Protein nutrition of growing lambs

1. Responses in growth and rumen function to supplementation of a low-protein-cellulosic diet with either urea, casein or formaldehyde-treated casein

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1. The effects of supplementation of a cellulose-based diet with either urea, casein or formaldehyde-treated (HCHO)-casein were studied in growing lambs. Responses were measured in terms of growth rate, food intake and food conversion ratio.

2. In Expt 1 lambs were given free access to a basal diet containing (g/kg) oat-hulls (700) and solka floc (300) (containing 5 g nitrogen/kg dry matter (DM)) supplemented (g/kg basal diet) with either urea (25), untreated casein (75), HCHO-casein (75) or combinations of these. Food intake was increased on average by 27% above that on the basal diet by the addition of either urea, casein, HCHO-casein plus urea. Urea plus HCHO-casein when given as a combined supplement further increased food intake on average by 60% above that on the basal diet. Supplements of either urea, casein, HCHO-casein or casein or casein plus urea changed a mean live-weight loss of 40 g/d on the basal diet to a mean live-weight gain of 56 g/d. Urea plus HCHO-casein further increased lamb growth to 112 g/d.

3. In Expt 2 lambs were given free access to the basal diet (plus 25 g urea/kg diet) used in Expt 1. In this experiment the content of insoluble and soluble casein in the diets was varied by the addition of HCHO-casein and untreated casein of 0, 150; 50, 100; 100, 50 and 150, 0 g/kg basal diet respectively. Maximum lamb growth (141 g/d) was obtained with a supplement of 25 g urea plus 100 g HCHO-casein and 50 g casein/kg.

4. The growth responses to these supplements suggest a requirement for soluble N by the rumen micro-organisms to maximize rumen fermentation, and for maximum growth rate on this diet a further requirement for amino acids produced by protein which has escaped degradation in the rumen.

5. Fermentation and the absorption of nutrients were examined in Expt 3 in lambs fitted with simple 'T'-shaped cannula in the duodenum and ileum, and fed *ad lib.* one of the diets: a basal diet of oat hulls and solka floc, or the basal diet supplemented (g/kg) with either urea (25), urea (25) plus casein (150), or urea (25) plus HCHO-casein (150). The rates of production of volatile fatty acids (VFA), methane and microbial cells were measured using isotope-dilution techniques. The apparent absorption of nutrients was determined by differences in the quantity of those nutrients in digesta at the duodenum and ileum.

6. Supplements of urea, urea plus casein and urea plus HCHO-casein increased organic matter (OM) intake in lambs by 65% above that on the basal diet. OM digestibility was unchanged by the form of nitrogen supplementation. The rates of production of all fermentation end-products varied directly with voluntary food intake.

7. Rumen methane production remained constant at 0·09 mol methane/MJ metabolizable energy (ME) intake on all diets, which represented an 11% loss of digestible energy (DE). Hindgut methane production was highest on the urea-supplemented diet.

8. The rate of VFA production (mol/MJ ME intake) in the rumen was highest on the diet supplemented with urea in comparison with the basal, urea plus casein and urea plus HCHO-casein diets (which were not significantly different). The molar proportions of the individual VFA in rumen fluid were not significantly different between diets except for the branched chain and higher fatty acids which were highest in proportion with the urea plus casein diet.

9. The loss of energy in the faeces, urine or as methane in expired air was not influenced by the form of N supplementation. DE and ME were greater on the supplemented diets, as a result of the increased OM intake of these diets.

10. There was no effect of the form of N supplementation on OM digested in the rumen, small intestine or large intestine. Of an increase in OM intake, apparently 55% was digested in the rumen (of which 19% was incorporated into rumen micro-organisms) and 26% disappeared in the small intestines. The apparent digestibility of OM for all diets was 0·67.

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There are many recent studies which show that under certain production and feeding conditions, the supply of microbial protein does not meet the ruminants amino acid requirements for production. Under these conditions, supplementation with small amounts of a dietary-protein which is not fermented in the rumen but is digested in the small intestines has increased voluntary food intake (Egan, 1965; Egan & Moir, 1965; Egan, 1977), growth rate (Ørskov, 1970; Preston & Willis, 1970), wool growth (Reis & Schinckel, 1963), and improved the efficiency of food utilization (Ørskov *et al.* 1973).

In Australia more than 90% of ruminants are grazed on pasture (Weston & Hogan, 1973) which at certain times of the year may be low in protein (< 0·5% N/kg dry matter (DM)) and high in cell wall content. In sheep and cattle given these diets, DM intakes are low and the animals characteristically decline in body-weight. In general, supplementation of these diets with non-protein nitrogen (NPN) has not significantly improved production (Loosli & McDonald, 1968; Leng *et al.* 1973). Egan (1965) and Egan & Moir (1965) however have shown with mature wethers given dry low quality cellulose diets that OM intake could be substantially increased by providing amino acids post-ruminally. Therefore as part of a study to evaluate the role of NPN and rumen-undegraded dietary proteins in the nutrition of ruminants given such low-protein–cellulose diets, experiments were designed to investigate whether in growing lambs given these diets responses could be obtained to (1) feeding N to the rumen micro-organisms (2) feeding the animal a protein which is not degraded in the rumen, or both. The results from these studies indicated that supplementation of a low-protein–cellulose-based diet with such a protein increases feed intake and live-weight gain.

In order to study the effects in the animal of such supplements, measurements were made of the rates of production of fermentation end products in the rumen and of the passage and absorption of nutrients from the digestive tract. Lambs were fed *ad lib.* a basal low-protein–cellulose diet supplemented with urea, and either urea plus casein or urea plus formaldehyde-treated (HCHO)-casein. The effect of supplementation of these diets with NPN and a rumen-undegraded protein appeared to be solely due to their effect on appetite.

EXPERIMENTAL

Experimental design (*Expts* 1 *and* 2)

Two 42 d feeding trials were carried out using Border Leicester x Merino wether lambs (approximately 4 months of age) housed in an animal house in single pens on slatted flooring. All lambs were vaccinated with a multi-dose vaccine against clostridial infection (TVL Vaccine; Tasman Vaccine Laboratory (Aust) Pty Ltd, Victoria, Australia)and drenched every second week to control intestinal parasites (Nilzan; ICI Australia Ltd, 1 Nicholson St, Melbourne). The lambs were given lucerne chaff *ad lib.* in group pens for 2 weeks before commencement of the growth experiment.

Expt 1

Groups of five lambs (initial weight 26·3 ± 0·58 kg) were randomly allocated to one of the six experimental diets. All animals were given free access to a basal diet of oat hulls and solka floc (700:300) (a purified wood cellulose obtained from H. M. Goddard, 63 Trafalgar Avenue, Lindfield, NSW, 2070) to which supplements (5 g/kg oat hull, solka floc mixture) of sodium chloride, sodium sulphate and minerals (Susta-Vet-R, Bristol Veterinary Products, 345 Pacific Highway, Crows Nest, NSW, 2065) were added. The treatment groups included the basal diet (1) supplemented (g/kg basal diet) with 25 urea (2), 75 HCHO-casein (3), 75 casein (4), 25 urea plus 75 HCHO-casein (5), or 25 urea plus

75 casein (6). The lactic casein was purchased from Girgarrie Cheese Co. Pty Ltd, 177 McAuley Road, Melbourne, 3000 and the HCHO-casein prepared at a CSIRO Laboratory (P.O. Box 239, Blacktown, NSW) by the methods of Ferguson *et al.* (1967).

To facilitate handling, the NaCl, Na_2SO_4, minerals and relative amounts of urea, casein and HCHO-casein for each diet were mechanically mixed with the solka floc in 5 kg quantities. At 08.00 hours on each day the lambs were offered 100 g food in excess of that consumed on the previous day.

Expt 2

In order to determine the optimum requirement for HCHO-casein to support maximum growth rate in lambs on the low protein diet used in Expt 1, isonitrogenous supplements were given in which the proportion of HCHO-casein and untreated casein was varied.

Eight lambs (initial weight $24{\cdot}5 \pm 0{\cdot}52$ kg) were allocated at random to each of the five experimental treatments. All were given free access to a basal diet of oat-hulls and solka floc (700:300). This basal diet included (g/kg oat-hull, Solka-Floc mixture) 5 NaCl, 5 Na_2SO_4, 5 minerals and 25 urea. The treatment groups included the basal diet (A), supplemented (g/kg basal diet) with 150 casein (B), 100 casein plus 50 HCHO-casein (C), 50 casein plus 100 HCHO-casein (D) or 150 HCHO-casein (E).

Measurements

Daily food intake was recorded for each animal and adjusted for DM content after a representative feed sample had been dried at 100° for 48 h. The lambs were weighed each week before feeding in order to reduce variation associated with rumen fill.

Expt 3

Eight 1-year-old Border Leicester x Merino wethers (approximately 35 kg live weight) were fitted with a rumen cannula and single 'T'-shaped cannula in both the proximal duodenum (50 mm posterior to the pylorus) and in the terminal ileum (200 mm anterior to the ileo-caecal junction) (see Hogan, 1964). The animals were held in metabolism cages under continuous lighting and given free access to water.

Diets and feeding

The diets were selected from those used in Expts 1 and 2 as being the ones most critical for studying the responses. The diets were: A, a basal diet of oat-hulls and solka floc (700:300) plus (g/kg oat hull, solka floc mixture) 5 NaCl, 5 Na_2SO_4, and 5 minerals; B–D, the basal diet plus (g/kg basal diet): B, 25 urea; C, 25 urea and 150 casein; D, 25 urea and 150 HCHO-casein. The diets contained 5, 17, 33 and 32 g N/kg DM respectively. The diets were given in amounts which allowed *ad lib.* intake using automatic feeders which offered feed at hourly intervals. The amount offered was adjusted daily, each animal being given 100 g in addition to its previous day's intake.

Experimental design

To achieve four replicates per treatment, the experiment was conducted in two parts. In the first part, two animals were randomly allocated to each of the treatments. At the end of this experimental period, the animals were reassigned to the treatment groups and the experiment repeated after an adjustment period of 4 weeks.

Procedures

Food refusals and faeces and urine outputs were measured daily for each animal over a 7 d period (days 1–7). Urine was preserved with 20 ml of a mixture of mercuric chloride in glacial acetic acid (1 g/l).

Methane production rate was measured using the isotope dilution technique described by Murray *et al.* (1976). $^{3}H\text{-}CH_4$ was infused into the rumen (1 μCi/min) for 8 h and expired gas and rumen gas samples collected over the final 4 h of infusion. Similarly, rumen VFA production rate was measured by isotope dilution (see Weller *et al.* 1967). A solution containing 0·5 μCi and 1 μM[U$-^{14}$C]acetate/ml was infused into the rumen (1·2 ml/min) for 8 h and rumen contents sampled over the final 4 h of infusion.

The movement of digesta through the duodenum and ileum was estimated by reference to ^{51}Cr-EDTA (Downes & McDonald, 1964) and ^{103}Ru-labelled tris (1, 10-phenanthroline) ruthenium (II) chloride (Tan *et al.* 1971). These markers were infused into the rumen (0·34 ml/min, 5 μCi ^{105}Ru and 30 μCi ^{51}Cr/ml) for 15 d (days 9–23). Four samples (each 150 ml) of duodenal digesta were taken at intervals of 6 h on days 13 and 19. As collection of intestinal contents has been shown to influence the flow of digesta early in a collection period (Harris & Phillipson, 1962), collections were made on every third day. Ileal digesta samples were taken on days 16 and 22. The four samples of duodenal and ileal digesta for each day were pooled for each sheep and then assayed for ^{51}Cr and ^{103}Ru-P (see Tan *et al.* 1971).

Analytical methods

DM content was determined by heating samples at 100° in a forced air oven to a constant weight. OM content was subsequently determined after the samples were ignited at 600° for 3 h. N was determined by the Kjeldahl oxidation procedure (Association of Official Agricultural Chemists, 1960). The heat of combustion of dried samples was determined in an adiabatic bomb calorimeter. The apparent digestibility of gross energy (GE) (digestible energy; DE) was calculated as energy in food minus energy in faeces; metabolizable energy (ME) was DE minus energy in urine plus methane. Total VFA was estimated by steam distillation (Annison, 1954) and proportions of individual VFA measured using a gas–liquid chromatograph (Erwin *et al.* 1961).

Estimation of radioactivity in individual VFA

The radioactivity of VFA was determined on the acids separated using liquid–liquid partition chromatography. The column was prepared using 5 g silic acid (Mallinchrodt 100 mesh AR) mixed with 3·0 ml 0·25 M-sulphuric acid and washed into the 250 mm column (10 mm i.d.) with equilibrated hexane (hexane equilibrated with 0·25 M H_2SO_4). Samples of rumen fluid (2 ml) were made alkaline with 10 M sodium hydroxide and dried under vacuum over concentrated H_2SO_4. The dried sample was acidified with 10 M H_2SO_4, mixed with 1 g silic acid and added to the top of the silic acid column. Butanol in equilibrated hexane (120 ml/l; 70 ml) was used as the carrier and allowed to run through the column. The first 5 ml of eluate was discarded and the following 60 ml collected. Two 5 ml samples of this were titrated with 0·02 M NaOH under carbon dioxide-free conditions, using bromothymol-blue in ethanol (2 ml/l) as indicator. A further 5 ml was pipetted into a scintillation vial, 5 ml toluene-2,5-diphenyloxazole (4 ml/l) and 1,4-bis-2-(5-phenyl-oxazolyl)-benzene (0·02 ml/l) scintillation mixture added and then assayed in a scintillation spectrometer (Tri-carb model 3320; Packard Instrument Co., P.O. Box 423, La Grange Ill. USA) using the channels-ratio method of Bruno & Christian (1961).

^{51}Cr and ^{103}Ru-P were estimated in the same sample using a gamma counter (model

3002 autogramma; Packard) with windows set to maximize count rate for each isotope with a minimum of overlap.

Statistical analysis

Differences between treatment effects were analysed statistically by analysis of variance (Snedecor & Cochran, 1968). The relation between DM intake and live-weight change of lambs in each group was obtained by regression analysis. Differences in slope and elevation of these regression equations for lambs in Expt 1 and Expt 2 were compared by analysis of covariance (Snedecor & Cochran, 1968).

As much of the difference between diets in Expt 3 was reflected in differences in food intake, treatment effects were examined statistically after the results were adjusted to the same food intake by covariance analysis (Snedecor & Cochran, 1968). Food intake was calculated as the mean intake of the 2 d before, plus the day of measurement. The ME intake for any period was the product of DM intake for the period and the ME content of the diet for each animal estimated during the first 7 d of the experimental period.

Relationships between food intake and the variable under consideration were obtained by regression analysis.

RESULTS

Expts 1 and 2

In both experiments, individual animals on some diets lost weight over the experimental period. Consequently, food conversion ratio (FCR) (kg DM/kg live-weight gain) was calculated from the mean values presented in Tables 1 and 2, rather than from values for individual animals.

Expt 1

The diets 1, 2, 3, 4, 5 and 6 contained on average 940 g DM/kg and 5, 17, 14, 15, 25 and 25 g N/kg DM respectively. Mean DM intake (g/d) and live-weight change (g/d) for each treatment are given in Table 1. Supplements of either urea, casein, HCHO-casein or urea plus casein significantly ($P < 0.01$) stimulated DM intake to 27% above that on the basal diet. The combined supplement of urea and HCHO-casein further significantly ($P < 0.01$) stimulated voluntary food intake to 60% above that on the basal diet. Similarly, maximum live-weight gain (112 g/d) was in response to a combined supplement of urea plus HCHO-casein.

Expt 2

The diets A, B, C, D and E contained an average 940 g DM/kg and 17, 33, 32, 32 and 32 g N/kg DM respectively. Mean DM intake (g/d) and live-weight change (g/d) for each treatment are given in Table 2. There was no significant difference in DM intake for each diet but live-weight change was significantly increased above that on the basal diet at all levels of HCHO-casein supplementation. Maximum live-weight change (141 g/d) was in response to supplements (g/kg) of either 100 HCHO-casein and 50 untreated casein, or 150 HCHO-casein.

There was no significant difference in the slopes or elevations of the regression equations of live-weight change (g/d) on DM intake (g/d) for Expt 1 and Expt 2. Therefore the regression equation of Y (average daily gain, g/d) v. X (DM intake, g/d) for results from both experiments is:

$$Y = 0.52\ (\pm 0.051)\ X - 293\ R^2\ 0.92, \text{ residual SD } 15.2.$$

Table 1. *Expt 1. Mean dry matter (DM) intake (g/d), initial live weight (kg) and live-weight change for lambs given a basal diet of oat-hulls and Solka-Floc* ad lib. *and supplemented with either urea, casein or formaldehyde-treated (HCHO)-casein*

(All values are the means of five observations)

	Diet*						
	Basal	Basal + urea	Basal + HCHO-casein	Basal + casein	Basal + urea + HCHO-casein	Basal + urea + casein	SEM
DM intake (g/d)	507[a]	630[ab]	654[b]	657[b]	807[c]	642[ab]	47
Initial live wt (kg)	25·9	26·0	25·4	27·0	27·5	26·3	1·41
Live-wt change (g/d)	−40[a]	29[b]	67[b]	71[b]	112[c]	55[b]	14
Food conversion ratio (DM intake/live-wt gain)	–	22·1	9·8	9·2	7·2	11·7	–

a, b, c Values within the same horizontal row with different superscripts are significantly different.
* For details, see p. 293.

Table 2. *Expt 2. Mean dry matter (DM) intake (g/d), initial live weight (kg) and live-weight change (g/d) for lambs given a basal diet of oat-hulls, Solka-Floc and urea (25 g/kg) and supplemented with combinations of casein or formaldehyde-treated (HCHO)-casein*

(All values are the means of eight observations)

	Diet*					
	A Basal	B Basal + 15% casein	C Basal + 5% HCHO-casein 10% casein	D Basal + 10% HCHO-casein 5% casein	E Basal + 15% HCHO-casein	SEM
DM intake (g/d)	634	695	726	830	776	49
Initial live wt (kg)	24·6	24·8	24·6	24·4	24·3	1·30
Live-wt change (g/d)	14[a]	60[ab]	88[b]	141[c]	109[bc]	18
Food conversion ratio (DM intake/live-wt gain)	44·4	11·6	8·2	5·9	7·1	–

a, b, c Values within the same horizontal row with different superscripts are significantly different.
* For details, see p. 293.

Expt 3

OM intake and apparent digestibility

Urea, urea plus casein and urea plus HCHO casein supplements to a basal diet of oat hulls and solka floc significantly ($P < 0.05$) increased OM intake (g/d) in comparison with that on the basal diet. There was however no significant difference in intakes of OM on these supplemented diets. Supplements of either urea, urea plus casein or urea plus HCHO-casein did not significantly alter OM digestibility (see Table 3).

Methane production

Rumen methane production (mol/MJ ME) was not significantly altered by the form of N supplement (see Table 4, Fig 1). The quantity of methane produced in the hind-gut and

Table 3. *Mean values for organic matter (OM) and nitrogen (N) intake (g/d), and apparent OM and N digestibility in sheep given a basal low-protein diet (A), basal plus urea (B) basal plus urea and casein (C) and basal plus urea and formaldehyde-treated (HCHO)-casein (D)*

(Mean values adjusted to a common intake of OM or metabolizable energy (ME) (MJ/d) by covariance analysis are also given; all values are the means of four observations)

	Diet†				Significance of difference between means		SEM
	A	B	C	D			
Intake							
OM	599[a]	933[b]	953[b]	1073[b]	*		108
N	1·9[a]	15·0[b]	32·4[c]	36·9[c]	**		2·94
Digestibility							
OM	0·52[a]	0·59[b]	0·63[b]	0·61[b]	*		0·02
N	0·36[a]	0·73[b]	0·80[b]	0·71[b]	**		0·05
					Significance of difference between		SE of difference of adjusted means
					slopes	means	
Digestibility							
OM (adjusted for OM intake)	0·51	0·60	0·64	0·62	NS	NS	0·045
N (adjusted for ME intake)	0·24[a]	0·73[b]	0·79[b]	0·69[b]	NS	**	0·009

a, b Values within the same horizontal row with different superscripts are significantly different.
NS, Not significant, * $P < 0·05$, ** $P < 0·01$. † For details, see p. 294.

excreted via the lungs, however, was significantly ($P < 0·05$) greater on the urea supplemented diet. Although there was a significant difference between diets in hind-gut methane production, there was considerable variation within animals on the same diet.

VFA production

Total VFA production (mol/d) was significantly increased by all forms of N supplementation, although when differences in ME intake between diets were accounted for by covariance, total VFA production (mol/MJ ME) was only increased on the urea supplemented diet (Table 4, Fig. 2). Acetate production (mol/d) was increased by all forms of N supplementation, whereas propionate production (mol/d) was only increased by the urea supplement. Butyrate production (mol/d) was not significantly altered by any of the N supplements. After differences in ME intake were accounted for there was no significant difference between diets in the production (mol/MJ ME) of acetate, propionate or butyrate (Table 4).

The regressions of Y (VFA production rate, mol/d) (pooled for diets that did not have significantly different production rates) *v.* X (ME intake, MJ/d) were as follows:

Diet	VFA	Regression equation	R^2	Residual SD
ABCD	Acetate	$Y = 0·12\ (\pm 0·03)\ X + 0·99$	0·47	0·42
ABCD	Propionate	$Y = 0·06\ (\pm 0·02)\ X + 0·34$	0·39	0·23
ABCD	Butyrate	$Y = 0·02\ (\pm 0·008)\ X + 0·07$	0·30	0·32

Mean total VFA concentration (mmol/l) and molar proportions (mol/100 mol) of the individual VFAs are given in Table 5. The molar proportions of the individual VFA in rumen fluid were not significantly different between diets except for the branched chain

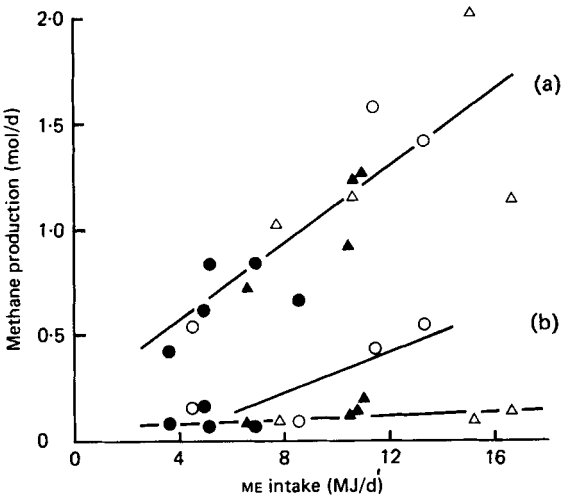

Fig. 1. Methane production (mold/) in relation to metabolizable energy (ME) intake (MJ/d) in sheep given A, the basal (●), B, the basal plus urea (○), C, the basal plus urea and casein (▲) and D, the basal plus urea and formaldehyde-treated (HCHO)-casein (△) diets. Regression equations (± SE of the regression coefficients) of Y (methane production, mol/d) *v.* X (ME intake; MJ/d) for (a) the rumen and (b) the hind gut methane which was excreted via the lungs are:

			R^2	Residual SD
(a)	$Y = 0{\cdot}20 + 0{\cdot}09\ (\pm 0{\cdot}016)X$	all diets	0·68	2·46
(b)	$Y = 0{\cdot}07 + 0{\cdot}04\ (\pm 0{\cdot}003)X$	diets A,C,D	0·16	0·04
	$Y = 0{\cdot}05\ (\pm 0{\cdot}02)X - 0{\cdot}20$	diet B	0·82	0·13

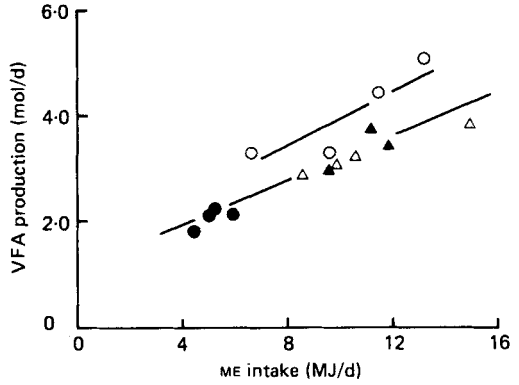

Fig. 2. Total volatile fatty acid production (mol/d) in the rumen of sheep given A, the basal (●), B, the basal plus urea (○), C, the basal plus urea and casein (▲) and D, the basal plus urea and formaldehyde-treated (HCHO)-casein (△) diets in relation to ME intake (MJ/d). The regression equations (± SE of the regression coefficients) of Y(VFA production, mol/d) *v.* X (ME intake, MJ/d) are:

		R^2	Residual SD
Diets ACD	$Y = 0{\cdot}21\ (\pm 0{\cdot}018)X + 1{\cdot}09$	0·94	0·19
Diet B	$Y = 0{\cdot}25\ (\pm 0{\cdot}100)X + 1{\cdot}49$	0·75	0·51

Table 4. *Mean values for the production (mol/d) of methane in the rumen, and in the hind gut (excreted via the lungs), and total and individual volatile fatty acid production (mol/d) in lambs given a basal low-protein diet (A), plus urea (B) and either urea plus casein (C) or urea plus formaldehyde-treated (HCHO)-casein (D)*

(All values are the means of four observations; values adjusted to a common metabolizable energy (ME) intake (MJ/d) by covariance analysis are also given)

	Diet†				Significance of difference between means		SEM
	A	B	C	D			
Rumen methane	0·68	1·06	1·04	1·34	NS		0·20
Hind gut methane excreted via the lungs	0·09	0·31	0·13	0·11	NS		0·06
Acetate	1·38^{a}	2·52^{b}	2·30^{b}	2·17^{b}	**		0·19
Propionate	0·54^{a}	1·10^{b}	0·89^{ab}	0·88^{ab}	*		0·11
Butyrate	0·16	0·37	0·25	0·23	NS		0·04
Branched chain and higher VFA	0·02^{a}	0·09^{a}	0·25^{b}	0·05^{a}	**		0·023
Total VFA	2·10^{a}	4·07^{b}	3·68^{b}	3·32^{b}	**		0·28
					Significance of difference between slopes	means	SE of the difference of adjusted means
Rumen methane	1·1	1·0	0·9	1·0	NS	NS	0·21
Hind gut methane excreted via the lungs	0·2^{ab}	0·3^{b}	0·1^{a}	0·1^{a}	NS	NS	0·08
Acetate	2·0	2·4	1·8	1·9	NS	NS	0·29
Propionate	0·6	1·1	0·8	0·8	NS	NS	0·24
Butyrate	0·3	0·3	0·2	0·2	NS	NS	0·12
Branched chain and higher VFA	0·06^{a}	0·08^{a}	0·24^{b}	0·03^{a}	NS	**	0·04
Total VFA	2·9^{a}	3·9^{b}	3·1^{ab}	3·0^{a}	NS	*	0·37

a, b Values within the same horizontal row with different superscripts are significantly different.
NS, Not significant. * $P < 0·05$, ** $P < 0·01$. † For details, see p. 295.

Table 5. *Mean volatile fatty acid (VFA) concentrations (mmol/l) and molar proportions (mol/100 mol) in rumen fluid of sheep fed a low-protein basal diet (A), basal plus urea (B), basal plus urea and casein (C), and basal plus urea and formaldehyde-treated (HCHO)-casein (D)*

(All values are the means of four observations)

		Individual VFA proportions (mol/100 mol)					
Diet†	Total VFA (mmol/l)	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate
A	57·3	65·5	25·7	0·5^{a}	7·9	0·3^{a}	0·2^{a}
B	85·8	62·0	27·6	0·5^{a}	8·8	0·8^{a}	0·5^{a}
C	71·8	62·6	23·7	2·1^{b}	7·0	3·7^{b}	1·9^{b}
D	57·8	64·3	27·2	0·6^{a}	7·2	0·5^{a}	0·2^{a}
SEM	9·98	2·64	2·88	0·16	1·07	0·71	0·26
Significance of difference between means	NS	NS	NS	**	NS	*	**

a, b Values within the same vertical column with different superscripts are significantly different.
NS, Not significant. * $P < 0·05$, ** $P < 0·01$. † For details, see p. 295.

Table 6. *Mean flow of organic matter (OM; g/d) at the duodenum and ileum and in the faeces and flow of total digesta (kg/d) at the duodenum and ileum of lambs given a low basal-protein diet (A), plus urea (B) and either urea plus casein (C) or urea plus formaldehyde-treated (HCHO)-casein (D)*

(All values are the means of eight observations; values adjusted to a mean OM intake (or mean dry matter intake for total digesta flow at the duodenum and ileum) by covariance analysis are also given)

	Diet†				Significance of difference between means	SEM
	A	B	C	D		
OM at						
Duodenum	350[a]	545[b]	539[b]	709[c]	**	56
Ileum	332	376	338	408	NS	51
Faeces	289	382	347	412	NS	48
Total duodenal digesta	6·66[a]	9·69[b]	9·53[b]	10·44[b]	*	0·82
Total ileal digesta	3·66[a]	5·83[b]	4·43[ab]	5·42[b]	*	0·53

					Significance of difference between		SE of difference of adjusted means
					slopes	means	
OM at							
Duodenum	510	542	498	549	NS	NS	49
Ileum	375	366	324	324	NS	NS	40
Faeces	421	360	314	333	NS	NS	34
Total duodenal digesta	9·3	9·7	9·0	8·1	NS	NS	0·54
Total ileal digesta	5·1	5·7	4·2	4·4	NS	NS	0·61

a, b Values within the same horizontal row with different superscripts are significantly different.
NS, Not significant.
* $P < 0.05$, ** $P < 0.01$.
† For details, see p. 298.

and higher fatty acids which were highest in proportion with the urea plus casein diet. The regression of Y (total VFA production, mol/d) *v.* X (concentration of total VFA, mmol/l) was as follows:

$$Y = 0{\cdot}03\ (\pm 0{\cdot}01)\ X + 1{\cdot}58 \qquad \text{residual SD} = 0{\cdot}49$$

OM digestion

The flows of OM (i.e. dietary plus microbial plus endogenous) (g/d) at the duodenum, ileum and in the faeces are given in Table 6. After correction for differences in OM intake there was no significant difference between diets in the flow of OM (g/unit OM intake) at any of these sites (Table 6). Similarly, the flow of total digesta (kg/unit DM intake) past both the duodenum and ileum was not significantly different between diets (Table 6). The regression equations with regression coefficients (±SE) of Y (OM flow, g/d) *v.* X (OM intake, g/d) were as follows:

		R^2	Residual SD
(*a*) duodenal OM (all diets)	$Y = 61 + 0{\cdot}55\ (\pm 0{\cdot}048)\ X$	0·82	82·5
(*b*) ileal OM (all diets)	$Y = 97 + 0{\cdot}29\ (\pm 0{\cdot}040)\ X$	0·66	69·9
(*c*) faecal OM (all diets)	$Y = 62 + 0{\cdot}33\ (\pm 0{\cdot}042)\ X$	0·81	44·0

The quantity of OM apparently digested (Y) in the various regions of the gastro-

Table 7. *Mean values for energy intake (MJ/d) and energy loss (MJ/d) in faeces, urine and methane in sheep given a basal low-protein diet (A), basal plus urea (B), basal plus urea and casein (C) and basal plus urea and formaldehyde-treated (HCHO)-casein (D)*

(All values are the means of four observations)

	Diet†				Significance of difference between means	SEM
	A	B	C	D		
Energy intake	10·9[a]	16·9[ab]	17·8[b]	20·2[b]	*	1·97
Faecal energy	5·3	7·2	6·5	7·9	NS	0·93
Digestible energy	5·6[a]	9·7[b]	11·2[b]	12·3[b]	**	1·19
Methane	0·7	1·1	1·1	1·1	NS	0·16
Urine	0·2	0·3	0·5	0·4	NS	0·09
Metabolizable energy	4·7[a]	8·3[b]	9·7[b]	10·7[b]	**	1·08

a, b Values within the same horizontal row with different superscripts are significantly different.
NS, Not significant.
* $P < 0·05$, ** $P < 0·01$.
† For details, see p. 299.

intestinal tract was given by the difference between the respective regression equations as follows:

Y (rumen) = OMI − OM in duodenal flow,
Y (small intestine) = OM in duodenal flow − OM in ileal flow,
Y (large intestine) = OM in ileal flow − OM in faeces,
digestible OM (DOM) = OMI − OM in faeces.

Algebraic subtraction of the appropriate regression equations given above provided equations for the relationship between OMI and the amount of OM apparently digested in the rumen (R), small intestine (SI) and large intestine (LI). These were as follows:

$$R = 0·45\,OMI - 61,\quad SI = 0·26\,OMI - 36,\quad LI = 35 - 0·04\,OMI,\quad DOM = 0·67\,OMI - 62.$$

These equations indicate that for each additional 100 g OM intake/d, 45 g OM was digested in the rumen and a further 26 g OM was digested in the small intestine. However, a gain of 4 g OM apparently occurred in the hind gut. The apparent OM digestibility for all diets was 0·67.

DE and ME intakes

Both DE and ME (MJ/d) intakes on the supplemented diets were significantly ($P < 0·01$) greater than on the basal diet, however there was no significant difference between diets in the loss of energy (MJ/d) in faeces, in urine, or as methane (Table 7). On these diets rumen methane production represented an 11% loss of DE. The relationships between DE, ME and GE intakes, obtained by regression analysis, indicated that DE was 63% of GE intake, and ME was 90% of DE intake.

DISCUSSION

Expt 1

The results from this experiment indicate that for growth, lambs given a low-protein–cellulose diet have a requirement for both NPN and a protein which is not degraded in the rumen. Supplementation with urea alone supported a small increase in lamb growth

(69 g/d) above that on the basal diet, suggesting that in lambs consuming the unsupplemented basal diet the rumen micro-organisms were N deficient. That soluble proteins are almost completely fermented in the rumen (McDonald & Hall, 1957) and provide little or no amino acids *per se* for the animal is suggested by the similarity of live-weight responses to both casein (a soluble protein) and urea supplements. Provision of extra ammonia in the rumen from the casein (above that supplied by urea) apparently did not further stimulate microbial growth (insofar as this could be detected by a change in live weight) and hence it could be assumed that the microbial requirements for NH_3-N were met from the dietary urea.

Formaldehyde treatment of casein has been shown to increase the availability and absorption of amino acids to the animal (Faichney & Weston, 1971). The live weight response to the HCHO-casein supplement indicated that the supply of amino acids from microbial protein was insufficient to meet the amino acid requirements of the growing lamb. This response to HCHO-casein only occurred when urea was also supplied indicating the requirement for a readily available (soluble) source of N in the rumen to provide NH_3 for microbial growth, and a requirement for rumen-undegraded protein to augment the supply of amino acids from microbial protein.

Expt 2

In this experiment designed to determine the optimum level of HCHO-casein in the diet, supplements of both urea and casein supported growth rates similar to those in lambs given the same supplements in Expt 1. Lambs given a diet containing 200 g crude protein (N × 6·26)/kg DM (diet B) were apparently amino acid deficient as indicated by the growth responses to similar levels of dietary crude protein but where the protein was apparently not fermented in the rumen. The optimal HCHO-casein content of these diets was 100 g/kg (diet D), which supported a mean average daily gain of 141 g/d.

From the combined relations between live-weight change and food intake for both studies, it is apparent that the NPN supplement alone could not support maximum food intake and that supplementation of this low-protein diet with a rumen-undegraded protein had a greater effect on appetite. This supports the concept of a positive feedback relationship between the availability of amino acids relative to the animal's requirements and voluntary food intake which also applies to diets based on starch and sugar (Ørskov *et al.* 1973; Preston, 1972). Although the diet given here was not highly lignified, it is apparent that the first limitation to appetite in ruminants given many low-protein foods is the quantity of absorbed amino acids and not primarily rumen distension.

Associated with the increased food intake with supplementation with a rumen-undegraded protein was a decrease in the calculated FCR (see Tables 1 and 2), although the lowest of these values are much higher than those reported by Ørskov *et al.* (1974). In the latter study, lambs given barley–fish meal diets gained weight at 430 g/d with an FCR of 2·2:1.

The term FCR was originally used to quantitate the efficiency of food utilization in single stomached animals where the digestibility of the ration was approximately 0·80. In ruminants however, the digestibility of diets consumed may vary between 0·40 and 0·80. Also, FCR is affected by the level of food intake relative to maintenance. Therefore, to reduce some of this variation, the regressions of live-weight gain (g/d) on digestible DM intake (DDMI, g/d) have been calculated for Expt 1 and Expt 2 and for results from lambs given barley diets supplemented with fish meal (Ørskov *et al.* 1974). As there was no significant difference in slope or elevation of these equations, the regression of Y (live-weight gain, g/d) *v.* X (DDMI, g/d) for both diets was:

$$Y = 0{\cdot}79\ (\pm 0{\cdot}28)X - 235,\ R^2\ 0{\cdot}98,\ \text{residual SD } 14{\cdot}4.$$

From this relation it was apparent that even though DDMI and growth rates were much higher in the lambs fed the barley diet, an efficiency of utilization of digestible DM above maintenance of 0·79 was common to all diets (i.e. an FCR of 1·3:1). It would appear therefore that the rate of live-weight gain depends largely on those factors which influence the intake of digested nutrients by the animal, rather than the efficiency of utilization of digested nutrients within the animal.

Expt 3

This experiment was designed to determine the effects of feeding either NPN, a rumen-undegraded protein, or both, on rumen function and nutrient availability in the animal. In preliminary studies it was found that young lambs (approximately 20 kg live-weight) with the combinations of cannulas necessary to obtain the information needed were difficult to maintain, since food intake was erratic and the lambs either failed to grow or lost weight. For these reasons it was necessary to use older and heavier lambs than in the previously reported growth studies. Since the necessary criterion for this experiment was that the animals were fed *ad lib*, food intake for individual animals varied between days. Also, the form of N supplement increased food intake to different extents and so a covariance analysis technique combined with a regression approach was adopted to assist in interpreting the data.

Voluntary food intake

In this study, the major effect of supplementation of the low-protein diet with urea, urea plus casein or urea plus HCHO-casein was to increase OMI by 65% above that on the basal diet. Concomitant with the increase in food intake was an increase in the production of VFA and methane in the rumen and absorption of nutrients from the digestive tract.

Rumen fermentation

The effects of N supplementation on rumen fermentation (as indicated by the production of VFA and methane) and the digestion of OM suggested that fermentation on the basal diet was limited by a deficiency of soluble N. On the basal diet, only 35% of the DOM was apparently digested in the rumen in comparison with 39% with the urea-, urea plus casein-, and urea plus HCHO-casein-supplemented diets. However, when differences in food intake were accounted for, a constant proportion (45%) of additional OM consumed on each diet was apparently digested in the rumen. The rate of methane production (mol/MJ ME) in the rumen also remained constant on all diets, which further suggests that fermentation may not have been limited by a deficiency of soluble N. However, when HCHO-casein was given as the sole N supplement, recycling of plasma urea-N to the rumen was unable to supply sufficient N for microbial growth, since maximum feed intake and growth rate was only supported by a supplement of both NPN and HCHO-casein (Table 1).

OM digestion

Of the additional OM consumed on each diet, 45% was apparently digested in the rumen and 20% apparently digested in the small intestines. From microbial protein production values for these diets (see Kempton *et al.* 1979) and assuming 1 g microbial N was contained in 12 g OM (Hungate, 1966), apparently 55% of the OM intake was calculated to be fermented in the rumen when an allowance was made for microbial OM (19% of that apparently fermented) flowing from the rumen. These results taken together suggest that supplementation of the low-protein–cellulose diets used in this study with any of the N supplements had no effect on the fermentation of OM in the rumen, despite the effect of stimulating food intake.

It was concluded that growing lambs given low-protein–cellulose diets have a requirement

for amino acids in addition to those supplied from microbial protein digested in the small intestines. Furthermore, the effect of supplementation of these diets with a rumen undegraded protein was directly attributable to an increased food intake.

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