

Supplemental protein degradation, bacterial protein synthesis and nitrogen retention in sheep eating sodium hydroxide-treated straw

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1. Alkali (sodium hydroxide)-treated wheat straw was given to six rumen- and abomasal-cannulated sheep to study the rumen degradation of cotton-seed meal (CSM) and barley (B), and the effects of these supplements on nitrogen retention and efficiency of bacterial protein synthesis were measured.

2. N degradation, using porous synthetic (nylon) bags incubated within the rumen (P), and in vivo measurement determined from the abomasal flow of N (V), distinguished quantitatively between the two supplements. Estimates of P , corrected for fractional outflow rates/h (FOR), underestimated estimates of V when FOR of undegraded protein from the rumen (k) of 0.05 and 0.08 were used. Estimates of V for CSM and B were 70.9 and 80.8% respectively.

3. Intakes of alkali-treated straw were not affected by the supplements. Intakes of digestible organic matter (DOM) for the diets comprising alkali-treated straw alone (W), straw plus CSM (WC) and straw plus barley (WB) were 477, 575 and 590 g/d respectively ($P < 0.05$) and organic matter (OM) apparently digested in the rumen (OMADR) was 339, 399 and 435 g/d respectively ($P < 0.05$).

4. On W, WC and WB respectively, flows at the abomasum were 11.0, 14.0 and 13.3 g/d for bacterial N ($P < 0.05$) and 0, 2.8 and 0.5 g/d for dietary supplemental N; g bacterial N/kg OMADR were 32.4, 35.6 and 30.9 ($P > 0.05$) and N balances were 2.37, 4.27 and 3.29 g/d ($P < 0.05$) on the respective treatments. It was suggested that supplements increased total OM intake as a result of increased OM digested in the rumen rather than OM flow from the rumen.

Chemical treatment of straw increases its edibility and digestibility (Jackson, 1977; Kellaway *et al.* 1978). These improvements in forage quality are only apparent when sufficient rumen-degradable nitrogen (RDN) is supplied (Ørskov & Grubb, 1978; Ørskov, 1982). The positive effects of protein supplements on the growth of animals consuming low-quality forages may be predictable in terms of an additional energy intake (Srisankarajah & Kellaway, 1982) or may be attributable to an increase in the proportion of nutrients absorbed as essential amino acids (Gill & Beever, 1982). The latter factor is a function of both the extent of rumen protein degradation and microbial protein production. Recent systems for estimating protein requirements (e.g. Burroughs *et al.* 1975; Journet & Verite, 1977; Agricultural Research Council (ARC), 1980), rely on accurate prediction of both the yield of microbial protein and the proportion of food protein escaping to the small intestine. The objectives of the present experiment were to measure yields of bacterial protein and dietary protein degradation in the rumen of sheep fed on sodium hydroxide-treated straw, alone (W) or supplemented with cotton-seed meal (WC) or barley (WB).

Alkali-treated straw, which has a very low content of protein and reasonably high energy content is an eminently suitable diet for measuring dietary protein degradation as there is minimal bias attributable to N flow at the abomasum from dietary sources other than the

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test protein. A preliminary account of part of this work was reported by Amaning-Kwarteng *et al.* (1984).

MATERIALS AND METHODS

Animals

Six two-tooth Merino × Border Leicester wethers, weighing about 35 kg live weight, and fitted with permanent rumen cannulas (64 mm diameter) and simple abomasal cannulas, were housed in individual metabolism cages under continuous illumination.

Diets and feeding regimen

Straw from a crop of semi-winter wheat, *Triticum aestivum* (cv. Shortim), was hammer-milled (30 mm screen) and was sprayed with NaOH solution (45.6 g NaOH in 113 g water/kg). This was followed by a mineral solution supplying (g/kg) 79 water, 14.2 nitrogen, 1.0 sulphur, 0.9 phosphorus and (mg/kg) 1.8 copper and 0.11 cobalt and limestone sprinkled to supply (g/kg) 1.0 calcium. 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. At 28 d before the start of the study the animals had been removed from a pasture of ryegrass (*Lolium rigidum*) and clover (*Trifolium repens*), kept together in a pen and fed on alkali-treated straw. Thereafter, animals were put in individual metabolism cages and given the experimental diets. During a preliminary period of 14 d, animals were offered alkali-treated straw at 20% in excess of their intake the previous day. *Ad lib.* straw intake reached a constant level between days 10 and 12 of this period. Subsequently, alkali-treated straw was supplied in buckets, automatically at 2-h intervals at 0.9 times *ad lib.* intake. This level of intake was determined for each period. The supplemented wethers received, once daily and before the straw, 150 g supplement of either cotton-seed meal (CSM) or barley (B). The diets W, WC and WB constituted the experimental treatments. Water was available *ad lib.*

Experimental design and time schedule

Immediately before the digesta flow and digestibility studies, three of the six wethers were used to study the disappearance of N from the protein supplements as outlined for nylon-bag measurements. Thereafter, the three dietary treatments were randomly allocated to the six wethers in two 3 × 3 Latin squares. Each diet was offered to each sheep for 26 d per period. The time schedule followed during each period was as follows: preliminary feeding (days 1–14) followed by infusion of marker (days 15–22). Faecal and urine samples were collected over 5 d (days 19–23) and rumen and abomasal contents over 3 d (days 20–22). Infusion of markers was terminated on day 23 for rumen-dilution-rate measurement. Rumen pool size was determined on day 26.

Nylon-bag N degradation measurements

Rate and extent of supplemental N degradation was estimated using porous synthetic (nylon) bags incubated within the rumen (Mehrez & Ørskov, 1977). Nylon bags (80 × 170 mm, 25 μm pore size), containing 5 g air-dried samples each of CSM and B, were incubated in the rumen of three sheep (three bags/sheep per supplement) eating alkali-treated straw at 0.9 times *ad lib.* intake plus 150 g daily of a mixed supplement containing, by weight, 50 parts CSM and 50 parts B. Sets of three bags of both supplements were incubated concurrently in the rumen of each of the three sheep. The bags were withdrawn at 0, 3, 6, 9, 12, 24, 36 or 48 h. 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°. Residues were analysed for N. Degradation of N was estimated by the percentage disappearance of N in the supplement from the bags.

Markers and infusate preparation

Dual markers used were CrEDTA for the liquid phase and acid-detergent lignin for particulate matter. Originally it had been intended to use ytterbium as the particulate-matter marker but while rumen dilution rates for Yb were reasonable, there was near-zero digestion in the stomach when Yb was used as a dual marker with CrEDTA to calculate abomasal digestion flow.

Analysis of samples for lignin concentration gave an average precision of 2.6% which according to Faichney & White (1983) is acceptable for the analytical method used. Faecal lignin output rather than feed lignin intake was used to calculate the daily intake of marker lignin. Several workers (Porter & Singleton, 1971; Egan *et al.* 1975; Faichney, 1980; Neutze, 1985) have stressed that any possible digestion of lignin or solubilization of lignin as a lignin-carbohydrate complex (Gaillard & Richards, 1975) occurs primarily in the rumen. They have also demonstrated quantitatively the similarity between abomasal lignin flow and faecal lignin output. In the present study, for daily faecal output (Y) *v.* daily lignin intake (X), regressions for the three treatments did not differ significantly ($P > 0.05$) in slope and intercept and the combined regression was:

$$Y = 2.39 + 0.92X \quad (n\ 18, r^2\ 0.989, S_{Y.X}\ 0.6948).$$

The slope of the equation (SE 0.0243) was significantly less than 1 ($P < 0.05$), suggesting significant lignin digestion in the digestive tract. The positive intercept (SE 1.2403) which indicates artifact lignin formation, was not significantly greater than zero ($P > 0.05$) and did not fully compensate for lignin digestion (means for faecal lignin output and feed lignin intake were 49.0 and 50.5 g/d respectively). We used faecal lignin as the solid marker for determining abomasal digesta flow.

CrEDTA solution was prepared by mixing and boiling together for 1 h solutions of Na₂EDTA (20 g/300 ml water) and CrCl₃.6H₂O (14.2 g/200 ml water). Water (400 ml) and 1 M-calcium chloride (4 ml) were added to the solution when cooled. About 10 ml NaOH (400 g/l) was then added to bring the pH to 6.5. The CrEDTA solution contained 2.77 g chromium/l. Yb solution was prepared by dissolving 6.94 g Yb(NO₃)₃.6H₂O/l water to give a Yb concentration of 2.77 g/l.

Infusate was prepared by mixing together 57.6 g each of CrEDTA and Yb solutions with 196.8 g water. This was infused into the rumen via the permanent cannula using a multichannel peristaltic pump at a constant rate of 13 ml/h. The infusion rate supplied 160 mg Cr and 160 mg Yb/kg dry matter intake (DMI). A priming dose of 150 ml infusate was put into the rumen before the commencement of infusion to bring the marker concentrations in the rumen close to their final equilibrium concentrations (Faichney, 1975).

Digestibility measurements

Total daily faecal output was collected into polyethylene bags attached to the sheep. Faeces were dried at 50° and bulked for each animal over the 5 d faecal collection period.

Urine was collected with a rubber cap fitted over the prepuccial fold and held in position with the aid of a light webbing harness. A rubber tube led from the cup into a receptacle containing 6 M-hydrochloric acid to maintain pH between 2 and 2.5. A 10% portion of each daily output was sampled and bulked for each animal over 5 d and stored at -10° for N analysis.

Sampling and handling of rumen and abomasal digesta

A total of twelve rumen and abomasal samples each were collected over the 3 d digesta collection period. Collections were made at intervals distributed throughout the 24 h cycle. Samplings were made at 08.30, 14.30, 20.30 and 02.30 hours on day 20; at 10.30, 16.30,

22.30 and 04.30 hours on day 21 and at 12.30, 18.30, 00.30 and 06.30 hours on day 22. A rumen sample (about 85 ml) was removed each time with a suction pump and filtered through a nylon gauze to remove coarse particles. The remaining bulked rumen fluid was kept at 5° for bacterial isolation after being acidified to pH 2.

About 80 ml abomasal digesta were obtained at each sampling. The bulked sample was stored at -10°.

Dilution rate and rumen pool measurement

Seven separate rumen samples were collected at 90 min intervals after stopping the infusion pump (see p. 558). Total rumen samples were taken each time from four different sites of the rumen, mixed together and a 200 g subsample taken and stored at -10° for dry matter (DM), Cr and Yb assay.

Rumen pool size was determined by manually emptying the entire rumen contents at the end of each period. Digesta were weighed, mixed, subsampled, stored at -10° and later analysed for DM and lignin.

Estimation of bacterial N 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 value for each sample calculated.

Chemical analyses

Abomasal samples were centrifuged at 2400 g for 20 min to obtain liquid-rich fractions. Feed, faeces, rumen, total abomasal samples and corresponding liquid-rich fractions were analysed for DM by drying in a forced-draught oven to a constant weight at 50°. Organic matter (OM) was determined as DM minus the residual ash obtained after heating at 550° for 16 h. N was analysed by a micro-Kjeldahl technique. Acid-detergent lignin and neutral-detergent fibre were determined by the method of Goering & Van Soest (1970). Cr and Yb concentrations in faeces, rumen and abomasal samples were measured by atomic absorption spectrophotometry. DAPA in whole digesta and bacterial fraction was determined using ion-exchange chromatography (TSM Amino Acid Autoanalyzer; Technicon Equipment Ltd, Sydney).

Calculations

Nylon bag N degradability estimates. The relation between percentage N degradation (\bar{P}) and period of incubation (t ; h) was described by the exponential equation:

$$\bar{P} = a + b(1 - e^{-ct}) \quad (\text{Ørskov \& McDonald, 1979}), \quad (1)$$

where a , b and c are constants fitted by an iterative least-squares procedure so as to estimate the rapidly-soluble fraction (a), the degradable fraction (b) and the rate-constant (c) for the degradation of b .

Effective degradation of supplements. Before the constants for N degradation measured with nylon bags can be applied *in vivo*, it is necessary to predict the fractional outflow rate (FOR) of the N source from the rumen. Three different FOR values of undegraded dietary protein from the rumen used by Rooke & Armstrong (1983), k 0.02, 0.05 and 0.08 /h, were assumed and used to correct for the passage of proteins from the rumen of sheep. The second and third k values were similar to the average FOR of Yb and CrEDTA respectively measured in the present experiment (Table 3). Using the constants a , b and c from the nylon-bag estimates of degradability and the assumed k values, the effective N degradation (D) was calculated from the equation:

$$D = a + bc/(c + k) \quad (\text{Ørskov \& McDonald, 1979}). \quad (2)$$

Outflow rates. Lignin turnover rates from the rumen were estimated from the ratio, daily lignin intake:lignin concentration in the rumen. Estimates of rumen FOR of CrEDTA and Yb were calculated from the slopes of dilution curves plotted from ln Cr and Yb concentrations respectively *v.* time (Shipley & Clark, 1972).

Digesta flow. The true composition of digesta was mathematically reconstituted from Cr and lignin concentrations in total digesta and in liquid-rich fraction. The flow of digesta from abomasum was corrected to 100% recovery of daily dose Cr input and calculated by using eqns (1)–(3) from Faichney (1975).

Bacterial N flows. 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; and bacterial N flow calculated by multiplying total digesta N flow by the proportion of bacterial N in digesta.

OM digestibility. OM apparently digested in the rumen (OMADR) was considered equal to OM intake minus abomasal OM flow. Percentage digestible OMADR was calculated by expressing OMADR as a percentage of OM apparently digested in the entire gastrointestinal tract.

Supplemental N escape. Feed plus endogenous N passage to the small intestine was assumed equal to total N leaving abomasum minus bacterial N components. Escape of supplemental N was calculated as the difference in abomasal passage of feed plus endogenous N between test and control diets (Zinn & Owens, 1983), and *in vivo* degradation of N in the supplements (*V*) calculated as the difference between intake and escape of supplemental N.

Statistical analysis

Values pertaining to digesta flow and digestibility studies were subjected to an analysis of variance for two 3×3 Latin squares. Interactions between treatments and squares were not significant and the effect of treatment was tested against the residual mean square (4 df). Treatment means were compared on the basis of the least significant difference (Steel & Torrie, 1980). Variations in the constants of eqn (1) pertaining to nylon-bag N-degradation measurements were analysed as a randomized complete block design with subsamples. The coefficient of variation (CV) for between-bags within a treatment and a sheep, was calculated from the subsampling error mean square. The CV for experimental error was calculated from residual error mean square (see Amaning-Kwarteng *et al.* 1986).

RESULTS

Supplemental protein degradation

The constants relating to nylon-bag degradation of the two supplements are shown in Table 1. The percentage of potentially-degradable N ($a+b$) was 84 for CSM and 97 for B. Of this degradable N, about 15 and 3% were instantly soluble from CSM and B respectively. CSM appeared to be degraded at a faster rate (32%) than B although this difference was not significant ($P > 0.05$). *In vivo* degradation of the supplements was high with B degradation being 14% higher than CSM (Table 5).

Effective degradability (*D*) values calculated for CSM and B are compared with corresponding estimated *in vivo* (*V*) degradation in Table 2. *D* values based on values for *k* of 0.05 and 0.08 underestimated the *V* values. They were 13 and 24% respectively for CSM and 23 and 36% respectively for B, lower than the corresponding *V* values. When it was assumed that *k* is 0.02, however, estimates of *D* were similar to the *V* values. Both techniques ranked the degradabilities of the two supplements in the same order.

Table 1. Constants in equation $\bar{P} = a + b(1 - e^{-ct})$, where \bar{P} is percentage of nitrogen degraded after time t (h) from cotton-seed meal and barley incubated in nylon bags within the rumen of sheep given sodium hydroxide-treated straw (at $0.9 \times$ ad lib. intake) plus 150 g mixed supplement†/d

(Values are means of three sheep \times three bags/sheep; n 9)

	Coefficients			100 - (a + b)
	<i>a</i>	<i>b</i>	<i>c</i>	
	Soluble	Degradable	Fractional degradation	Undegradable
Cotton-seed meal	12.5	71.4	0.112	16.1
Barley	2.8	94.6	0.085	2.6
SED (2 df)	0.45	4.32	0.0103	1.03
Statistical significance of difference	*	*	NS	*
Coefficient of variation (%)				
Between bags within treatment and sheep	1.97	0.13	11.28	12.01
Experimental error	12.60	11.10	22.29	23.49

SED, Standard error of difference based on experimental error mean square; NS, not significant.

* $P < 0.05$.

† A mixture of 500 g cotton-seed meal and 500 g barley/kg.

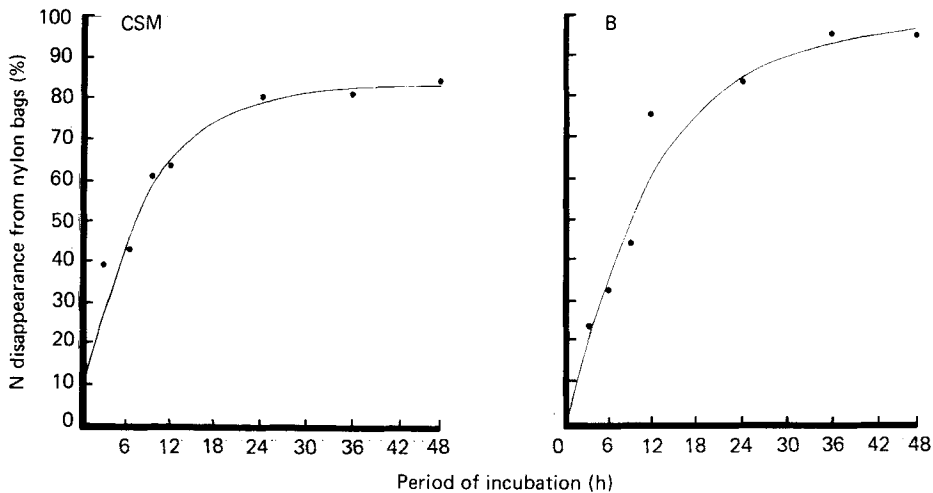


Fig. 1. Disappearance of nitrogen from cotton-seed meal (CSM) and barley (B) incubated in nylon bags in the rumen of sheep given sodium hydroxide-treated straw plus 150 g of a mixed supplement (see p. 558) over 48-h periods. The lines shown were drawn from fitted equations of the form $\bar{P} = a + b(1 - e^{-ct})$, where \bar{P} is the percentage N which disappeared from the bag at time t (h); a , b , c are constants using the coefficients given in Table 1. Values are means of three sheep \times three bags per sheep. For details, see Ørskov & McDonald (1979).

Table 2. A comparison between calculated effective degradability (D^*) and estimated *in vivo* degradation (V) in sheep given sodium hydroxide-treated straw plus 150 g cotton-seed meal (CSM) or barley (B)/d

Supplement ...	CSM	B
Calculated D values		
Fractional outflow rates (/h)†		
k 0.02	73.1	79.4
k 0.05	61.9	62.4
k 0.08	54.2	51.5
Estimated (V) values (Table 5)	70.9	80.8

* $D = a + bc/(c + k)$, using constants in Table 1.

† Rates suggested by Rooke & Armstrong (1983).

Table 3. Dry matter intake (DMI), rumen contents and fractional outflow rates (FOR) from the rumen of sheep given sodium hydroxide-treated straw alone (W) or with supplements of cotton-seed meal (WC) or barley (WB)

(Values are means of six sheep per treatment)

Diet ...	W	WC	WB	SED (4 df)
Straw DMI (g/d)	904	901	882	33.5
Total DMI	904 ^a	1041 ^b	1022 ^b	33.8
Total rumen dry matter	677 ^a	797 ^b	769 ^b	28.8
Total rumen liquid (l)	4.52	5.10	4.53	0.297
FOR (/h)				
Lignin	0.033	0.032	0.034	0.0041
Ytterbium	0.052	0.050	0.046	0.0031
CrEDTA	0.091 ^a	0.086 ^b	0.082 ^b	0.0018

^{a, b} Means in horizontal rows with unlike superscript letters were significantly different ($P < 0.05$).

Intakes, digestion and N retention

Effect of supplements on intake, rumen pool size and FOR from the rumen are given in Table 3; OM digestion and N retention in Table 4. The higher total DMI by supplemented sheep was associated with larger DM pools in the rumen of these sheep without any significant change in total OM leaving the rumen and FOR of lignin and Yb.

There was no significant change in OM digestibility of the diet as a whole when supplements were fed, but there was an increase in OMADR ($P < 0.05$).

Intake of total digestible OM and N retention were both increased by supplements (21 and 80% respectively by CSM and 24 and 39% respectively by B); the effect of B on N retention was, however, not significant.

Bacterial protein synthesis

DAPA concentrations in rumen bacteria isolated from sheep given supplements were 11 and 9% lower with CSM and B respectively than without supplements (Table 5). Both supplements increased the flow of bacterial N from the abomasum, but only CSM significantly increased ($P < 0.05$) efficiency of bacterial protein synthesis.

Table 4. *Organic matter (OM) digestion and nitrogen utilization in sheep given sodium hydroxide-treated straw alone (W) or with supplements of cotton-seed meal (WC) or barley (WB)*

(Values are means of six sheep per treatment)

Diet...	W	WC	WB	SED (4 df)
Total digesta leaving abomasum (kg/d)	15.7	14.6	13.5	2.13
OM (g/d)				
Ingested (g/d)	777 ^a	906 ^b	895 ^b	26.4
Leaving abomasum (g/d)	438	507	460	30.4
In faeces (g/d)	300	332	305	42.8
Apparently digested in rumen (OMADR)	339 ^a	399 ^b	435 ^c	10.8
Apparent digestibility (%)	61.4	63.4	65.9	3.15
Percentage of OM apparently digested in rumen	71.1	69.5	73.7	6.60
Digestible OM intake (g/d)	477 ^a	575 ^b	590 ^b	23.9
Digestible OM in dry matter (%)	52.8 ^a	55.2 ^b	57.7 ^b	0.74
N				
Ingested from straw (g/d)	17.0	16.7	16.6	0.57
Ingested from supplement (g/d)	—	9.60	2.60	—
Excreted in faeces (g/d)	4.72 ^a	5.99 ^b	4.99 ^{ab}	0.454
Excreted in urine (g/d)	9.91 ^a	16.10 ^b	10.92 ^a	0.908
Apparent digestibility (%)	72.3 ^a	77.3 ^b	74.0 ^{ab}	1.75
N retention (g/24 h)	2.37 ^a	4.27 ^b	3.29 ^a	0.342

^{a, b} Means in horizontal rows with unlike superscript letters were significantly different ($P < 0.05$).

DISCUSSION

Degradation of supplemental dietary protein

In a study involving heifers also eating NaOH-treated straw (K. Amaning-Kwarteng and R. C. Kellaway, unpublished results), we observed the *in vivo* degradation of a different batch of CSM to be 35.3%. The difference in degradation of the batches of CSM used in the cattle experiment and the present experiment could be partly due to species variation in rumen outflow rates as well as factors intrinsic to the supplements such as source, harvesting, storage and processing. Problems of measurement involved in the estimation of supplemental protein degradation could have, however, contributed substantially to the discrepancy in the degradability values. The undegraded portion of supplemental protein was, in both studies, not measured directly but calculated after partitioning the total N flow into bacterial, endogenous and dietary fractions. Since the N from the undegraded supplement was only a small fraction of the total N flow at the abomasum (3 and 15% for B and CSM respectively in the present study) and, given the limitations of the steps leading to this estimation (such as the fact that protozoal N contribution was not accounted for), the accuracy of this measurement is limited. For example, Harrison *et al.* (1979) estimated that protozoal N may contribute about 20% of the total microbial amino acids in duodenal digesta. If protozoal N contributed 10 or 20% of bacterial N then, using the same assumptions and calculations as in Table 5, the calculated *in vivo* degradabilities of the protein supplements would become 74 or 77% for CSM and 88 or 100% for B respectively.

In spite of the limitations and uncertainties inherent in the measurement, the *in vivo* technique identified differences in the degradability of CSM and B in the present experiment. The application of the *in vivo* method is, however, limited by being unsuitable for the assessment of large numbers of ingredients at a time. Also, the single degradation value

Table 5. Bacterial protein synthesis and *in vivo* supplemental protein degradation in sheep given sodium hydroxide-treated straw alone (W) or with supplements of cotton-seed meal (WC) or barley (WB)

(Values are means of six sheep per treatment)

Diet ...	W	WC	WB	SED (4 df)
Intakes				
Total N (g/d)	17.0 ^a	26.3 ^b	19.2 ^a	1.31
g N/kg organic matter apparently digested in rumen (OMADR)	50.1 ^a	65.9 ^b	44.1 ^a	4.32
Diaminopimelic acid concentration (mg/g N)				
Rumen bacteria	40.1 ^a	35.6 ^b	36.4 ^{ab}	1.55
Abomasal digesta	32.9 ^a	25.8 ^b	29.7 ^c	1.03
Percentage bacterial N in digesta N	82.0	72.5	81.6	4.27
Total N leaving abomasum (g/d)	13.5 ^a	19.3 ^b	16.3 ^c	0.95
Bacterial N leaving abomasum (g/d)	11.0 ^a	14.0 ^b	13.3 ^b	0.79
Bacterial yield (g N/kg OMADR)	32.4 ^a	35.6 ^b	30.9 ^a	1.05
Supplemental N degradation				
Dietary plus endogenous N leaving abomasum (g/d)*	2.5 ^a	5.3 ^b	3.0 ^{ab}	0.88
Supplemental N leaving abomasum (g/d)†	—	2.8	0.5	—
Supplemental N degradation (%)	—	70.9	80.8	—

a, b, c Means in horizontal rows with unlike superscript letters were significantly different ($P < 0.05$).

* Total N flow – bacterial N flow.

† Dietary plus endogenous N flow of supplemented diet minus dietary plus endogenous N flow of control diet.

placed on each material is applicable only to the particular feeding situation of the experiment.

The nylon-bag method also identified differences between CSM and B. A significantly larger proportion of CSM was predicted to escape rumen degradation [$100 - (a + b)$] than B. The results, which agree with the observations of Ørskov *et al.* (1981), showed that a protein with a smaller *c* and a larger *b* (as with B) degrades more slowly but, with time, will be degraded to a greater extent than a protein with a larger *c* and a smaller *b* (as with CSM) (see Fig. 1).

D values based on *k* values similar to the FOR of particulate matter and liquid phase of digesta underestimated degradation compared with the *in vivo* values. The use of a smaller *k* value, however, gave estimates of *D* similar to those determined *in vivo*. This suggests that the FOR of CSM and B might have been different from FOR of Yb and CrEDTA, i.e., the proteins might have moved independently of particulate matter and liquid phase of digesta. Mathers & Miller (1981) observed a similarity between the effective N degradation based on the nylon-bag method and observed *in vivo* measurements, but only when a *k* value of 0.046 was assumed, this value being substantially lower than their measured dilution rates. A possible additional source of the disparity between *D* and *V* values in the present experiment is the fact that by feeding a mixture of CSM and B during the nylon-bag measurements (see Table 1) there could have been an interaction between the two supplements which could have influenced the disappearance of N from the bags, but such an interaction would not have been present during the *in vivo* measurements. The large differences in protein degradation (based on both *in vivo* and nylon-bag measurements) observed in the present study emphasize that there cannot be a single value for degradability of protein supplements (Ganev *et al.* 1979; Hughes-Jones, 1979; Rooke & Armstrong, 1983).

Intake and digestion

Protein supplements either have a synergistic effect (when given at moderate levels) or a 'substitution' effect (when given above 25% of total DMI) on the intake of low-quality roughages (Crabtree & Williams, 1971). In situations where over 90% of ruminants are grazed on pasture, as in Australia (Weston & Hogan, 1973), it is ideal for a supplement to maintain or increase intake of low-quality forages. In the present study we examined the synergistic effects of CSM and B supplementation at about 13% of total DMI. Similar effects which have been measured in our laboratory were reviewed by Kellaway & Leibholz (1983). They reported that responses to dietary N supplements when intake of RDN was non-limiting showed a weighted (for animal numbers) mean of only 3.7% increase in forage intake ($P > 0.05$). In the present study supplements effected a non-significant depression ($P > 0.05$) of 0.3–2.4% in straw DMI. This supports the conclusion of Kellaway & Leibholz (1983) that when RDN is non-limiting, protein supplements given at moderate levels have negligible effects on roughage intake.

Protein supplements would enhance microbial activity in the rumen and hence enable the animal to consume more feed if essential nutrients, such as N and S, were limited in the rumen. The levels of RDN, S and other essential minerals sprayed on the alkali-treated straw were designed to provide optimal requirements for microbial growth (ARC, 1980). It was, however, assumed that requirements for preformed amino acids would be supplied by endogenous proteins (MacRae & Reeds, 1980), much of which would be accounted for as sloughed epithelial cells from the rumen wall (Kennedy & Milligan, 1980). If the availability of amino acids were a limitation to appetite, as suggested by Kempton & Leng (1979) and Kempton *et al.* (1979), and protein supplements increased total DMI by the supply of amino acids to rumen microbes, then the endogenous supply of protein could not have met the optimal value for non-protein-N:amino acid of 72:25 (Maeng *et al.* 1976) for microbial growth.

Weston (1979) described the factors associated with the removal of OM from the rumen as partly due to digestibility and absorption of fermentation products and partly due to propulsion of particulate matter from the reticulo-rumen. Our results suggest that supplements increased total OM intake by increasing digestion and absorption of fermentation products rather than OM flow from the rumen.

The ARC (1980) adopted a value of 65% for the percentage of digested OMADR for sheep and cattle. This value was derived from a wide range of diets. With low-quality forages, percentage OMADR values reported include 89 (Hunter & Siebert, 1980), 73 (ARC, 1980) and 77 (Sriskandarajah & Kellaway, 1984). Our observed values in the present experiment are within the reported range for low-quality forages. The digestible OM in dry matter (DOMD) values observed by us when NaOH-treated straw was given alone and with supplements are similar to the values reported by the Ministry of Agriculture, Fisheries and Food (1975) for hays from grasses of low and moderate digestibilities respectively. Sriskandarajah & Kellaway (1982) obtained higher values of 60–64% for cattle given NaOH-treated straw when calculation of DOMD was based on lignin ratio. This could partly be due to overestimation of faecal lignin, as Sriskandarajah & Kellaway (1982) dried faecal samples at 60°. Van Soest (1964) reported that feed lignin could be overestimated by 20% by drying at 60° compared with 50°.

Microbial protein synthesis

Concentrations of DAPA in both bacterial isolates and abomasal digesta decreased with supplementation. This agrees with the results of Whitelaw *et al.* (1984). Changes in DAPA concentration with change in diet is associated with changes in microbial population and

activity (El-Shazly *et al.* 1961; Campling, 1966; Hungate, 1966). Recent investigations have shown that differences in DAPA concentrations exist also between different animals in a given dietary situation (Dufva *et al.* 1982). Whitelaw *et al.* (1984) observed large between-diet, between-animals and within-animal variations in DAPA concentrations (17.2–37.3 and 11.3–40.4 mg DAPA/g N for digesta and bacterial isolates respectively). Much of the variations were attributed to unstable conditions within the rumen, which resulted in unrepresentative sampling. The reasonably high precision of DAPA concentrations observed in our study (CV 7.2% compared with 26.7% reported by Ling & Buttery (1978) for bacterial samples) may be attributable to the sampling schedule followed during this experiment (see p. 559).

The proportion of bacterial N in abomasal N also decreased with supplementation. When diets have a high RDN:undegraded dietary N (UDN) supply, a high proportion of abomasal N is of microbial origin (Sriskandarajah & Kellaway, 1984). In this experiment the RDN:UDN supply was 9.87, 5.05 and 7.98 for W, WC and WB respectively. The corresponding values for percentage bacterial N in the abomasum were 82.0, 72.5 and 81.6, thus supporting the positive relation between the two factors. Variations in the reported proportions of microbial N in digesta N have also been associated with the microbial marker used. The usual explanation for the lower DAPA-based value compared with estimates based on RNA or ³⁵S is the inability of the DAPA method to estimate protozoal protein. Notable exceptions to the usual ranking of DAPA and ³⁵S are those reported by Siddons *et al.* (1979, 1982).

Bacterial N leaving the abomasum was significantly increased by supplementation. Teather *et al.* (1980) also observed a marked stimulatory effect on rumen bacterial populations *in vivo* in cattle given maize silage when soya-bean protein was supplied. Whether this effect was due to an additional supply of amino acid (or peptides) or of branched-chain volatile fatty acids to the rumen microbes or to some other factors is not easy to ascertain. Evidence by Gill & Beever (1982), however, suggested that the synthesis of microbial protein in silage-fed animals could be limited by low amino acid availability.

Wide ranges of efficiency of microbial protein synthesis values have been reported in the literature (for references, see ARC, 1980). For example, Hogan & Weston (1971) reported a range of 32–55 g microbial N/kg OMADR of sheep given ground NaOH-treated straw at three levels of intake. We observed a mean value of 33 g bacterial N/kg OMADR. With the inclusion of protozoal N the mean value would become 36 or 39 g microbial N/kg OMADR if protozoal N were assumed to be 10 or 20% respectively of bacterial N. Tamminga (1979), Theurer (1982) and Sriskandarajah & Kellaway (1984) have discussed the relative importance of other factors such as digesta flow marker, isolation technique, feed intake, dietary N concentration or sources and feed ingredient processing on efficiency of microbial N synthesis.

N utilization

N economy of all diets showed a positive daily N balance. The significantly larger amount of N retained on WC compared with W and WB was probably a function of more efficient rumen production of bacterial protein or a greater proportion of dietary N escaping degradation in the rumen, or a combination of both. The results suggest that WC has the potential to support greater live-weight gains than W or B.

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