Rumen degradation and fractional outflow rates of nitrogen supplements given to cattle eating sodium hydroxide-treated straw

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1. Six rumen and abomasal cannulated heifers were used to study the effects of intake on the fractional outflow rates (FOR) of chromium-mordanted cotton-seed meal (Cr-CSM) and meat meal (Cr-MM), CrEDTA, ytterbium and lignin from the rumen. Values of FOR of Cr-CSM and Cr-MM were combined with values of nitrogen disappearance from the protein supplements, placed in porous synthetic (nylon) bags and incubated within the rumen (P), to calculate effective degradation (D) of CSM and MM when fed to heifers eating sodium hydroxide-treated straw. Also, N degradation in vivo (V) was measured as the difference between abomasal N flow and the sum of flows of microbial and endogenous N.

2. FOR were positively related to intake and differences between supplements were significant ($P < 0.01$). FOR pertaining to high and low intakes respectively were 0·073 and 0·052 for Cr-CSM, 0·082 and 0·071 for Cr-MM, 0·030 and 0·023 for lignin, 0·082 and 0·073 for CrEDTA and 0·044 and 0·035 for Yb.

3. A rise of 28·8 and 13·4% in FOR of Cr-CSM and Cr-MM respectively, associated with an increase in intake from maintenance to 1·5 times maintenance, resulted in 10·7 and 2·2% reductions in D, 24 h after feeding, for CSM and MM respectively.

4. With the exception of CSM at the high intake, estimates of V were underestimated by D and were 8·6–25·0% greater than the D values when time of incubation (t) = ∞. The two techniques, however, ranked the degradation of the two supplements in the same order at both levels of intake.

5. Underestimation of V by D may be attributable to underestimation of P, overestimation of FOR (both resulting in underestimation of D) or overestimation of V due to biases associated with the estimation of this part of the comparison. The relative importance of these factors remains to be determined.

Recent recommendations on protein requirements for ruminants, e.g. Agricultural Research Council (1980), are based on the concepts of rumen-degradable protein (RDP) to satisfy the requirements of microflora, and undegradable dietary protein (UDP) plus microbial protein to meet the requirements of the animal. In order to apply such recommendations, the RDP and UDP contents of dietary proteins have to be known. The nylon-bag technique has been used to estimate rates of rumen degradation of proteins (Mehrez & Ørskov, 1977). However, the effective degradation (D) of proteins is determined by their retention time in the rumen as well as their rate of degradation in the rumen (Ørskov *et al.* 1980). Ganev *et al.* (1979) observed that increasing the intakes of a basal diet leads to increased fractional outflow rate (FOR) of protein supplements which would result in underestimation of the effective degradation of supplements given with the basal diet. This was confirmed by Sriskandarajah *et al.* (1981). Most of the studies in this area have involved soya-bean and fish meals (Mansbridge & Ørskov, 1980; Eliman & Ørskov, 1984), with a few observations on cotton-seed meal (CSM) and rape-seed meal (Lindberg, 1982) and casein (Sriskandarajah

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et al. 1981). There do not appear to have been any quantitative measurements made on meat meal (MM). The basal diets in most of these studies have been hay or straw. In the present study, values of nitrogen disappearance from CSM and MM placed in porous synthetic (nylon) bags and incubated within the rumen (*P*), were combined with estimates of FOR from the rumen of the protein supplements to calculate *D*. These estimates of *D* were compared with in vivo measurements of protein degradation in the rumen (*V*).

Alkali-treated straw was selected for use in the present study because of its very low content of protein, high edibility and reasonably high energy content. With these attributes, there was a minimal source of bias attributable to N flow at the abomasum from dietary sources other than the test protein. A preliminary report of some of this work was reported by Amaning-Kwarteng *et al.* (1984).

MATERIALS AND METHODS
Nylon-bag measurements

The relation between percentage N disappearance (*P*) and time of incubation (*t*) is exponential and can be described by the equation:

$$P = a + b(1 - e^{-ct})$$

(Ørskov & McDonald, 1979). The constants *a*, *b* and *c* are measures of the rapidly-soluble N fraction, the degradable N fraction and the rate constant for the degradation of *b* respectively, and are particular to each protein and basal diet. To obtain the values to estimate these constants, CSM and MM, ground through a 2·5 mm screen, were incubated in nylon bags (80 × 170 mm, 25 μm pore size) within the rumen of three heifers (B, D and F) fitted with large rumen cannulas (100 mm diameter). The animals were given a basal diet of alkali-treated straw (see below) at 0·9 times *ad lib.* intake plus 500 g of a mixed supplement containing (by weight) 50 parts CSM and 50 parts MM. Air-dried CSM or MM (5 g) were incubated in a bag. Sets of three bags of both supplements were incubated concurrently in the rumen of each of the three heifers. The bags were withdrawn after 0, 3, 6, 9, 12, 24, 36 or 48 h of incubation. After rinsing lightly under running tap-water for 3 min the bags were dried in a forced-draught oven for 8 h at 60°, followed by *12 h at 100°*. Dry matter (DM) and N (by a microKjeldahl technique) were determined on the residues. Degradability of N was estimated by the percentage disappearance of N in the supplement from the bags. The constants *a*, *b* and *c* were calculated by an iterative least-squares procedure. The nylon-bag measurements were made immediately before the in vivo measurements.

In vivo measurements

Animals. Six Friesian heifers (A, B, C, D, E and F), aged 30 months, weighing about 290 kg live weight and fitted with large rumen cannulas (100 mm diameter) and simple abomasal cannulas were housed in individual pens. Heifers (B, D and F) were the same animals used in the nylon-bag measurements.

Diets and feeding regimen. Straw from a crop of semi-winter wheat, *Triticum aestivum* (*cv.* Shortim), was hammer-milled (30 mm screen) and sprayed with sodium hydroxide solution (44 g NaOH in 113 g water/kg) followed by a mineral solution supplying (g/kg): 79 water, 14 N, 1 sulphur and 1·5 phosphorus and (mg/kg): 4 copper, 0·1 cobalt and sprinked with limestone supplying 2 g calcium/kg. Straw was treated in 250-kg batches in a trailer mixer fitted with spray nozzles, and was stored in hessian bags for at least 7 d before feeding. Treated straw was given in buckets, automatically, at 3-h intervals at high (0·9 times *ad lib.*) and low (0·6 times high) levels of intake. These intakes were equivalent to 1·5 times

maintenance and maintenance requirements respectively. The *ad lib.* level of intake was determined during a preliminary period of 14 d when animals were offered 20% in excess of their intake of the previous day. The heifers received once daily, a 500 g supplement of either CSM or MM. The four dietary treatments tested were: high intake, CSM; high intake, MM; low intake, CSM; low intake, MM. Each of the four diets was offered to each heifer in an incomplete Latin-square design over four periods, so that there was a total of six observations per treatment mean. During each period, measurements of digesta markers and abomasal flows were made after 14 d of preliminary feeding. This was followed by measurements of outflow rates of Cr-CSM and Cr-MM.

Digesta markers and abomasal flow measurements. The quantity and composition of true digesta flowing from the abomasum and rumen outflow rates of liquid and particulate matter were determined by reference to CrEDTA as the liquid-phase marker, and ytterbium as the particulate marker. CrEDTA was prepared as described by Binnerts *et al.* (1968) and sprayed on alkali-treated straw to supply 200 μg Cr/kg. Yb marker was prepared by dissolving ytterbium nitrate in water and sprayed to supply 150 mg Yb/kg straw. The marked straw was fed to the six heifers for 13 d after the preliminary period. During the last 3 d, twelve samples each of rumen and abomasal digesta were collected at intervals distributed throughout the 24 h cycle. Bulked rumen and abomasal samples were stored at $^-10°$. At the end of the period the feeding of marked straw was terminated and rumen contents sampled six times within the following 32 h.

Outflow rates of Cr-CSM and Cr-MM. Quantitative observations were made of the effect of intake on FOR of Cr-mordanted CSM (Cr-CSM) and MM (Cr-MM) from the rumen of heifers eating NaOH-treated straw. Cr was complexed with CSM and MM according to Udén *et al.* (1980). Sodium chromate was applied at 139 g/kg DM of protein supplement. The Cr complex, after reduction with ascorbic acid, was thoroughly washed until free of soluble green matter and dried at 65°. The Cr-CSM and Cr-MM were used to determine outflow rates of the untreated supplements from the rumen. During this measurement, which lasted 13 d, heifers were given unmarked alkali-treated straw. On day 10 during this measurement, heifers were given a single meal (22–36 g) of either Cr–CSM or Cr–MM which was thoroughly mixed with the day's protein supplement. This was generally consumed within 5 min. The entire rumen contents were emptied manually at 2, 5, 8, 11 and 26 h after administering the Cr-mordant: they were then weighed, mixed and subsampled. Samples were analysed for DM, Cr and lignin (Goering & Van Soest, 1970). The rate of Cr dilution was used to estimate FOR of Cr-CSM and Cr-MM. Lignin turnover rate was estimated from the ratio, daily lignin intake: lignin concentration in the rumen.

Before feeding Cr-CSM and Cr-MM to heifers, DM recovery of both Cr-mordants was compared with those of the corresponding untreated supplements. Air-dried samples (5 g) were incubated in nylon bags in the rumen of animals B, D and F for 3 and 50 h (three bags/sample per animal).

Effective N degradability

The constants *a*, *b* and *c* from the nylon-bag measurements were combined with those of in vivo measurements of FOR to calculate *D* of CSM and MM from the equation of Ørskov & McDonald (1979):

$$D = a + [bc/(c+k)](1 - e^{-(c+k)t}),$$

D was calculated for each of the rate-constants and *k* was appropriate to the outflow rates of CSM and MM when the basal diet was given at high and low levels of intake. The constants *a*, *b* and *c* obtained from the nylon-bag measurements were used in the prediction of *D* at both high and low levels of intake (Ørskov & McDonald, 1979; Ørskov *et al.* 1981).

Chemical analyses and calculations

For estimation of bacterial protein in abomasal digesta, rumen bacteria were isolated from the rumen fluid samples according to Hutton *et al.* (1971). The N and 2, 6-diaminopimelic acid (DAPA) contents of dried bacteria and total digesta were determined and the respective N:DAPA for each sample calculated. The proportion of bacterial N in digesta was determined as the DAPA:N of whole digesta divided by the DAPA:N of the bacterial fraction.

Rumen fluid was filtered through nylon gauze to remove coarse particles. Abomasal samples were centrifuged at 2400 *g* for 20 min to obtain liquid-rich fractions. Feed, rumen, total abomasal samples and corresponding liquid-rich fractions were analysed for DM by drying in a forced-draught oven to constant weight at 50°. N was analysed by a microKjeldahl technique. DAPA in whole digesta and bacterial fractions was determined using ion-exchange chromatography (TSM Amino Acid AutoAnalyzer; Technicon Equipment Ltd, Sydney). Cr and Yb concentrations in rumen and abomasal samples were measured by atomic absorption spectrophotometry after being subjected to wet digestion (Sriskandarajah & Kellaway, 1984). FOR of liquid and and particulate matter were determined with reference to Cr and Yb dilutions respectively.

True digesta were mathematically reconstituted from Cr and Yb concentrations in total digesta and in liquid-rich fractions. The flow of digesta from the abomasum was corrected to 100% recovery of the daily dose of Cr input and calculated by using eqns (1)–(3) from Faichney (1975).

Estimates of V were calculated as total abomasal N flow minus the sum of bacterial, endogenous and straw N flows. Endogenous N was calculated as 4 g/kg DM intake (DMI) (Hart *et al.* 1982), and straw N flow as 1·73 g/kg DMI (J. Spragg and R. C. Kellaway, unpublished results).

Statistical analyses

Variations in the constants of the equation $P = a + b(1 - e^{-ct})$ pertaining to nylon-bag measurements were analysed as a randomized complete block design with subsamples. The effect of the supplement was tested against residual mean square (2 df). Both the coefficients of variation based on the variation between bags within a treatment and a heifer and that based on experimental error are presented. They were calculated from subsampling and residual mean squares respectively as $\sqrt{(EMS)}/\overline{X}$, where $\overline{X}$ is the grand mean and EMS is the experimental error mean square (Steel & Torrie, 1980).

The values pertaining to rumen characteristics and digesta kinetics were examined by analysis of variance for an incomplete Latin-square design. Effects of intake, supplement and intake × supplement interaction were tested against residual mean square (12 df). The least significant difference ($P < 0.05$) was used to compare treatment means (Steel & Torrie, 1980).

RESULTS

Nylon-bag measurements

Table 1 shows the coefficients describing digestion kinetics of N in nylon bags. MM had a larger soluble fraction ($P < 0.01$), a smaller degradable fraction ($P < 0.05$) and a higher rate of degradation than CSM. The 45% difference in fractional degradation constant was of biological significance although not statistically significant ($P > 0.05$). The coefficients of variation based on EMS were very large, especially that of *c*.

Mean values of DM recovery and standard deviations are presented in Table 2. Cr treatment reduced percentage DM loss of CSM in bags from 29 to 1·0 after 3 h and from 64·1 to 5·4 after 50 h of incubation. The corresponding reduction in MM DM loss due to Cr treatment was from 31·3 to 3·2% after 3 h and from 41·9 to 7·1% after 50 h of incubation.

Table 1. *Constants in equation* $\bar{P} = a + b(l + e^{-ct})$, *where* $\bar{P}$ *is percentage nitrogen disappearance after time-periods* (t, *hours*) *of incubation, from cotton-seed meal* (*CSM*) *and meat meal* (*MM*) *incubated in nylon bags within the rumen of heifers given sodium hydroxide-treated straw* (*at 0·9 times* ad lib. *intake*) *plus 500 g mixed supplements*†

(Values are means of three heifers × three bags/heifer; *n* 9)

Coefficients..	*a* Soluble	*b* Degradable	*c* Fractional degradation	$100 - (a+b)$ Undegradable
Supplement‡				
CSM	7·26	65·33	0·066	27·41
MM	29·65	33·76	0·120	36·59
Standard error of difference (2 df)§	0·965	6·207	0·0209	5·748
Statistical significance	**	*	NS	NS
Coefficient of variation (%)§ based on:				
Variations between bags	1·820	1·079	6·7787	2·284
Experimental error	11·096	26·576	47·7885	38·107

NS, not significant.

* $P < 0.05$, ** < 0.01.

† 50 parts by weight CSM and 50 parts by weight MM.

‡ Both supplements were incubated at the same time within the rumen of the same animals.

§ See statistical analysis, p. 390.

Table 2. *Percentage dry matter recovery of untreated* (*U*) *and chromium-treated* (*T*) *cotton-seed meal* (*CSM*) *and meat meal* (*MM*) *incubated in nylon bags within the rumen of heifers given sodium hydroxide-treated straw* (*at 0·9 times* ad lib. *intake*) *plus 500 g mixed supplement**

(Values are means and standard deviations† for three heifers × three bags/heifer; *n* 9)

Period of incubation (h)	CSM				MM			
	U		T		U		T	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3	71·1	2·19	98·9	1·66	68·7	3·17	96·8	1·76
50	35·9	2·93	94·6	2·27	58·1	4·57	92·8	2·05

* 50 parts by weight CSM + 50 parts by weight MM.

† SD (2 df) calculated for three heifers per treatment mean using average values of three bags per heifer.

In vivo measurements

Mean values of DM intake, rumen contents and FOR are given in Table 3. Outflow rates of CrEDTA, Yb, lignin, Cr-CSM and Cr-MM were higher at the high feed intake than at the low feed intake ($P < 0.01$). FOR of Cr-CSM was significantly less than that of Cr-MM.

Tables 4 and 5 respectively show the estimates of *D* for CSM and MM calculated relative to measured *P* and FOR values. Compared with the measured *P* values, the fitted ($\bar{P}$) estimates do not appear to fit well at the beginning of the time-scale. There was only a slight and insignificant increase in *D* (2·9–4·9% in CSM and 0·32–0·43% in MM) between 24 h and the final value when $D = a + bc/(c + k)$. A rise in *k* (28·8% for CSM and 13·4% for

Table 3. *Dry matter (DM) intake (DMI), rumen contents and fractional outflow rates (FOR) from the rumen of heifers given a basal diet of sodium hydroxide-treated straw at two levels of intake plus a 500 g supplement (S) of either cotton-seed meal (CSM) or meat meal (MM)*

(Values are means of six heifers per treatment)

S...	CSM		MM		Standard error of difference† (12 df)	Statistical significance		
Intake...	High	Low	High	Low		I	S	I × S
Total DMI (kg/d)	7·15	4·46	7·18	4·50	0·017	**	NS	NS
Total rumen DM (kg)	6·39	4·77	6·06	4·82	0·185	**	NS	NS
Total rumen fluid (l)	48·6	46·5	48·8	41·6	2·45	NS	NS	NS
Outflow rates (/h)								
Chromium supplement	0·073	0·052	0·082	0·071	0·0016	**	**	**
Lignin	0·029	0·022	0·030	0·023	0·0010	**	NS	NS
CrEDTA	0·081	0·072	0·082	0·074	0·0022	**	NS	NS
Ytterbium	0·044	0·034	0·044	0·036	0·0020	**	NS	NS

High, 0·9 times *ad lib.* intake; low, 0·6 times high intake; NS, not significant.
** *P* < 0·01
† Interaction means.

Table 4. *Effective nitrogen degradability (D) of cotton-seed meal calculated from percentage N disappearance from nylon bags at different time-periods (t) of incubation and in vivo fractional outflow rate measurements*

Period after incubating a supplement (*t*, hours)	Fractional outflow rate (/h)...	Percentage disappearance of N from nylon bags after time *t*		Calculated effective N degradation after time *t* (D)	
		Experimentally determined (*P*)	Fitted ($\overline{P}$)	High intake	Low intake
		0	0	0·073	0·052
3		23·84	18·99	17·84	18·15
6		28·74	28·62	24·81	25·80
9		35·63	36·52	29·40	31·17
12		42·17	43·00	32·43	34·93
24		53·54	59·19	37·18	41·65
—		—	72·59	38·28	43·80

P, values were means of three heifers × three bags per heifer; $\overline{P} = a+bl-e^{-ct}$, using constants in Table 1. The equation was constrained such that $a+b$ did not exceed 100 and $100-(a+b)$ was the undergradable fraction;

$$D = a+[bc/(c+k)](1-e^{-(c+k)t}),$$

assuming Ørskov & McDonald (1979) is appropriate. The constants from the nylon-bag measurements were used in the calculation of D for both high (0·9 times *ad lib.* intake) and low (0·6 times high intake) levels of intake (for details, see p. 389).

Table 5. *Effective nitrogen degradation* (D) *of meat meal calculated from percentage* N *disappearance from bags at different time-periods* (t) *of incubation and in vivo fractional outflow measurements*

Period after incubating a supplement (t, hours)	Fractional outflow rate (/h)...	Percentage disappearance of N from nylon bags after time t		Calculated effective N degradation after time t (D)	
		Experimentally determined (P)	Fitted ($\overline{P}$)	High intake	Low intake
		0	0	0·082	0·071
3		44·58	39·86	38·76	38·90
6		48·83	46·98	43·74	44·12
9		50·74	51·95	46·45	47·06
12		52·20	55·41	47·93	48·72
24		57·11	61·51	49·55	50·64
—		—	63·41	49·71	50·86

P, values were means of three heifers × three bags per heifer; $\overline{P} = a + b(1 - e^{-ct})$, using constants in Table 1. The equation was constrained such that $a + b$ would not exceed 100 and $100 - (a + b)$ was the undegradable fraction;

$$D = a + [bc/(c + k)](1 - e^{-c + k)t}),$$

assuming Ørskov & McDonald (1979) is appropriate. The constants from the nylon-bag measurements were used in the calculation of *D* for both high (0·9 times *ad lib.* intake) and low (0·6 times high intake) levels of intake (for details, see p. 389).

MM) due to an increase in intake from maintenance to 1·5 times maintenance resulted in reductions of 10·7 and 2·2% in *D* (24 h after feeding a supplement) for CSM and MM respectively.

Estimates of protein degradation calculated from abomasal digesta flow (Table 6) show that MM was 32% more degraded than CSM and that degradation was significantly higher ($P < 0.01$) at the low level of intake than at the high level of intake. Mean differences between MM and CSM were 20·2 and 19·1 at low and high intakes respectively with this interaction between supplements and intake being non-significant ($P > 0.05$).

Comparison of calculated D *and estimated* V *values*

A summary of the estimated *V* values compared with the calculated *D* values is shown in Table 7. With the exception of CSM at the high level of intake, calculated *D* values underestimated the *V* values. There was a strong heterogeneity of variance for the data obtained by the two methods compared and, for this reason, statistical comparison was not possible. The two methods, however, ranked the degradability of the supplements in the same order at both levels of intake.

DISCUSSION

The generally high coefficient of variation based on EMS was due, at least in part, to the small number of animals used. The excessively large coefficient of variation of *c* makes the statistical comparison of the two treatments for this index unreliable. The position of the bags in the rumen could be responsible for the relatively high between-bag variation in *c*. In this experiment, a weight of 45 g was attached to the bags in order to anchor them

Table 6. *Calculations of in vivo degradation of cotton-seed meal (CSM) and meat meal (MM) from digesta flow at the abomasum of heifers given a basal diet of sodium hydroxide-treated straw at two levels of intake plus 500 g CSM or MM/d*

(Values are means of six heifers per treatment)

Supplements (S)...	CSM		MM		SED	Statistical significance†	
Intake (I)...	High	Low	High	Low	(12 df)	I	S
Abomasal nitrogen flow (g/d)							
Total	136·8	89·7	137·0	91·8	3·38	**	NS
Bacterial	78·0	49·0	80·0	55·1	3·14	**	NS
Endogenous‡	27·3	17·4	27·6	17·2	1·71	**	NS
Straw§	11·0	6·7	11·1	6·6	0·18	**	NS
Supplemental N (g/d)							
Intake	31·7	31·7	40·1	40·1			
Flow at abomasum‖	20·5	16·6	18·3	12·9	2·50	NS	NS
Degraded	11·3	15·1	21·8	27·2	2·04	*	**
Degraded as percentage of intake (24 h after intake)	35·3	47·6	54·4	67·8	3·89	**	**

High, 0·9 times *ad lib.* intake; low, 0·6 times high intake; SED, standard error of difference for interaction means; NS, not significant.

　† I × S interactions were not significant.

　‡ Calculated as 4 g/kg dry matter intake (Hart *et al.* 1982).

　§ Calculated as 1·73 g/kg dry matter intake (J. Spragg, unpublished results).

　‖ Total abomasal N flow − (bacterial + endogenous + straw) N flow.

Table 7. *Comparison of estimated percentage in vivo nitrogen degradation (V) and calculated effective N degradability (D) of cotton-seed meal (CSM) and meat meal (MM) given to heifers eating sodium hydroxide-treated straw at two levels of intake plus 500 g CSM or MM/d*

(Values are means of six heifers (for *V* values) or three heifers (for *D* values) and their respective standard deviations)

Level of intake	Supplement	Estimated in vivo N degradation		Calculated effective N degradability		$D \times 100$
		(V)*	SD	(D)†	SD	V
High	CSM	35·3	1·65	38·28	0·58	108·0
	MM	54·4	2·01	49·71	0·91	91·4
Low	CSM	47·6	2·28	43·80	0·63	92·0
	MM	64·8	2·90	50·86	0·65	75·0

　High, 0·9 times *ad lib.* intake; low 0·6 times high intake.

　* From Table 6.

　† From Tables 4 and 5 when period of incubation (t) = ∞.

in the ventral sac where digestion is most rapid (Miles, 1951). Mehrez & Ørskov (1977), however, found that this procedure had no effect in reducing variation between bags. Significant differences in N degradability between soya-bean meal (SBM) and CSM were observed by Hughes-Jones (1979). The constants *a*, *b* and *c* were 9·5, 77·8 and 0·08 for SBM and 29·2, 36·5 and 0·063 for CSM respectively. Differences in coefficients for CSM observed

by us and Hughes-Jones (1979) may be attributable to differences in the extent of heating during processing as well as differences in the basal diet; the coefficients are affected by the passage rate of the basal diet (Ganev *et al.* 1979). The effects of processing on N degradation have been substantiated by Chapman & Norton (1982) and, more recently, by Freer & Dove (1984). The former authors observed that fresh, frozen forage samples had significantly greater *c* values than dried samples. Comparing the degradations of N from sunflower meals and rapeseed meals, the latter workers found that there was as much difference between two batches of sunflower meal as there was between either of them and rapeseed meal.

Increases in the rate of passage of Cr-treated supplements from the rumen with increase in intake have been reported by Ganev *et al.* (1979), Mansbridge & Ørskov (1980) and Lindberg (1982) for Cr-CSM, Cr-fish meal (FM) and Cr-rapeseed meal (Cr-RSM). Our results provide a quantitative description of the effect.

The significant difference in FOR between Cr-CSM and Cr-MM suggests that values obtained with one protein supplement might not be applicable to others. Mansbridge & Ørskov (1980) observed small differences between Cr-FM and Cr-soya-bean meal (Cr-SBM) outflow rates. Also, Lindberg (1982) observed small differences between Cr-CSM, Cr-SBM and Cr-RSM outflow rates at both maintenance and 3 times maintenance levels of intake. Larger and significant differences observed in the present study may be due to the highly significant difference in the soluble fractions of the two supplements (Table 1). Differences in solubility would result in differences in the distribution of the two supplements between the rumen liquid and particulate phases.

The range of FOR of Cr supplements obtained in the present study is similar to that of Sriskandarajah *et al.* (1981) who studied $H^{14}CHO$-casein in wethers given NaOH-treated straw (0·048–0·083/h) and those of Eliman & Ørskov (1984) who used Cr-FM in dairy cows in early lactation and gave a mixed diet (0·066 − 0·091/h). Our values are, however, higher than those obtained by Lindberg (1982) who studied Cr-CSM, Cr-SBM and Cr-RSM in dry cows (0·034–0·062) and Eliman & Ørskov (1984) when they used Cr-FM and Cr-SBM in sheep given long, dried grass (0·008–0·038). These results suggest that the basal diet has a major effect on FOR and show that a constant FOR of protein supplements from the rumen cannot be assumed in the calculation of *D* in the rumen when different basal diets are given.

Reports relating level of intake to liquid and particulate FOR have not been consistent. No significant changes in liquid FOR were observed in sheep given three levels of intake of a hay–grain diet (Hodgson *et al.* 1976). Similarly, no increase in FOR of rumen solids was observed when the intake of the basal diet was increased (Waldo *et al.* 1965; McAllan & Smith, 1976). Direct positive relations, such as those observed in the present study, have been reported by Minson (1966), Owens *et al.* (1979), Evans (1981 *a, b*) and Warner (1981). In a series of experiments, Grovum & Williams (1973, 1977) observed an increased FOR from the rumen, small intestine, large intestine, caecum and colon with increased feeding level. This phenomenon is probably associated with voluntary water consumption as water intakes generally parallel DM intake.

FOR of CrEDTA observed in the present study was lower than that of 0·093/h reported by Sriskandarajah & Kellaway (1984) for cattle eating a similar diet of NaOH-treated straw. Major differences in experimental procedure between the two studies are that steers were given NaOH-treated straw *ad lib.* by Sriskandarajah & Kellaway (1984) and received no supplemental energy, whereas the heifers in the present study were given a maximum of 0·9 times *ad lib.* intake and also received supplements of either CSM or MM. Decreases in rumen fluid FOR with decreased DM intake were reported by Evans (1981 *a*). Decreased rumen fluid FOR with increased grain or energy content of the diet have also been reported in sheep (Grubb & Dehority, 1975) and in cattle (Prange *et al.* 1979). It is possible that

concentrate feeding may also cause reduced solid FOR. Such a phenomenon could be due to alterations in the microbial population.

Reduction in D with increases in FOR of the supplement agrees with the observation of Ørskov et al. (1981) who calculated that D of SBM would be reduced from 89·1% at FOR of 0·01/h to 67·7% at FOR of 0·04/h. In the present study, 24 h after feeding supplements, D of CSM decreased from 41·65 at FOR of 0·052/h to 37·18 at FOR of 0·073/h. The corresponding decrease in D of MM was from 50·64 at FOR of 0·071 to 49·55 at FOR of 0·082/h. Our observed responses were smaller than those of Ørskov et al. (1981), probably due to the lower degradabilities of CSM and MM than that of SBM in the rumen. At 24 h after feeding, there was no difference between P of CSM and MM. However, the corresponding values of D were significantly different. Higher outflow rates caused lower degradability. In the present study the effect of higher FOR of MM was confounded by its substantially higher c with the net result that MM was effectively more degraded than CSM. Mehrez & Ørskov (1977) observed that both the extent of protein degradation and its rate of degradation should be considered when comparing the degradabilities of different protein sources. Ganev et al. (1979) concluded that while rumen outflow of potentially-degradable material has importance, the rate of degradation has the greater effect. Values of D, 24 h after feeding a supplement, were not different from the D values predicted for $t = \infty$. This is of great practical value and supports the observation of Ørskov & McDonald (1979) that 24 h is a sufficiently appropriate incubation time for protein supplements in nylon bags in the rumen when studying the percentage N disappearance from the bags. The use of the same constants (a, b and c) in the calculation of D at both high and low intakes (as by Ørskov & McDonald, 1979; Ørskov et al. 1981) assumes that the level of feeding does not affect these estimates of nylon-bag measurements. There has not been any quantitative information, however, to substantiate this assumption. This is a relevant area which needs investigation.

The method for estimating V also identified differences in the degradabilities of the two supplements and provided information about the rumen metabolism of the proteins. The application of this method is, however, limited because it is unsuitable for the assessment of large numbers of ingredients at a time. Also, the single degradation value obtained for each material is applicable only to the particular feeding situation. A change in feeding level will alter the V values. The technique further suffers from the disadvantage that errors present in the determination of the microbial N is, by subtraction, transferred to the generally much smaller undegraded dietary N. Estimates of microbial N have also varied considerably between different microbial markers used. For example, Punia & Leibholz (1984) found microbial N based on ^{35}S to be 21% higher than that based on DAPA. The usual explanation is that DAPA takes no account of the protozoal N contribution in the digesta. Sampling of the gut contents distal to the rumen to estimate total N flow at the abomasum, combined with errors in estimating endogenous N flow, are additional sources of variation. Any variation in digesta flow-rates from the abomasum would change N retention time and therefore estimates of N degradation. We minimized the effect of this source of variation by sampling at regular intervals throughout the 24 h cycle.

Attempts by earlier workers to make direct comparisons between nylon-bag degradability measurements and V have yielded inconsistent results (Chapman & Norton, 1982, 1984; Freer & Dove, 1984). The disparity could be attributed to the fact that the P values were not corrected for FOR of the supplemental protein from the rumen. Chapman & Norton (1984) used three techniques, namely nylon-bag, ^{35}S-microbial-labelling and ^{15}N-plant-labelling procedures, to compare P and V of pangola grass (*Digitaria decumbens*) and siratro (*Macroptilium atropurpureum*). Estimates of V based on the two isotope-labelling techniques gave higher values than those with the nylon bags. The nylon-bag and the in vivo

techniques also ranked the degradabilities of the two feeds differently. It is note-worthy that the P values were not corrected for FOR. Similar results were obtained by Mathers & Miller (1981) when P and V of dietary protein in the rumen of sheep given diets containing chopped lucerne (*Medicago sativa*) and rolled barley were compared. V values were determined using a ^{35}S-labelling procedure. When a k value was assumed from the work of Ørskov & McDonald (1979) and used to correct for FOR in their study, Mathers & Miller (1981) observed a remarkable similarity between D and V values.

We compared D with V estimates observed from analysis of abomasal digesta (Zinn *et al.* 1981). The similarity between estimated V and calculated D in the order they ranked the degradability of the two supplements at both levels of intake suggests that the nylon-bag technique is a useful tool for comparing the degradability of proteins when the P values are corrected for FOR.

Our results agree with the findings of Armstrong & Brookes (1981), Rooke *et al.* (1983) and Rooke & Armstrong (1983) who observed that the nylon-bag method ranked different supplemental proteins in the same order of degradabilities as did the observed in vivo methods even though the D values underestimated the V values. Underestimation of V by D may be attributable to the underestimation of P, overestimation of FOR (both resulting in underestimation of D) or overestimation of V due to biases associated with the estimation of this part of the comparison. The relative importance of these factors, as well as the possibility that the model of Ørskov & McDonald (1979) might not be a sufficiently good approximation of in vivo degradation, remains to be determined. The attraction of the nylon-bag method lies in the ability to follow sequentially the rate of protein degradation as well as study the nutritive value of feed residues after rumen incubation in order to estimate the qualitative contribution of undegradable protein to the abomasum.

The results of the present study suggest that a constant outflow rate of protein supplements cannot be assumed in the calculation of effective protein degradation in the rumen. The large differences in N degradation between CSM and MM (with both V and D) confirm that there cannot be a single value for the degradability of N in different protein supplements (Rooke & Armstrong, 1983).

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