Rumen bacterial protein synthesis and the proportion of dietary protein escaping degradation in the rumen of sheep

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1. The effect of supplementing barley diets with urea (U), extracted, decorticated groundnut meal (GNM) or Peruvian fish meal (PFM) on rumen bacterial protein synthesis and the proportion of undegraded food protein passing to the duodenum of sheep has been examined.

2. Three wethers were given isonitrogenous, isoenergetic diets containing (g/kg dry matter (DM)): U 20, GNM 106 or PFM 78, the crude protein (nitrogen × 6·25) contents being 139, 145 and 148 respectively. The sheep were fed hourly, the mean daily intake of DM being 0·634 kg.

3. Rumen bacterial protein synthesis was determined using ^{35}S and diaminopimelic acid (DAPA) as bacterial markers and polyethylene glycol (PEG) and chromic oxide as markers of digesta flow. Rumen volatile fatty acid (VFA) production rate was determined by a continuous infusion of $[1\text{-}^{14}C]$acetate.

4. ^{35}S and DAPA gave similar estimates of the proportion of bacterial N in the trichloroacetic acid-precipitable nitrogen of the rumen digesta, the mean value being 0·86. The VFA production rate did not vary significantly between diets, the mean being 5·8 mol/24 h. The flow of bacterial N from the rumen was calculated from the PEG and Cr_2O_3 estimates of flow and the ^{35}S and DAPA estimates of the proportion of bacterial N in the rumen. ^{35}S and DAPA gave similar values (mean 12·5 g/24 h) and Cr_2O_3 gave a slightly lower value (11·5 g/24 h) than PEG (13·5 g/24 h). Dietary effects, averaged over the four methods, were not significant; the values were 13·0, 13·4 and 11·0 g/24 h for the U, GNM and PFM diets respectively.

5. Duodenal samples were taken from two 12 h continuous collections from re-entrant cannulas and the DM flow adjusted to total recovery of Cr_2O_3. The mean recovery of Cr_2O_3 at the duodenum was 0·798. The rates of flow of DM were 0·296, 0·311 and 0·334 kg/24 h and of non-ammonia-N (NAN) 13·5, 15·2 and 15·4 g/24 h on the U, GNM and PFM diets respectively.

6. The concentrations of the essential amino acids in duodenal digesta were generally higher with the PFM diet than with either of the other two diets. The flow of most amino acids through the duodenum was generally higher on the PFM and GNM diets than on the U diet.

7. The energetic efficiency of bacterial protein synthesis was calculated to be 2·1 g bacterial N/mol VFA or 28 g bacterial N/kg organic matter fermented in the rumen.

8. From the estimates of bacterial N flow from the rumen and NAN flow through the duodenum it was calculated that 0·22 and 0·69 of the supplemental N from GNM and PFM respectively passed through the rumen undegraded.

The amino acids presented to the small intestine of the ruminant animal are derived mainly from microbial protein synthesized in the rumen and dietary protein that has escaped degradation in the rumen. The synthesis of microbial protein is limited by the amount of energy made available by anaerobic fermentation (Walker, 1965). The rate of degradation of a protein in the rumen and conversely the residual contribution to amino acids reaching the duodenum, was shown by McDonald (1952) and Henderickx & Martin (1963) to be related to its solubility. Although microbial protein appears to be of relatively constant amino acid composition (Purser & Buechler, 1966), variations in the amount of dietary proteins reaching the duodenum would be expected to cause some changes in the amino acid pattern presented for absorption (Little *et al.* 1968).

Growth trials and nitrogen balance studies have shown Peruvian fish meal (PFM) to be superior to either extracted, decorticated groundnut meal (GNM) or urea (U) (Whitelaw

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Table 1. *Composition (g/kg dry matter) of diets containing supplements of urea (U), extracted, decorticated groundnut meal (GNM) or Peruvian fish meal (PFM)*

	U	GNM	PFM
Rolled barley	697	711	720
Chopped barley straw	100	100	100
Molassine meal*	50	50	50
Urea	20	—	—
Groundnut meal	—	106	—
Peruvian fish meal	—	—	78
Maize starch	76	—	—
Milled barley straw	24	—	24
Vitamins and minerals†	30	30	25
Chromic oxide	3	3	3

* Molasses-sphagnum moss (75:25, w/w); The Molassine Company Ltd, London SE1D 0PU.

† The vitamin–mineral mixture contained (g/kg): NaCl 150, limestone flour 250, $CaHPO_4$ 250, MgO 40, $FeSO_4.2H_2O$ 5, ZnO 3, $CoSO_4.7H_2O$ 0·1, Na_2MoO_4 1·0, stabilized vitamin A equivalent to retinol 0·105 and (mg/kg) stabilized vitamin D_3 equivalent to cholecalciferol 1·25 and stabilized vitamin E supplying DL α-tocopheryl acetate 40. The mix was made to 1 kg with toasted cereal.

et al. 1961; Miller, 1968). These nitrogen supplements differ widely both in solubility in 1 M-sodium chloride and in amino acid composition. In a comparison of herring meal and GNM, Whitelaw & Preston (1963) suggested that both solubility and amino acid composition were important factors influencing the utilization of N.

In this paper an experiment is described in which an attempt has been made to quantify the importance of these two factors. Isonitrogenous supplements of U, extracted decorticated GNM and PFM to a barley-based diet were compared for their effect on microbial protein synthesis and passage of amino acids to the duodenum of sheep. A preliminary report of part of this experiment was given by Miller (1973).

EXPERIMENTAL

Animals and diets

Three Finnish Landrace × Clun wethers which had been early weaned and given an all-concentrate barley diet in an isolated environment to prevent the establishment of protozoa in their rumens were fitted with ruminal and re-entrant duodenal cannulas (Bruce *et al.* 1966). Significant populations of ciliate protozoa reappeared in the rumen at intervals during the experiment but were effectively removed by treatment with dioctyl sulpho-succinate (Abou Akkada *et al.* 1968).

The diets were designed to be isonitrogenous and isoenergetic and to be of approximately equal fibre content. Chopped barley straw was included to stimulate the flow of digesta to the small intestine (Phillips & Dyck, 1964). The composition of each diet is shown in Table 1. The pelleted diets were fed hourly, the mean daily intakes of dry matter (DM) being 637, 631 and 635 g and of N 14·3, 14·8 and 15·2 g on the U-, GNM- and PFM- supplemented diets respectively.

Experimental design and sampling procedures

Design. The three diets were given to each sheep in turn according to a randomized block design. The sequence of treatments was allocated at random to each sheep separately. The sequences were: sheep 85 GNM, PFM, U; sheep 110 U, PFM, GNM; sheep 175 U, GNM, PFM. Each experimental period lasted 14 d and was preceded by a 15 d dietary adaptation period.

Digestibility. The apparent digestibilities of DM, gross energy and N were determined from faecal collections over the first 5 d of the experimental period.

Volatile fatty acid (VFA) production rate. This was determined by the method of Leng & Leonard (1965) on both days 6 and 7. A solution containing [1-^{14}C]acetate (60 μCi, 60 μmol/l) was infused into the rumen at 9 ml/h for 7 h after a priming dose of 10 ml. Six samples of rumen digesta were obtained over the final 3 h of each infusion and were analysed for DM, VFA concentration, VFA specific radioactivity, VFA ratio and ammonia concentration.

Rumen turnover and bacterial protein synthesis. Rumen fluid turnover rate was determined by a continuous infusion of polyethylene glycol (PEG, molecular weight 4000) and rumen microbial crude protein (N × 6·25) synthesis was estimated from the incorporation of infused ^{35}S (Roberts & Miller, 1969). From days 8 to 13 a solution containing 6 g PEG/l and 3·5–6·0 μCi $Na_2{}^{35}SO_4$/l was infused into the rumen at 40 ml/h. Four samples of approximately 500 ml rumen digesta were obtained at 3·5 h intervals on both days 10 and 11 using a vacuum pump. Each sample was mixed and subsampled and the remainder rapidly returned to the rumen. These samples were analysed for DM and PEG and were also used to prepare a microbial fraction and trichloroacetic acid (TCA)-precipitated fraction, both of which were analysed for N, radioactivity and diaminopimelic acid (DAPA). The TCA precipitate was also analysed for chromic oxide.

Preparation of bacterial fractions and TCA precipitates. Rumen contents were centrifuged at 2000 g for 10 min. The precipitate was washed with 10 ml physiological saline (9 g NaCl/l and centrifuged again. The combined supernatant fractions were then centrifuged at 22000 g for 20 min and the resultant bacterial pellet was washed once with 5 ml water, once with 5 ml acetone and then dried at 80°. TCA-precipitated fractions were prepared by adding TCA to rumen contents to give a final concentration of 100 g TCA/l. This was centrifuged at 2000 g for 10 min and the precipitate washed twice with 5 ml water and once with 5 ml acetone before being dried at 80°.

Duodenal collections. Collection of duodenal digesta was carried out for 12 h periods on both days 12 and 13 by a manual technique similar to that described by Bruce *et al.* (1966). Every 2 h a sample representing 0·05 of the total digesta flow by volume was obtained. This was replaced by an equal volume of physiological saline and the digesta returned through the distal cannula over the succeeding 2 h period. The digesta returned to the duodenum during the first 2 h period had been collected over a similar period on the preceding day and stored overnight at −20°. For each 12 h collection period the samples were bulked to give one sample for each consecutive 4 h period and a further one sample representing the whole 12 h period. Four samples of rumen fluid were also taken at 4 h intervals for pH measurement. The 4 h samples of duodenal digesta were immediately analysed for NH_3. The remainder was dried in a vacuum oven at ambient temperature before analysis for N, Cr_2O_3 and amino acids.

Analytical methods

N was determined by the method of Varley (1966), NH_3 by the Conway micro-diffusion technique described by McDonald (1948), Cr_2O_3 by the method described by Owen *et al.* (1967) adapted to 1:5 scale for digesta samples, and PEG by the method of Hydén (1955).

VFA concentration and specific radioactivity. The total VFA concentration was determined by steam distillation and titration to pH 7·0 with 0·01 M-sodium hydroxide using an autotitrator and glass electrode (Pye, Cambridge) as described by Owen *et al.* (1968). When the titration was completed an additional 0·1 of the titre was added, the solution was evaporated under reduced pressure and the salts of the VFA were transferred to a scintillation vial and dried in a vacuum oven. Distilled water (1 ml) and scintillation fluid (15 ml of

a solution of 10 g 2-(4-tert-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole (butyl-PBD) in 1 l toluene and 500 ml Triton X-100) were added and the radioactivity was determined in a spectrometer (Tri-Carb model 3320; Packard Instrument Ltd, Wembley, Middx) using an external standard for quench correction. Total VFA production rate was calculated from the ratio, radioactivity infused:radioactivity/mol total VFA.

VFA molar ratio. This was determined by the method of Erwin *et al.* (1961) modified as described by Owen *et al.* (1968) and responses were integrated using a Chromalog Type I (Kent Instruments Ltd, Luton, Beds).

^{35}S. Between 0·02 and 0·05 g sample in a scintillation vial was oxidized by the method of Jeffay *et al.* (1960). The vials were placed in an electrically-heated aluminium block and the temperature gradually raised to 260–280°. The residual sulphate ash was dissolved in 1 ml water and mixed with 15 ml scintillation fluid.

Amino acids. Samples were hydrolysed for 24 h under reflux, in constant-boiling hydrochloric acid with continuous passage of N through the solution. Norleucine was used as an internal standard and the analyses were made on an amino acid analyser Model BC-200 (Bio-Cal Instruments, St Albans, Herts).

DAPA. This was analysed by a method similar to that of Hutton *et al.* (1971). Samples containing 0·04–0·5 mg DAPA were hydrolysed in 10 ml 6 M-HCl in firmly-capped McCartney bottles at 110° for 24 h. The filtered hydrolysate was evaporated to a very small volume under reduced pressure, washed three times with water to lower the HCl concentration and finally made to 10 ml with 0·2 M-sodium citrate buffer, pH 2·0. DAPA (in 0·5–4·0 ml portions) was separated on a 200 × 9 mm column of Amberlite CG 120 mesh II (BDH Ltd Poole, Dorset) at 46° using 2 M-citrate buffer, pH 3·66 and determined with acid ninhydrin reagent using a continuous flow method. The extinction was determined at 418 nm in a Vitatron recording colorimeter fitted with a logarithmic converter and integrator (Fisons Ltd Loughborough, Leics). Recovery of known amounts of DAPA added to both bacterial and TCA-precipitated digesta preparations averaged 95·5 ± 1·5%. Since the recovery was similar for both types of material and the ratio, DAPA in the TCA-precipitate:DAPA in the bacterial preparation, was unaffected by these small losses, no correction was made.

RESULTS

The results were analysed statistically by analysis of variance with complete partitioning of the sources of variation. The test for significance of a main effect was done by the *F* test against the error mean square computed by combining the appropriate interaction sums of squares which contained the random variable (sheep) and according to the rule described by Sokal & Rohlf (1969). Differences between means within a main effect were tested for significance by Duncan's multiple range test as modified by Harter (1960). The analyses of variance of fractional values were carried out on their angular transformations. Where dietary effects were tested *v.* sheep × diet interaction mean square with only four degrees of freedom a significance level of $P = 0·10$ was adopted to distinguish between a true and a random effect.

Rumen metabolism

Rumen pH. Within each day the pH varied significantly ($P < 0·001$) with time of sampling, the mean values with their standard error averaged over all days for the four sampling times (on days 12 and 13) being 6·03, 5·99, 5·92 and 5·89 (SEM ± 0·023, 51 df). The pH also varied more between the 2 d of sampling within each period ($P < 0·01$) than it did on any 1 d but there was no consistent bias to either the first or second day. The sheep × diet interaction was also greater than the variation due to day of sampling ($P < 0·01$) and was therefore used

Table 2. *Effect of diets containing supplements of urea (U), groundnut meal (GNM) or Peruvian fish meal (PFM) on dry matter (DM) and ammonia-N content of rumen digesta, flow of digesta and trichloroacetic acid-precipitable nitrogen (TCA-N) from the rumen and on VFA production rate in wethers*

Diet	DM content (g/kg)	Ammonia-N (mg/kg)	Liquid flow from rumen (l/24 h)	DM flow from rumen (g/24 h)	TCA-N flow from rumen calculated from:		VFA concentration (mmol/kg)	VFA production rate (mol/24 h)
					Liquid flow (g/24 h)	DM flow (g/24 h)		
U	118	251	5·26	341	16·8	11·6	61	6·8
GNM	110	146	4·69	374	16·1	15·1	55	5·3
PFM	135	223	3·52	314	15·7	13·6	60	5·5
SEM	8·3	42·4	0·536	14·6	3·07	0·69	7·1	0·33
df	4	4	4	4	4	4	4	9

for testing sheep and dietary effects. There were no significant differences in rumen pH between sheep or between diets, the over-all mean ($\pm$ SEM) being $5·96 \pm 0·048$, 4 df.

VFA ratios and production rates. As for pH, the molar ratios of acetic, propionic and butyric acid varied more between days than within days and the sheep × diet interaction was greater than the variation due to day of sampling. Diet effects tested *v.* sheep × diet interaction (4 df) were not significant. The mean ratios were acetic $0·59 \pm 0·025$, propionic $0·23 \pm 0·020$, butyric $0·14 \pm 0·011$ and higher acids $0·04$. The variation in VFA production rate attributable to the sheep × diet interaction was smaller than the variation due to day of measurement and a pooled estimate of variation with 9 df was obtained. The VFA production rate was greater with the U diet than with either the GNM or PFM diets ($P < 0·05$). The values are given in Table 2.

Rumen ammonia concentration. Variation attributable to the sheep × diet interaction was greater ($P < 0·001$) than that due to day of sampling. Diet effects, tested *v.* sheep × diet interaction (4 df) were not significant. The mean value for each diet is shown in Table 2.

DAPA content of microbial preparations. The variation attributable to the sheep × diet interaction was greater ($P < 0·001$) than variation between samples obtained either on successive days or within a day. No consistent effect of sheep or diet could be discerned and the mean value was 33 mg DAPA/g bacterial N with a coefficient of variation, based on the sheep × diet interaction term, of $21·9 \%$. Because of this variation, the proportion of bacterial N in the total digesta N was calculated for each sample of digesta and not from the average DAPA content. Furthermore, the DAPA content of the bacterial preparation differed considerably from values (mg DAPA/g N) reported in the literature, e.g. 41 (Hogan & Weston, 1970), 48 (Chamberlain *et al.* 1976), 52 (Hutton *et al.* 1971) 34 to 71 (Ling & Buttery, 1978).

Proportion of TCA-precipitable N of bacterial origin. The values obtained by the three procedures are given in Table 3. All three methods indicated that a very high proportion of the N was present as bacteria. The values calculated using either ^{35}S or DAPA as a marker of bacterial cells in the rumen did not differ but the ^{35}S method was quicker, easier and the results more reproducible than the measurement of DAPA. Although there were no statistically significant dietary effects the lowest value was obtained with the PFM diet. The method involving determination of DAPA:N in the duodenal digesta gave some impossibly high values.

This may be accounted for if free DAPA, resulting from intra-ruminal lysis of bacteria,

Table 3. *The effect of (a) method of estimation and (b) dietary supplement of urea (U), groundnut meal (GNM) or Peruvian fish meal (PFM) on the proportion of trichloroacetic acid-precipitable nitrogen of bacterial origin in ruminal or duodenal digesta of wethers*

(Mean values with their standard errors)

(a) Method of estimation

	Bacterial marker		
	^{35}S in rumen	DAPA in rumen	DAPA in duodenum*
Mean	0·88	0·82	1·03
SEM	0·025	0·036	0·076
Coefficient of variation†	8·6	13·1	21·0
df	4	4	4

(b) Diet

U	GNM	PFM	SEM	df
0·93^a	0·85^{a,b}	0·78^b	0·038	4

a, b, Values with different superscripts differed significantly ($P < 0.10$). DAPA, diaminopimelic acid.
* Not used in comparison of dietary effects.
† Based on the variance attributable to the sheep × diet interaction (4 df) when the value for each sheep period was determined as the average of samples representative of 12 h periods on each of two successive days.

Table 4. *The effect of method of estimation and of dietary supplements of urea (U), groundnut meal (GNM) or Peruvian fish meal (PFM) on the flow of bacterial N from the rumen (g/24 h) of wethers*

Bacterial marker		SEM	df
^{35}S	DAPA		
12·88	12·06	1·007	22

Flow-rate marker		SEM	df
PEG	Chromic oxide		
13·46	11·48	1·007	22

Diet			SEM	df
U	GNM	PFM		
13·0	13·4	11·0	1·27	4

DAPA, diaminopimelic acid; PEG, polyethylene glycol.

was present in duodenal digesta. Nikolić & Jovanović (1973) have similarly reported higher DAPA:N values in some rumen digesta samples than in bacterial isolates. In addition the bacterial isolate was obtained from the rumen on days 10 and 11 whereas the duodenal digesta was obtained on days 12 and 13 of each period. Variation in DAPA concentration in the bacteria with time could contribute to the error. Results obtained by this procedure were not used in further calculations.

Flow-rates of liquid, DM *and TCA-N from the rumen.* As shown in Table 2, the PFM diet was associated with a high DM content in the rumen digesta and a low rate of passage from the rumen of both liquid and DM, determined respectively from rumen concentrations of PEG and Cr_2O_3. Multiplication of these flow-rates of liquid and DM from the rumen by

Table 5. *Flow-rate of digesta and dry matter* (DM) *through the duodenum of wethers during continuous 12 h collection*

(Mean values with their standard errors)

	0–2	2–4	4–6	6–8	8–10	10–12	SEM	df
			Period (h)					
Volume collected (ml/2 h)	514	286	299	386	275	326	30·6	85

	0–4	4–8	8–12	SEM	df
		Period (h)			
Volume collected (ml/4 h)	801	685	601	28·7	34
DM flow (g/4 h): absolute value	51·93	39·27	34·54	2·075	34
Value corrected for total recovery of chromic oxide	53·58	52·38	52·79	1·353	34

	1	2	SEM	df
		Day		
Volume collected (ml/12 h)	1953	2221	118·3	8
DM flow (g/12 h): absolute value	121·08	131·58	7·395	8
Value corrected for total recovery of chromic oxide	154·83	159·29	1·700	8

the concentration of TCA-N in the digesta gave the two estimates of TCA-N passing to the duodenum.

The values calculated from the DM flow-rate were lower and less variable than those calculated assuming all the N passed from the rumen at the same rate as the liquid. The flow rates of N from the rumen calculated from the Cr_2O_3 values were lower for the U diet than for the GNM diet ($P < 0.05$), the values for the PFM diet being intermediate between the other two.

Flow of bacterial N from the rumen. Four estimates were obtained by multiplying the two estimates of N flow by the two estimates of the proportion of N contributed by the bacteria. The values are given in Table 4. There were no differences attributable to the use of either bacterial marker; estimates based on DM flow were lower than those based on liquid flow; differences due to diets, averaged over the four methods of estimation, were not significant but the highest value obtained with the GNM diet was 1·21 times that of the lowest value obtained with the PFM diet.

Duodenal digesta

Duodenal pH. There were no significant differences within each day, between the 2 d of sampling within each period, or between the diets, the over-all mean ($\pm$ SEM) being 2·77 $\pm$ 0·027, 54 df.

Flow of digesta. The mean flow-rates of digesta through the duodenum during the 12 h continuous collections are shown in Table 5. The only significant variation in the volume of digesta collected occurred within days. The 4 h collection volumes decreased throughout the 12 h experiment ($P < 0.001$). In particular the flow-rate in the first 2 h collection was higher than in any subsequent 2 h period ($P < 0.01$). The movement of DM in the first 4 h collection was significantly higher ($P < 0.001$) than in either the 4–8 h or the 8–12 h collection but this significance was removed when each value was corrected for total recovery of the Cr_2O_3. The mean daily recovery of Cr_2O_3 did not vary significantly between sheep, between diets or between the 2 d of sampling within each period, the over-all mean being 0·798 with 95% confidence limits of 0·657–0·938 (10 df).

Table 6. *Effect of dietary supplements of urea (U), groundnut meal (GNM) or Peruvian fish meal (PFM) on the flow-rate (g/24 h) of dry matter (DM), nitrogen and non-ammonia-N (NAN) through the duodenum of wethers*

Diet ...	U	GNM	PFM	SEM	df
DM	296	311	334	15·8	4
N	14·2	15·8	16·0	0·58	8
NAN	13·5	15·2	15·4	0·58	8

Table 7. *Concentration of amino acids (g/16 g N) in the duodenal digesta of wethers given diets supplemented with urea (U), groundnut meal (GNM) or Peruvian fish meal (PFM)*

(Mean values of six observations with their standard errors)

Amino acid	Diet U	GNM	PFM	SEM	df	PFM v. U	PFM v. GNM	GNM v. U
Aspartic acid	10·85	10·33	10·74	0·626	4	NS	NS	NS
Threonine	5·15	5·72	5·88	0·165	10	**	NS	**
Serine	5·34	4·79	5·31	0·240	10	NS	NS	NS
Glutamic acid	11·89	12·69	11·99	0·730	10	NS	NS	NS
Proline	2·70	3·57	3·09	0·385	4	NS	NS	NS
Glycine	4·56	5·15	5·28	0·204	10	**	NS	*
Alanine	5·73	6·20	6·59	0·218	10	**	NS	NS
Cystine	1·05	1·26	1·15	0·121	10	NS	NS	NS
Valine	5·40	5·42	5·89	0·113	10	**	**	NS
Methionine	2·24	2·37	2·82	0·115	10	***	**	NS
Isoleucine	5·20	5·12	5·67	0·152	10	*	**	NS
Leucine	5·91	6·42	7·11	0·321	4	*	NS	NS
Tyrosine	4·54	4·64	4·66	0·158	10	NS	NS	NS
Phenylalanine	4·98	5·17	5·38	0·076	10	***	*	NS
Lysine	6·57	6·81	8·08	0·189	10	****	****	NS
Histidine	2·47	2·04	2·30	0·401	4	NS	NS	NS
Arginine	4·27	5·45	5·42	0·440	4	NS	NS	NS

The "Diet" heading spans the U, GNM, PFM columns; "Statistical significance of difference between diets:" spans the PFM v. U, PFM v. GNM, GNM v. U columns.

NS, not significant. $* P < 0.10$, $** P < 0.05$, $*** P < 0.01$, $**** P < 0.001$.

The mean quantities of DM, N and non-ammonia-N (NAN) which passed through the duodenum on the three diets are shown in Table 6. There were no significant dietary effects on the flow of DM or N but a slightly smaller quantity ($P < 0.05$) of NAN passed through the duodenum on the U diet than on either of the other two diets.

Concentration of amino acids. The concentration of each amino acid in the hydrolysed duodenal digesta on each diet is shown in Table 7. Each value is the mean of six 12 h samples. The large standard errors of the mean for aspartic acid, proline, leucine, histidine and arginine were due to the significant sheep × diet interactions. The duodenal concentrations of the essential amino acids were generally higher on the PFM diet than on either of the other two diets. Tryptophan was not measured.

Flow of amino acids through the duodenum. The flow of each amino acid through the duodenum on each diet is shown in Table 8. The sheep × diet interaction mean square was used as the error mean square for the tests for dietary effects on the flow of aspartic acid, serine, glutamic acid, proline, leucine, histidine and arginine; as a result of the high SEM in each instance the differences between diets were not statistically significant. The duodenal flow of each of the other amino acids was generally significantly higher on the PFM and GNM diets than on the U diet but in most instances there was a smaller difference between

Table 8. *Flow of amino acids (g/24 h) through the duodenum of wethers given diets supplemented with urea (U), groundnut meal (GNM) or Peruvian fish meal (PFM)*

(Mean values of six observations with their standard errors)

| | Diet | | | | | Statistical significance of difference between diets: | | |
Amino acid	U	GNM	PFM	SEM	df	PFM *v.* U	PFM *v.* GNM	GNM *v.* U
Aspartic acid	9·45	10·28	10·76	0·564	4	NS	NS	NS
Threonine	4·57	5·70	5·91	0·219	10	**	NS	**
Serine	4·76	4·76	5·35	0·396	4	NS	NS	NS
Glutamic acid	10·36	12·69	11·96	1·192	4	NS	NS	NS
Proline	2·48	3·58	3·17	0·533	4	NS	NS	NS
Glycine	4·02	5·12	5·28	0·165	10	****	NS	****
Alanine	5·07	6·15	6·61	0·221	10	****	NS	***
Cystine	0·90	1·25	1·13	0·130	10	NS	NS	NS
Valine	4·83	5·40	5·92	0·196	10	***	*	*
Methionine	2·00	2·38	2·83	0·158	10	***	*	NS
Isoleucine	4·66	5·08	5·69	0·248	10	**	NS	NS
Leucine	5·27	6·41	7·12	0·579	4	NS	NS	NS
Tyrosine	4·03	4·60	4·69	0·237	10	NS	NS	NS
Phenylalanine	4·45	5·13	5·38	0·213	10	**	NS	**
Lysine	5·85	6·77	7·96	0·300	10	****	**	*
Histidine	2·29	2·03	2·32	0·505	4	NS	NS	NS
Arginine	3·88	5·45	5·43	0·643	4	NS	NS	NS

NS, not significant.   * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$, **** $P < 0.001$.

Table 9. *Apparent digestibility of dry matter* (DM), *nitrogen and energy by wethers given diets supplemented with urea (U), groundnut meal (GNM) or Peruvian fish meal (PFM)*

(Mean values with their standard errors)

Diet ...	U	GNM	PFM	SEM	df
DM	0·781	0·765	0·759	0·0061	4
N	0·770	0·734	0·753	0·0065	4
Energy	0·761	0·773	0·762	0·0055	4

the PFM and GNM diets. However, significantly more valine ($P < 0.10$), methionine ($P < 0.10$) and lysine ($P < 0.05$) passed through the duodenum on the PFM diet than on the GNM diet.

Apparent digestibilities of DM, *N and energy.* The apparent digestibilities of DM, N and energy on the three diets are shown in Table 9. The apparent DM digestibility of the U diet was slightly higher ($P < 0.10$) than of the PFM diet and the apparent digestibility of N of the U diet was slightly higher ($P < 0.10$) than of the GNM diet.

DISCUSSION

The results for the apparent digestibility of N would seem to suggest the U diet to be superior to the GNM diet with the PFM diet intermediary. This order, however, does not reflect the relative values of these supplements in previous growth or N balance trials (Whitelaw *et al.* 1961; Miller, 1968). The detailed studies of the synthesis of microbial protein and passage of NAN and of amino acids to the duodenum provide an explanation for this discrepancy.

Rumen metabolism and estimates of the flow of bacterial N from the rumen. The lower proportion of bacterial N in the TCA-precipitable N of the rumen digesta of sheep given the PFM diet (Table 3) suggests that less PFM protein than GNM protein was degraded in

the rumen. The similarity of the values for the U and GNM diets suggest that the GNM protein was almost completely degraded. The DM flow-rate from the rumen (Table 2) and passage through the duodenum (Table 6) were determined on separate days from the Cr_2O_3: DM values in the rumen and duodenal digesta respectively. The estimated DM flow from the rumen averaged 1·09 times the duodenal flow. In part this may have been due to variation between days of measurement of flow from the rumen and through the duodenum. The most likely cause, however, was probably due to the sampling of rumen contents in which the Cr_2O_3:DM value was continuously changing. The difference was in the direction expected if the DM in the rumen was simultaneously undergoing fermentation to VFA, carbon dioxide and methane and also flowing to the duodenum. The average difference between the two methods of measuring DM flow-rate was 27 g/d which was equivalent to the presence in the rumen of a single hourly feed awaiting complete fermentation. It might be emphasized that the nature of the diet and method of feeding are considered important features of this method of obtaining estimates of DM flow from the Cr_2O_3:DM value in the rumen.

Bacterial cells are partly in suspension in the rumen fluid and partly closely associated with the solid phase that is undergoing fermentation. Assuming the bacteria pass from the rumen either with the liquid phase or with the solid phase gives two estimates of bacterial flow from the rumen which might be regarded as upper and lower limits respectively of the true situation (Table 4). The difference between the two values indicates the differential flow-rates of liquid and solid phases. Nevertheless, the two estimates are not very dissimilar and this again is probably due to the uniform nature of the digesta resulting from the type of diet and method of feeding. The best estimate of bacterial N flow was taken as the average of the values obtained using the two bacterial markers, ^{35}S and DAPA, and the two flow markers, PEG and Cr_2O_3 (Table 4).

Theoretically microbial growth in the rumen is limited by the amount of ATP liberated during the anaerobic fermentation. With in vitro studies Walker & Nader (1968) found microbial protein synthesis to be within the range 2·5–2·8 g N/mol theoretical yield of VFA. Hogan & Weston (1970) reported a mean bacterial yield in vivo with a variety of forage diets of 2·37 g N/mol VFA produced, the latter being estimated by isotope dilution. Walker et al. (1975), using ^{35}S, reported mean yields of 2·6 and 3·3 g microbial N/mol VFA for dried and fresh forages respectively. Kennedy & Milligan (1978), using ^{15}N, obtained yields of 2·6 to 3·6 g microbial N/mol VFA in sheep given brome grass pellets under warm and cold stress conditions respectively. We have used the same technique of infusing [^{14}C]acetate in order to measure the total VFA production rate. The assumptions involved and validation of the technique for roughage diets have been discussed by Leng (1970). However, the same assumptions may not be completely valid when the technique is applied to animals given concentrate diets (Boxall, 1971). Since we used defaunated animals our estimates of bacterial yield should be compared to other estimates of total microbial yield and, therefore, our estimates are expressed as microbial yield per mol VFA produced as shown in Table 10. The mean value of 2·1 g N/mol VFA is lower than the values reported for roughage diets. However, the VFA production rates in the present work, expressed as mol/kg of organic matter apparently digested in the rumen, were 18·9, 15·8 and 17·7 for U, GNM and PFM respectively. These values are considerably greater than corresponding values of 9·7 and 12·1 reported by Walker et al. (1975) for dried and fresh forages respectively, 12·2 for a flaked maize-dried grass diet (Harrison et al. 1975), 9·0 for grass silage (Beever et al. 1977) and 12·8 to 15·3 for pelleted brome grass (Kennedy & Milligan, 1978). Assuming the individual VFA are produced in proportion to their molar ratios in the rumen and applying stoichiometric relationships to the VFA production rates obtained in the present work, it is calculated that 648, 507 and 528 g anhydrous hexose equivalent should have been fermented on U, GNM and PFM diets respectively. Such values are considerably in excess of estimates

Table 10. *Calculated proportions of digestion apparently taking place in the rumen and relationship of microbial N flow from the rumen to estimates of energy supply in wethers given diets supplemented with urea (U), groundnut meal (GNM) or Peruvian fish meal (PFM)*

Diet ...	U	GNM	PFM
Proportion of DM digestion taking place before the duodenum	0·69	0·66	0·62
OM intake (g/24 h)	605	593	597
OM flow to duodenum (g/24 h)	246	257	287
Microbial OM flow to duodenum (g/24 h)	111	115	94
FOM in rumen (g/24 h)	470	451	404
Microbial N flow (g/24 h)	13·0	13·4	11·0
Microbial N (g/mol VFA)	1·9	2·4	2·1
Microbial N (g/kg FOM)	28	30	27

DM, dry matter; OM, organic matter; FOM, fermented organic matter.

of the amount of organic matter fermented (FOM) in the rumen (Table 10). The VFA production values must be regarded as unrealistically high and, therefore, the microbial yield/mol VFA as an underestimate. An alternative approach is to relate the flow of microbial N from the rumen to the FOM in the rumen. Allen & Miller (1976), reviewing published values concluded that total microbial yield averaged 32·6 g N/kg FOM while bacterial yield, estimated from DAPA in the presence of unknown quantities of protozoa, averaged from 25 to 28 g N/kg FOM for diets ranging from all concentrates to all roughages. Their own maximum microbial yield, on diets of barley-starch-U similar to those used in the current experiment, was estimated as 30 g N/kg FOM. Although the digestion of ash was not measured in the present experiment, reasonable approximations can be made from the results of Ørskov *et al.* (1971 *a, b*) and these were used to calculate the organic matter (OM) digested in the rumen. It was assumed that the ash content (g/kg) of the U diet was 50 and of the GNM and PFM diets was 60. The quantities of ash passing to the duodenum expressed as proportions of the dietary intakes were assumed to be 1·57, 1·42 and 1·26 for the U, GNM and PFM diets respectively. A correction was made for the contribution of microbial OM to the total OM passing through the duodenum by assuming that bacteria contain 117 g N/kg OM (Lindsay & Hogan, 1972). The calculations are shown in Table 10. The mean value of 28 g microbial N/kg FOM is a little lower than the mean of 32·6 in literature reviewed by Allen & Miller (1976). However, it is not as low as other values reported for high cereal diets. McMeniman *et al.* (1976), using RNA as the marker, reported a mean value of 22, while Ørskov *et al.* (1972) and Chamberlain *et al.* (1976), both using DAPA, reported yields of 24·9 and 18·3 g N/kg FOM respectively. The higher yields in the present study with high cereal diets may be due to the lack of protozoa and a reduced predation of bacteria. Alternatively N, or other factors, may have been limiting microbial growth in some of the other studies (McMeniman *et al.* 1976; Chamberlain *et al.* 1976). In the present study the rumen ammonia concentrations appeared to be adequate compared to estimated requirements (Satter & Slyter, 1974; Allen & Miller, 1976).

Compared with mainly roughage based diets, the slightly lower microbial yield obtained in this study may be a result of the low flow-rate of digesta (average 4·4 l/24 h) with the concentrate diets. In an experiment with synthetic diets where the average flow of digesta was 7·3 l/24 h Hume & Bird (1970) reported a mean value of 30 g microbial N/kg FOM. Low flow-rates of digesta from the rumen are probably associated with increased recycling of NH_3 within the rumen (Nolan & Leng, 1972) and the apparent increases in the yield of bacterial protein obtained by the continuous infusion of water (Roberts & Miller, 1969) or salt solutions (Harrison *et al.* 1975) into the rumen of sheep may well be the result of a reduction in the degradation of micro-organisms in the rumen.

Flow of N and amino acids through the duodenum. The variable volumes of digesta collected at the duodenum during the course of 12 h, despite hourly feeding, is a common finding associated with manual methods of collection and return of digesta (Hogan & Phillipson, 1960; MacRae & Armstrong, 1969). Correction of flow of DM for 100% recovery of Cr_2O_3 gave constancy of flow-rate over the 12 h period. The depressed flow-rate may affect some constituents more than others by, for example, prolonging the period of time spent under-going fermentation in the rumen. There is also the possibility that solid and liquid phases move at different rates through the abomasum and small intestine and the use of Cr_2O_3 may result in an underestimate of the flow of dissolved or finely divided DM moving with the liquid phase. However, MacRae *et al.* (1972) using chopped-grass diets and a similar collection technique observed highly-significant correlations between the flow of Cr_2O_3 and the flow of DM, OM, N, cellulose, hemicellulose and gross energy to the duodenum and ileum of sheep.

The quantities of NAN reaching the small intestine expressed relative to N intake were 0·95, 1·02 and 1·02 for the U, GNM and PFM diets respectively. The small effect of the protein supplement compared to the U supplement and the failure to find any effect of feeding the insoluble PFM compared to the soluble GNM were unexpected. Further experiments with very much higher food intakes approaching *ad lib.* conditions have revealed a marked difference in NAN flow on supplementing a basal diet with PFM and none on adding U (see Miller, 1973). The failure to demonstrate a large effect in this experiment is probably due to the low feeding level and the low flow-rate through the rumen.

The amino acid composition of the duodenal digesta and the flow of amino acids through the duodenum showed that the supplements were not without effect. The most striking differences between the amino acid compositions of GNM and PFM are the greater amounts of methionine, lysine, threonine and alanine in PFM and of glutamic acid, cystine and arginine in GNM. For the most part these differences were reflected in the amino acid composition of the duodenal digesta, especially when the PFM diet was fed and less so for the GNM diet.

Calculation of proportion of supplementary protein escaping degradation in the rumen. Subtraction of the estimated bacterial N flow from the rumen from the NAN flow through the duodenum leaves 0·5, 1·8 and 4·4 g N/d on the U, GNM and PFM diets respectively which is assumed to represent abomasal secretions of N and dietary N escaping fermentation. Assuming contributions of abomasal secretions and undegraded N from the common dietary components equal to the 0·5 g N/d observed with the U diet, 1·3 g/d of GNM-N and 3·9 g/d PFM-N escaped fermentation. Expressed relative to the supplemental N fed, 0·22 and 0·69 of the GNM-N and PFM-N respectively passed to the duodenum. The similarity of NAN flow to the duodenum on the GNM and PFM diets is accounted for by an extra 2·4 g bacterial N/d on the GNM diet compared to an extra 2·6 g N from dietary protein with the PFM diet. These results agree well with those of Hume (1974) who gave sheep semi-purified diets supplemented with, among other protein sources, fish meal and peanut meal and found that 0·27 and 0·71 of the peanut meal- and fish meal-N respectively passed to the duodenum.

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