & Smith (1983) and of Zinn & Owens (1983) can this decrease be unequivocally attributed to increasing DM intake; in the other experiments cited the composition of the diet was not kept constant as DM intake increased.

The effect of increasing DM intake on the apparent efficiency of microbial N synthesis within the rumen is much less consistent. In none of the experiments cited previously was a significant increase in the efficiency of microbial N synthesis noted, although Zinn & Owens (1983) reported a near significant ($P = 0{\cdot}07$) cubic effect of increasing DM intake upon the efficiency of microbial N synthesis while Rohr *et al.* (1979) observed that, on average, efficiency increased with DM intake. A similar trend was observed in the present experiment, i.e. increasing DM intake gave an increase in the efficiency of microbial N synthesis. However this was not significant as the effect was due almost entirely to the response of one of the four cattle. Thus, although the efficiency of microbial synthesis is increased when the dilution rate of the rumen liquid phase is increased by environmental conditions

(Kennedy & Milligan, 1978) or by manipulating rumen conditions, e.g. by the addition of buffer salts to the diet (Harrison *et al.* 1975), the effect of DM intake on the efficiency of microbial N synthesis appears to be limited, notwithstanding that increasing DM intake increases both rumen liquid and solid phase turnover rates (Evans, 1981 *a*, *b*). With reference to the scheme proposed by the ARC (1980, 1984) for evaluation of protein requirements for ruminants, the effect of a decrease in the proportion of the digestible OM apparently disappearing within the rumen as DM intake increases coupled with no change in the apparent efficiency of microbial N synthesis within the rumen would be to overestimate the amount of microbial protein synthesized within the rumen.

In the present experiment and in the studies of Nicholson & Sutton (1969), Tamminga *et al.* (1979) and Zinn & Owens (1983), the quantities of total non-ammonia-N entering the small intestine daily, expressed as a proportion of N intake, were increased as DM intake increased; these increases resulted from increased quantities of undegraded feed N entering the small intestine as DM intake increased. Thus, reduction of the extent of feed protein degradation when feed DM intake increased appeared to be an important factor regulating the quantities of protein entering the small intestine in the previously mentioned studies. In contrast, McAllan & Smith (1983) observed in young cattle no consistent increase in the quantities of total non-ammonia-N entering the small intestine/kg DM as DM intake increased.

Formaldehyde treatment of the soya-bean meal also increased the mean quantities of non-ammonia-N entering the small intestine/g N intake at both levels of DM intake. Although these increases were not statistically significant, they are in agreement with previous results from this laboratory (Rooke *et al.* 1983*a*). The values obtained using psf bags suspended in the rumen and in vivo in the present experiment show clearly that the response to HCHO-treatment of the soya-bean meal resulted from the reduction in rumen degradability of the soya-bean meal after HCHO-treatment. If it is assumed that the apparent digestibility of amino acids within the small intestine is 0·70 (ARC, 1980) and calculating that the ME content of the diet was 12·7 MJ/kg DM, then the quantities of amino acids absorbed from the small intestine/MJ ME intake were respectively 6·1 (LU), 6·9 (LT), 7·0 (HU) and 7·8 (HT) g amino acids/MJ ME intake for each of the four diets given. Armstrong (1981) has calculated the quantities of amino acids required for a range of highly productive ruminants to be between 6 and 10 g amino acids absorbed/MJ ME intake depending on the nature of the production. The additional amino acids absorbed as a result of the HCHO-treatment of the soya-bean meal would thus potentially increase the levels of production attainable for a given energy intake.

Many experiments have been done to obtain information about the rates of disappearance of feedingstuff N from psf bags within the rumen. However, to obtain a measurement of the degradability of feedingstuff N within the rumen it is necessary to combine this information with measurements of the rate of outflow of particulate material from the rumen (Ørskov & McDonald, 1979). Such measurements of outflow rate are usually made either directly from the decline in the concentration of a marker within the rumen or indirectly from the pattern of excretion of the marker in the faeces. In the present experiment, samples were obtained from the re-entrant cannula sited in the proximal duodenum before the bile duct. The reasons for so doing were first, that sampling from the rumen of animals given silage-based diets is difficult because of the dense raft of fibre floating on top of the rumen digesta and the small diameter of the rumen cannula (50 mm). Second, samples taken from the proximal duodenum have the advantage that they are representative of the digesta entering the small intestine and also, in contrast to faecal collections, the near continuous flow of digesta through the proximal duodenum allows discrete sampling of the digesta at specific times. Third, use of the mathematical approach outlined by Grovum & Williams

(1973), in which two exponential rate constants are calculated relating to the rates of outflow of a marker from the rumen and from the caecum and colon, can be avoided. The assignment of the two exponential constants as outlined by Grovum & Williams (1973) has been questioned by Ellis *et al.* (1979) in relation to diets based on long fibre and by Faichney & Boston (1983) in relation to pregnant ewes. Faichney & Boston (1983) also identified the abomasum as a third site for the retention of digesta within the gut. However, in the present experiment, no evidence was found for more than one exponential rate constant in digesta samples obtained from the duodenum.

The results in Table 8 show that, at both levels of DM intake, the markers used could be subdivided into one of three classes; Cr-mordanted fibre, RuP and ^{35}S-AA; Cr-mordanted soya-bean meal; CoEDTA. The fact that Cr-mordanted fibre, RuP and ^{35}S-AA had almost identical outflow rates at each level of DM intake was somewhat surprising. The Cr-mordanted fibre given to the animals in the present experiment was resistant to degradation by the rumen microflora; after 72 h rumen incubation in psf bags, 1·02 (SE 0·0004, $n = 4$) of the Cr-mordanted-fibre DM incubated remained in the bags and the Cr content of the fibre was unchanged. The fibre was given to the animals in the same form (20–50 mm length) as the silage from which it was prepared and thus the outflow rate for the Cr-mordanted fibre reflected the physical breakdown of the fibre by mastication or rumination to a size small enough to leave the rumen. Indeed the outflow rates measured for Cr-mordanted fibre in this experiment were similar to those obtained for Cr-mordanted forage-fibre by Colucci *et al.* (1982), Udén *et al.* (1982) and by Mader *et al.* (1984). Thus the outflow rates for Cr-mordanted silage fibre reported in the present experiment may indeed reflect those of the fibre part of the diets. If this is so then the RuP and ^{35}S-AA outflow rates are determined by the rate at which fibre leaves the rumen and not by the rate at which small particles leave the rumen. The outflow rate of small particles is reflected in the outflow rate of Cr-mordanted soya-bean meal. The fact that ^{35}S-AA, which presumably are of microbial origin, and RuP have near identical outflow rates is not in itself surprising as it has been found (Faichney & Griffiths, 1978; Faichney, 1980) that RuP associates preferentially with bacteria. A proportion of bacteria normally leave the rumen associated with the small particles and not with fibre (Faichney, 1980; Mugdal *et al.* 1982). The surprising fact is that the outflow rate of bacteria appears to be dependent on that of fibre. There appears to be no explanation for this observation at present.

The outflow rates of the markers (Table 8) in conjunction with the psf bag values (Table 7) were used to calculate estimates of degradability of the feedingstuffs according to Ørskov & McDonald (1979) and McDonald (1981). For the silage a mean value at each level of DM intake was calculated from the outflow rates of Cr-mordanted fibre, RuP and ^{35}S-AA; for barley and for the soya-bean meals the outflow rates determined for Cr-mordanted soya-bean meal were used in the calculation. In addition, similar calculations were made using the outflow rates proposed by the ARC (1984); 0·02/h for cattle fed near maintenance energy requirements and 0·05/h for growing and fattening cattle.

Values were calculated (Table 9(*a*)) for the apparent degradability for individual feedingstuffs determined using either disappearance rates from psf bags and experimentally determined outflow rates or those given by the ARC (1984). The values obtained were similar except for barley and the soya-bean meals at the low level of DM intake where the greater outflow rate measured in the experiment (0·048/h) resulted in lower values for apparent N degradability than those values calculated using the suggested ARC (1984) value of 0·02/h. These estimates for apparent N degradability of each individual feedingstuff determined using either experimentally determined or ARC (1984) outflow rates (Table 9(*a*)) were used to calculate values for N degradability for each of the four diets given and the values are shown in Table 9(*b*). It can be seen that at the lower level of DM intake the

Table 9. *The apparent nitrogen degradability of the individual components of the diets given to the cattle calculated from N disappearance rates from porous synthetic fibre (psf) bags using either experimentally-determined outflow rates or markers from the rumen or those proposed by Agricultural Research Council (ARC) (1984)*

(The degradabilities of the whole diets are also given, calculated both from psf bags and in vivo results)

	Degradability			
(*a*)	Low		High	
DM intake...				
Outflow rate*...	Measured	ARC (1984)	Measured	ARC (1984)
Silage	0·78	0·78	0·75	0·72
Barley	0·75	0·85	0·75	0·75
Untreated soya-bean meal	0·70	0·84	0·64	0·64
Formaldehyde-treated soya-bean meal	0·14	0·33	0·13	0·13

	Degradability			
(*b*)				
Diet...	LU	LT	HU	HT
In vivo	0·81	0·71	0·75	0·60
psf bags estimates†	0·74	0·55	0·71	0·54
psf bags estimates‡	0·82	0·65	0·70	0·53

DM, dry matter; LU, low-DM intake, untreated soya-bean meal; LT, low-DM intake, formaldehyde-treated soya-bean meal; HU, high-DM intake, untreated soya-bean meal; HT, high-DM intake, formaldehyde-treated soya-bean meal.

* For details, see p. 702.

† Calculated using measured outflow rates.

‡ Calculated using outflow rates proposed by ARC (1984).

values calculated using the measured outflow rates were lower than those based on the ARC (1984) outflow rates. Both sets of values calculated from psf bag measurements for the degradability of each diet were less than the values determined in vivo using ^{35}S as a marker for microbial protein except for diet LU when ARC (1984) outflow rates were used. Kennedy *et al.* (1982) also noted that N degradabilities based upon disappearance rates from psf bags and measured outflow rates based on RuP as a marker were less than those determined from measurements in vivo using ^{35}S as a marker of microbial protein despite the fact that no allowance was made for endogenous secretions in the calculations of in vivo degradability. There are no clear reasons for these differences. They may arise in part from the fact that feedingstuffs incubated in psf bags may be contaminated by rumen bacteria (Mathers & Aitchison, 1981; Rooke *et al.* 1984); furthermore, feedingstuffs in such bags are not subjected to mastication by the animal.

The conclusion to be drawn from the present study is that increasing DM intake of silage-based diets increases the rates of passage of different markers from the rumen. These increased rates of passage result in a depression of OM fermentation within the rumen and an increased passage of undegraded feedingstuff N to the small intestine. However, the apparent efficiency with which microbial N is synthesized within the rumen appears to be unaffected by increased DM intake. Values for feed N degradability calculated from N disappearance from psf bags incubated intraruminally and from outflow rates of particulate material from the rumen are lower than those measured from the flows of digesta entering the small intestine.

The authors wish to thank Messrs B. Brown, D. Smith and D. Robertson for care of the animals, the Agricultural and Food Research Council and the Deutsche Forschungemeinschaft for financial support. Thanks are also due to Dr J. C. Mathers for helpful discussions in the preparation of the manuscript and to Mr P. Oakley, Department of Geology, University of Newcastle upon Tyne for use of the X-ray spectrometer.

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Printed in Great Britain