The effect of dietary energy source on nitrogen metabolism in the rumen of sheep

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1. Seven isonitrogenous diets were prepared containing soya-bean meal and dried grass either unsupplemented, or supplemented with wheat starch or paper or equal mixtures of both.

2. The diets were allocated according to a balanced incomplete block design to seven Clun Forest wether sheep, each fitted with a re-entrant duodenal cannula.

3. After each sheep had received a diet for 6 d, daily samples of digesta were collected automatically for the next 3 d.

4. The amounts of dry matter, gross energy and major nitrogenous components consumed in the diet, passing into the duodenum and excreted in the urine and faeces were determined.

5. A novel method was applied to estimate the proportion of the total amino acids passing into the duodenum which was of microbial origin.

6. The amounts of microbial total amino acids synthesized were compared with the amounts of energy disappearing in the rumen for each of the seven diets tested. For the starch- and paper-containing diets an average of 14·7 g microbial total amino acid passed into the duodenum per MJ energy disappearing in the rumen, whilst for the diets containing either paper or starch alone the mean value, 6·1 g/MJ, was significantly lower ($P < 0.05$).

7. The results of the experiment suggested that the energy released from a mixture of starch and paper was utilized more efficiently for microbial protein synthesis than when the energy was provided by supplements of either paper or starch alone.

Hogan & Weston (1970) studied the relationship between the passage of nitrogenous materials to the duodenum and the nitrogen:digestible organic matter in the diet of sheep given *ad lib.* a number of pasture plants which differed widely in nutritive value. They concluded that protein synthesis in the rumen was frequently limited by the amount of biologically-useful energy that the rumen micro-organisms could obtain from the diets.

In the present study, the basis for this conclusion was broadened by studying the effect of different energy sources on the flow of nitrogenous materials to the duodenum, using semi-purified diets differing only in carbohydrate content. A preliminary experiment carried out on a single sheep has been reported previously (Offer, Evans & Axford, 1972*b*). A short report of the present study has been given by Offer, Evans & Axford (1975).

EXPERIMENTAL

Sheep and diets

Seven adult Clun Forest wether sheep, each previously fitted with a re-entrant cannula in the proximal duodenum, were housed in metabolism cages and were fed automatically at 2 h intervals. Water was available to the sheep at all times. A basal diet was compounded, providing (g/d): soya-bean meal 250, milled dried grass 150, mineral–vitamin mixture (ICI Cattle and Sheep; ICI Ltd, Alderley Park, Macclesfield) 30, molasses 80, chromic oxide 2.

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This basal diet was supplemented with (g/d) 300 or 600 wheat starch or paper or equal quantities of each (diets 300S, 600S, 300P, 600P, 150SP and 300SP respectively). The diets were pelleted before feeding.

Experimental design

The seven diets were fed to the seven sheep according to a balanced incomplete block design, in which each treatment was replicated three times. Each sheep received a diet for a period of 9 d. On the sixth day of each period, the re-entrant cannula was linked to an automatic device (Axford, Evans & Offer, 1971) collecting representative samples, which were a constant proportion of the digesta passing to the duodenum. Daily samples were taken for the next 4 d, freeze-dried and stored for subsequent analysis. Urine (preserved by acidification) and faeces were also collected. The daily dietary intake was recorded and any uneaten food was removed.

Chemical analysis

Analyses were carried out on bulk 3 d samples. Dry matter was determined by drying to constant weight at 105°. Gross energy (GE) was measured using a ballistic bomb calorimeter (Miller & Payne, 1959). Ammonia was determined by the method of Fawcett & Scott (1960), adapted to the AutoAnalyser (Technicon Instrument Co. Ltd, Basingstoke RG2 12YE, Hants). Samples for total N determination were digested on a semi-micro scale using the reagents of Siriwardine, Thomas, Evans & Axford (1966) and analysed using the AutoAnalyser. Total amino acids were measured by hydrolysis with 6 M-hydrochloric acid at 108° for 24 h under an atmosphere of N_2, followed by automated ion-exchange chromatography (Thomas, 1970). Cr_2O_3 was determined by a modification of the method of Stevenson & Clare (1963). Urinary allantoin was measured by a modification of the method of Pentz (1969).

RESULTS

The diets were designed to provide a N intake of 65 g/3 d. Soya-bean meal provided 85·3% and dried grass 14·7% of this amount. Table 1 gives the measured total N intakes and also the allocation of sheep to the seven diets. In four of the twenty-one experimental periods there were significant food refusals. All three sheep given diet 600P refused approximately 40% of the food provided. Sheep no. 6 also refused a large quantity of diet 300SP. In order to allow for these differences in intake all digesta, urine and faeces values were normalized to a standard N intake of 65 g/3 d, assuming a direct proportionality between intake and the measurement made.

Table 2 gives the corrected treatment mean values for the recovery of dietary Cr_2O_3 at the duodenum and faeces. Over the whole experiment, the recovery of Cr_2O_3 was (mean ± SE) 98·0 ± 3·0% at the duodenum and 93·0 ± 2·6% in the faeces. The recoveries in digesta samples for diet 300SP and in faecal samples for diet 300P differed significantly ($P < 0.05$) from 100%. On the evidence of previous experiments (Offer, Evans & Axford, 1972a), we are satisfied that Cr_2O_3 used as described, is an accurate marker for dry matter in digesta passing to the duodenum. In the present experiment, all flow values were corrected to give 100% recovery of the administered dose. Faecal values were similarly corrected.

The results were analysed statistically by standard methods for balanced incomplete blocks (Yates, 1936) to obtain treatment means free from variation due to differences between sheep.

The results for the digestion of dry matter and GE are given in Table 3. The over-all digestibilities were lowest for diets 300P, 600P and 300SP. The digestibilities of diets 300P and 300SP were significantly ($P < 0.05$) lower than those of the other diets except 600P. It

Table 1. *Intake of dietary total nitrogen (g/3 d) by sheep given diets differing in carbohydrate content*

		Sheep no.						
Diet* Supplement		1	2	3	4	5	6	7
Basal None		—	—	67·1	62·1	67·1	—	—
300S ⎱ Wheat		—	—	69·4	—	—	69·4	69·4
600S ⎰ starch		66·0	60·7	66·0	—	—	—	—
300P ⎱ Paper		63·1	—	—	63·1	—	—	63·1
600P ⎰		—	40·6	—	43·1	—	37·4	—
150SP ⎱ Wheat starch		—	63·4	—	—	63·4	—	63·4
300SP ⎰ and paper		65·8	—	—	—	66·2	34·4	—

* For details of diets, see p. 35.

Table 2. *Recovery (%) of dietary chromic oxide in digesta and faeces from sheep given diets differing in carbohydrate content*

(Mean values for three determinations/dietary treatment)

Diet*	Supplement	Duodenal digesta	Faeces
Basal	None	96·7	90·3
300S ⎱ 600S ⎰	Wheat starch	103·2 / 111·4	95·1 / 91·3
300P ⎱ 600P ⎰	Paper	98·5 / 94·3	85·2 / 94·6
150SP ⎱ 300SP ⎰	Wheat starch and paper	98·5 / 83·4	102·4 / 92·3
SE of means		7·2	6·3

* For details of diets, see p. 35.

Table 3. *Amounts of dietary dry matter (g/3 d) and gross energy (MJ/3 d) digested by sheep given diets differing in carbohydrate content*

(Mean values for three determinations/dietary treatment, adjusted to a standard intake of 65 g total nitrogen/3 d)

		Dry matter				Gross energy			
Diet*	Supplement	Intake	Flow to duodenum	Faeces	Apparent digestibility	Intake	Flow to duodenum	Faeces	Apparent digestibility
Basal	None	1310	755	286	0·768	22·3	10·0	4·2	0·796
300S ⎱	Wheat	2032	775	426	0·794	35·3	11·8	7·2	0·798
600S ⎰	starch	2769	1454	616	0·773	48·0	22·1	11·1	0·765
300P ⎱	Paper	2242	1446	913	0·589	39·3	20·1	15·6	0·600
600P ⎰		2830	1472	949	0·662	48·2	22·9	17·4	0·636
150SP ⎱	Wheat	2236	1740	679	0·700	40·0	26·3	11·6	0·715
300SP ⎰	starch and paper	2815	2263	1117	0·605	47·5	32·5	20·2	0·573
SE of means		—	100	85	0·034	—	1·8	1·6	0·037

* For details of diets, see p. 35.

Table 4. *Passage of nitrogenous compounds (g/3 d) and apparent absorption of total nitrogen entering the duodenum of sheep given diets differing in carbohydrate content*

(Mean values for three determinations/dietary treatment, adjusted to a standard intake of 65 g total N/3 d (45·9 g amino acid–N/3 d))

Diet*	Supplement	Flow to duodenum					Faeces		Urine	
		Total N	Total amino acid-N†	Non-ammonia-N	Ammonia-N	Ammonia-N (% total N)	Total N	Apparent absorption of total N	Total N	Allantoin-N
Basal	None	32·7	18·3	27·5	5·2	16·0	8·8	0·727	49·1	0·87
300S	Wheat starch	40·1	21·1	34·2	5·8	15·1	10·3	0·744	34·2	1·06
600S		72·9	41·3	68·8	4·1	5·5	21·8	0·669	24·0	1·76
300P	Paper	41·6	24·0	31·1	10·5	23·7	19·5	0·549	36·9	0·97
600P		44·9	23·6	37·2	7·7	17·7	17·4	0·589	36·6	0·73
150SP	Wheat starch and paper	53·5	32·6	43·8	9·7	17·9	14·8	0·723	42·4	1·05
300SP		67·5	44·2	57·6	9·8	15·0	19·7	0·719	29·9	1·57
SE of means		4·9	2·6	4·8	1·2	2·4	2·4	0·055	3·0	0·22

* For details of diets, see p. 35.
† Obtained by summing N contributions of individual amino acids.

Table 5. *Intake and flow (g/3 d) of amino acids to the duodenum in sheep given diets differing in carbohydrate content*

(Mean values for three determinations/dietary treatment, adjusted to a standard intake 65 g total nitrogen/3 d)

Amino acid	Total	Aspartic acid	Threonine	Serine	Glutamic acid	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine
Intake	339·3	36·7	23·5	16·1	65·1	17·1	17·9	16·1	6·8	15·2	27·6	14·6	18·4	11·3	22·1	24·3
Flow to duodenum																
Diet* Supplement																
Basal None	134·5	16·3	9·5	6·4	17·7	7·5	9·5	7·9	3·4	7·1	12·1	6·5	7·5	4·5	8·3	7·9
300S } Wheat starch	156·4	17·7	11·3	7·3	21·5	8·5	11·1	8·7	4·3	8·2	12·9	8·0	7·9	5·2	13·1	7·4
600S }	305·1	34·1	21·6	14·8	44·2	16·2	21·3	16·8	6·9	16·5	25·9	14·9	16·1	9·6	23·6	16·1
300P } Paper	177·4	19·9	12·4	8·7	24·4	10·6	13·3	10·2	4·3	9·1	14·7	9·0	9·1	5·9	14·1	8·3
600P }	174·8	17·8	14·5	8·5	22·9	10·5	13·0	10·4	3·9	9·2	14·9	9·0	9·7	6·1	13·1	7·7
150SP } Wheat starch and paper	235·7	23·8	15·5	12·8	33·5	13·8	16·3	13·3	5·4	12·9	20·3	12·6	12·4	9·1	21·0	11·2
300SP }	321·1	34·6	25·0	17·1	43·8	19·6	24·1	17·7	8·1	17·1	26·4	16·7	16·5	11·9	24·8	15·1
SE of means	18·8	2·6	1·8	0·8	2·4	1·2	1·7	1·5	0·3	0·9	1·4	1·0	1·1	0·6	2·0	1·3

* For details of diets, see p. 35.

Table 6. *The amino acid profiles of bacterial and endogenous constituents (g amino acids/kg total amino acids included in profile)*

Amino acid ...	Aspartic Acid	Threonine	Serine	Glutamic Acid	Glysine	Alanine	Valine	Methionine	Isoleusine	Leusine	Tyrosine	Phenyl-alanine	Histidine	Lysine	Arginine
Bacterial*	124	58·6	44·5	135	60·7	77·3	65·3	27·5	60·9	79·1	51·2	53·6	21·9	89·5	51·2
Endogenous†	130	75·4	122	117	55·9	31·9	69·3	13·9	100	79·6	80·7	67·0	7·6	28·9	25·8

* Bacterial profile is the mean of fifty-four profiles taken from: Berger, Purser & Cline (1968a, b), Burris, Bradley & Boling (1974), Hoeller & Harmeyer (1964), Hoogenraad & Hird (1970); Ibrahim & Ingalls (1972), Leibholtz (1972), Meyer, Bartley, Deyoe & Colenbrander (1967), Oltjen, Slyter, Williams & Kern (1971), Purser & Buechler (1966), Sharma & Ingalls (1974), Weller (1957), Williams & Dinusson (1973).
† Endogenous profile is that of bovine pepsinogen calculated from Chow & Kassell (1968).

Table 7. *Estimated contribution of microbial, dietary and endogenous amino acids (g/3 d) to the total amino acids in the duodenal digesta of sheep given diets differing in carbohydrate content*

(Mean values for three determinations/dietary treatment, adjusted to a standard intake of 65 g total nitrogen/3 d)

Diet*	Supplement	Microbial amino acids	Dietary amino acids	Endogenous amino acids
Basal	None	86·9	31·3	13·8
300S } 600S }	Wheat starch	{ 120·3 { 192·5	30·4 97·6	3·1 11·8
300P } 600P }	Paper	{ 133·5 { 125·2	33·8 31·7	7·3 13·0
150SP } 300SP }	Wheat starch and paper	{ 163·8 { 221·3	56·6 68·8	9·6 23·4
SE of means		25·2	9·6	5·2

* For details of diets, see p. 35.

should be noted that the value obtained for diet 600P was likely to be an over-estimate because of the correction applied to allow for the low intake of this diet.

Table 4 give values for the passage of nitrogenous compounds. There was a significant ($P < 0.05$) loss of total N between the mouth and the duodenum for the basal diet and diets 300S, 300P and 600P. The mean flows of total N at the duodenum were higher than the total N intakes for diets 600S and 300SP although these differences were not significant. Carbohydrate supplementation of the basal diet resulted in increased flows of non-ammonia-N and total amino acid-N to the duodenum. These increases were significant ($P < 0.05$) for diets 600S, 150SP and 300SP. The flow of total amino acid-N for diet 150SP was significantly higher than for diets 300S or 300P. The flows of non-ammonia-N and total amino acid-N for diet 600P were significantly lower than those obtained for diets 600S and 300SP.

The flow of ammonia-N to the duodenum was lowest for the basal diet and diets 300S and 600S. When expressed as a percentage of total N passing to the duodenum there was no significant difference between treatments except with diet 600S. The total N in the digesta flowing to the duodenum for diet 600S contained a much lower proportion of ammonia-N.

There were no significant differences in total N excreted in the faeces for diets 600S, 300P, 600P and 300SP, but each was significantly ($P < 0.05$) higher than the level for the basal diet. The apparent absorption of total N passing to the duodenum was lowest for diets 300P and 600P (basal diet supplemented with paper). On average, 67 % of the total N entering the intestine disappeared.

Supplementation of the basal diet with carbohydrate reduced the amounts of total N excreted in the urine. The lowest values were obtained for diets 600S and 300SP. The amount of allantoin-N excreted in the urine was increased by carbohydrate supplementation except for diet 600P. The values obtained for diets 600S and 300SP were significantly ($P < 0.05$) higher than for the basal diet.

Table 5 gives the intake and duodenal flow of amino acids. For each amino acid there was a large loss between the mouth and the duodenum when the basal diet was fed. Carbohydrate supplementation led to increased flow of amino acids. The highest flows were obtained for diets 600S and 300SP. The flows of methionine and histidine were significantly ($P < 0.05$) higher for diet 300SP than for diet 600S. For diet 300SP significantly ($P < 0.05$) more alanine and methionine passed to the duodenum than was consumed.
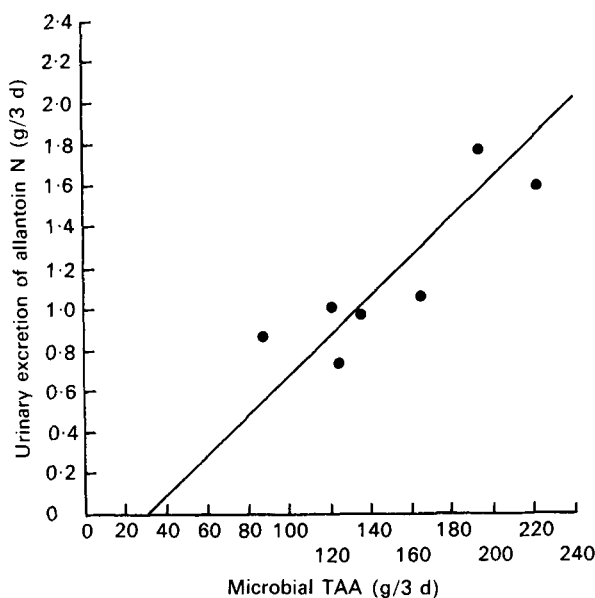
Fig. 1. Relationships between urinary allantoin N excretion (g/3 d) and the estimated microbial total amino acid (TAA) flow (g/3 d) at the duodenum in sheep given diets differing in carbohydrate content (for details of diets, see p. 35). r 0·84; $Y = 30·2 \ (\pm 27·2) + 103·8 \ (\pm 29·5) \ X$, where X is urinary allantoin N excretion, and Y is microbial TAA flow.

DISCUSSION

Estimation of microbial protein passing to the duodenum

The proportion and amount of duodenal total amino acids present as microbial protein was estimated using the method of Evans, Axford & Offer (1975). This method depends on the generation, by computer, of an amino acid profile by mixing in different proportions the known profiles of dietary, bacterial and endogenous constituents which are expected at the duodenum. The profiles used for bacterial and endogenous constituents are listed in Table 6. The best match of this to the composition of the duodenal digesta is obtained by an iterative process, which continues until the sum of squares of residuals is minimal. The computer program finds the proportion of total amino acids attributable to each constituent which minimizes the objective function:

$$\Sigma \ (\text{digesta actual} - \text{digesta calculated})^2.$$

The program carries out an organized search for the lowest value of this function. Constraints are applied by adding a penalty to the function whenever the search enters a forbidden zone. These zones exist because no dietary constituent of the digesta is permitted to exceed the intake of that constituent nor may any constituent be negative.

Table 7 gives the results of application of this method to the values obtained in the present experiment. On average, 69% of the flow of total amino acids at the duodenum was attributed to microbial protein. This value agrees closely with our calculated values for the soya-bean-meal–starch–cellulose diets studied by Goshtasbpour-Parsi, Ely, Boling, Alderson & Amos (1974). Application of the optimization method to the amino acid values given by these authors gave values of 69 and 67% microbial protein in the digesta for diets which contained 150 and 310 g soya-bean meal/kg respectively as the only protein source.

In the present experiment only small amounts of soya-bean-meal protein escaped degrada-

Table 8. *The energetic efficiency of microbial total amino acid production in sheep given diets differing in carbohydrate content*

(Mean values for three determinations/dietary treatment, adjusted to a standard intake of 65 g total nitrogen/3 d)

Diet*	Supplement	Energy disappearing before the duodenum		Microbial total amino acids:energy disappearing (g/MJ)
		MJ/3 d	Gross energy intake (%)	
Basal	None	12·2	55·9	7·6
300S 600S	Wheat starch	{ 23·5 { 25·9	67·0 55·5	5·8 6·8
300P 600P	Paper	{ 19·2 { 25·3	47·9 49·9	7·7 4·4
150SP 300SP	Wheat starch and paper	{ 13·7 { 15·0	34·9 30·7	13·0 15·7
SE of means		1·8	4·1	2·9

* For details of diets, see p. 35.

tion in the rumen. The amount of total amino acids passing to the duodenum attributable to the soya-bean meal was significantly greater ($P < 0.05$) than zero for diets 600S and 150SP only.

Although there were no significant differences between diets in the amounts (%) of total amino acids passing to the duodenum identified as microbial amino acids, no relationship was found between microbial and dietary or endogenous amino acid flow. There were, however, large differences in the total passage of microbial amino acids to the duodenum. Supplementation of the basal diet with 300 g each of paper and starch/d resulted in an increase in microbial total amino acids passing to the duodenum from 87 to 221 g/3 d. The duodenal flow of microbial amino acids for diets 600S, 150SP and 300SP was significantly greater ($P < 0.05$) than for the basal ration.

Results shown in Fig. 1 suggest that the excretion of allantoin in urine is related to the microbial protein synthesis in the rumen. Although allantoin arises from the catabolism of purines derived from microbial, dietary, and endogenous nucleic acids, there is a significant relationship ($P < 0.02$) between microbial amino acids passing to the duodenum and the excretion of allantoin in the urine. The large standard errors of the coefficients of the regression equation limit its use for predictive purposes.

Effect of energy source on amino acids passing to the duodenum

A comparison of the efficiency with which each carbohydrate source was utilized for microbial protein synthesis was made by calculating the amounts of microbial total amino acids passing to the duodenum per MJ food energy disappearing between the mouth and the duodenum.

The values of energy disappearing before the duodenum expressed as MJ/3 d and as a percentage of the GE intake are given in Table 8. When expressed as a percentage of GE intake, the highest value was that for the 300S diet, the values for the basal diet and for diets 600S, 300P and 600P were lower and the lowest values were those for diets 150SP and 300SP. These groups were distinguishable at $P < 0.05$. These values for preduodenal digestibility, when compared with those for over-all digestibility of energy previously described (Table 3), indicated a shift in the relative importance of different sites of digestion for the various diets. It should be remembered, however, that both the preduodenal and over-all

digestibilities for diet 600P may have been over-estimated due to the correction applied for the low intake of this diet.

Table 8 also gives the amounts of microbial total amino acids passing to the duodenum per MJ energy apparently disappearing between the mouth and the duodenum. The values for diets 150SP and 300SP (mean 14·4 g/MJ) were significantly higher ($P < 0·05$) than those for the other diets (mean 6·3 g/MJ).

The results of the present experiment suggested that the energy released from a mixture of starch and paper in the rumen was utilized more efficiently for microbial protein synthesis than when the energy was provided in supplements of either starch or paper alone. This may account for the synergistic effect of dietary constituents in promoting the flow of N to the duodenum reported by Offer *et al.* (1972*b*) and Chamberlain & Thomas (1974).

Values for microbial total amino acids:energy disappearing between the mouth and the duodenum for diets containing both starch and paper were similar to those which can be calculated from values reported for mixed and forage diets assuming that 1 kg apparently digested organic matter is equivalent to 18 MJ. The calculated values (g microbial protein synthesis/MJ organic matter apparently digested in the rumen) range from 3·7 to 7·3 (Liebholtz, 1972; Liebholtz & Hartmann, 1972), 17·5 to 21·3 (Lindsay & Hogan, 1972) and a value of 12·8 (Hogan & Weston, 1970). Liebholtz (1972) concluded that the efficiency of microbial protein synthesis in the rumen depends on protein source. This response probably depends on the ability of the different dietary proteins to maintain optimal ammonia concentrations in the rumen for microbial growth since we have found that supplementing a diet of chopped hay (670 g/kg) and milled barley (330 g/kg) with urea or ammonium acetate or ammonium lactate leads to increased energetic efficiency of microbial protein synthesis (Offer, Evans & Axford, 1976). The results of the present experiment suggest that the nature of the energy source also has a considerable effect.

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