The influence of levels of protein and starch in rations of sheep on the utilization of protein

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It has recently been well established that the overall utilization of protein by ruminants represents the result of two opposing processes of ammonia utilization: part of the ammonia produced by the decomposition of food protein may be excreted as urea in the urine, and thus lost to the animal, and part may be built into valuable microbial protein and utilized by the host animal. However, only a few assessments of the nutritive value of proteins supplied in different types of feeds have been conducted on the basis of the metabolic fate of proteins in the ruminant.

It was found in this laboratory (Tagari, Ascarelli & Bondi, 1962) that a decrease in solubility of different protein feeds as a result of heat treatment, such as the toasting of soya-bean meal, is accompanied by improved utilization (cf. Chalmers, 1961*b*; Sherrod & Tillman, 1962). The reason given for this was that highly soluble proteins such as those of untoasted soya-bean meal are degraded in the rumen to NH_3 at a rate too rapid for efficient utilization.

Two further aspects of this problem are considered in this paper. Supplying excess protein to cattle is typical of the feeding practice prevailing in many countries. This is so during the greater part of the year in Israel, where protein-rich feeds, such as leguminous forage are more plentiful during the winter than are carbohydrate-rich foods. Most practical rations that could be formulated to meet the energy requirements of ruminants for that season would of necessity include a considerable excess of protein. The influence of giving a surplus of proteins on the metabolic reactions occurring in the rumen has therefore been studied.

Different authors (McDonald, 1952; Williams, Nottle, Moir & Underwood, 1953; Chalmers, 1961*b*; Lewis, 1957, 1962; Phillipson, Dobson, Blackburn & Brown, 1962) noted that the addition of readily available carbohydrates to protein-rich diets considerably depressed the NH_3 concentration in the rumen liquor. We, therefore, made experiments to determine whether the utilization of surplus proteins could be improved by substituting starch for the carbohydrates of the roughage in the diet and whether the concentration of protein-breakdown products in the rumen, particularly the concentration of NH_3 in the rumen liquor and of urea in the blood, could be used for estimating the efficiency of protein utilization.

EXPERIMENTAL AND RESULTS

Animals and treatment

Lambs and adult male and female sheep of the Awassi breed were used. The content of digestible crude protein and the starch equivalent of the various foods in the diets are given in Table 1. The animals had free access to water and mineral lick.

Table 1. *Content of digestible crude protein and the starch equivalent of the feedstuffs used in the rations**

Feedstuff	Digestible crude protein (%)	Starch equivalent/kg
Rhodes hay (*Chloris gayana* Kunth)	5·2	0·288
Millet hay (Foxtail variety, *Setaria italica* Beauv.)	6·0	0·275
Cottonseed hulls, delinted	0·0	0·216
Sugar-beet pulp, dried	4·5	0·490
Soya-bean meal†	45·1	0·895
Groundnut meal	45·1	0·892
Maize gluten feed	33·4	0·897
Barley, crushed	7·3	0·714
Maize, crushed	7·2	0·748
Potato starch	0·0	1·000

* Calculated from the analyses of feeds produced in Israel and from digestibility coefficients obtained in this laboratory (Bondi & Neumark, 1960).

† Commercial untoasted soya-bean meal as specified by Tagari *et al.* (1962).

Standards for energy and digestible crude protein requirements accepted for sheep farming in Israel were used. For lambs, they were based on Woodman's (1954) figures, and for adult sheep on the protein standards of Brody (1945) and the energy standards of Woodman (1954), but adapted for local conditions. The values for energy requirements for maintenance were lowered by about 18% because of the lower requirements revealed in practical sheep farming in this country, which is in agreement with calorimetric measurements of Langlands, Corbett, McDonald & Pullar (1963) published after the conclusion of our experiments. A further 15% reduction in the energy requirement seems to be in order for animals subjected to balance experiments in metabolic cages, because of the lower requirements of resting animals. The standards used in calculating the rations are given in Table 2.

Table 2. *The feeding standards used for the sheep*

	Growing lambs		Adult sheep			
Weight of animal (kg)	35–40	40–45	40	50	60	70
Digestible crude protein (g/day)	110	120	54·0	63·6	72·4	81·2
Starch equivalent (kg/day)	0·70	0·75	0·37	0·43	0·48	0·54

The animals were fed twice daily. The morning ration, given at 07.30 h, contained the main protein foods and a large part of the energy-supplying foods, whereas the

afternoon ration, given at 15.30 h, usually contained hay and other roughages in amounts necessary to complete the energy and protein requirements. The animals were given the diet under investigation for a preliminary period of 14 days before rumen liquor and blood samples were withdrawn (Tagari *et al.* 1962). That period was sufficient to assure the adaptation of the ruminal micro-organisms to the assayed rations (cf. Stielau, 1960). Balance experiments were begun after the sampling had been completed.

The amount of digestible crude protein supplied by the diets used in the different experiments is given in Table 3 and is expressed as a percentage of the protein requirement contributed by the various foods. The amounts of the different feeds supplied can be calculated from the values in Tables 1–3 on the assumption that the energy content of the rations was adjusted to the theoretical requirements with cottonseed hulls, grain or starch.

Sampling. Samples of rumen liquor were removed by suction through a Polythene tube of internal diameter 5 mm. Samples of blood for urea estimation were withdrawn from the jugular vein before the morning feeding and also later, after different time intervals.

The analysis of rumen liquor and blood was carried out on samples withdrawn before the morning feeding and after several time intervals on each of 3 alternate days unless otherwise stated.

Determinations of NH_3 and other components of rumen liquor were carried out on samples withdrawn at feeding time and after fixed time intervals ranging between 1 and 3 h and extending to 8 h after feeding. Sampling blood for urea determinations was continued up to 14 h after feeding unless otherwise stated. This is the time interval during which the concentrations of the respective metabolites are still related to the feed administered (Lewis, 1960).

Artificial rumen technique. A modification of the procedure of Huhtanen, Saunders & Gall (1954) was used (cf. Tagari *et al.* 1962). The digestion was terminated by acidification with trichloroacetic acid (TCA) to a final concentration of 10% (w/v). The content of the dialysis tube was centrifuged (2400 *g*; 20 min) and the supernatant liquid was mixed with the fluid surrounding the dialysis tube. NH_3 and NH_2 groups were determined in portions of the mixed fluids.

Chemical methods

NH_3 in rumen liquor. Conway's (1957) method was used, after microbial activity had been stopped by the addition of TCA to a final concentration of 10% (w/v) and centrifuging at 2400 *g* for 10 min.

NH_2 groups. These were determined by the copper chelation procedure of Pope & Stevens (1939). This method, however, was found to be unsuitable for the analysis of rumen liquor, because of colour interference. It was only used for NH_2 determinations in the contents of the artificial rumen.

Total soluble nitrogen. Fresh rumen liquor, clarified by centrifuging at 30000 *g* for 30 min was digested by the usual Kjeldahl method, and the NH_3 formed determined by the Conway (1957) procedure.

Table 3 *Digestible crude protein (DCP) supplied in the different experiments by the various components of the rations, expressed as percentages of the theoretical protein requirement*

Feeding Expt no.	Stage of experiment	Treatment Symbol*	Treatment Description	Protein supplied (as % of theoretical requirement)	DCP supplied by feeds† At 07.30 h Soya-bean meal	Maize gluten feed	Crushed barley	Milled foxtail millet hay	At 15.30 h Millet hay	Sugar-beet pulp dried
1	1	a	—	50	—	—	—	5·8	44·2	—
	2	a	—	50	—	—	—	13·0	37·0	—
		b	—	100	50·0	—	—	13·0	37·0	—
		c	—	150	100·0	—	—	13·0	37·0	—
2	1	—	Control	100	50·0	—	—	13·0	37·0	—
		O	—	150	100·0	—	—	11·1	38·9	—
				175	125·0	—	—	9·1	40·9	—
				200	150·0	—	—	7·5	42·5	—
	2	—	12·5 % of the energy	100	50·0	—	10·7	—	39·3	—
		P	supplied by barley	150	100·0	—	10·8	—	39·2	—
				175	125·0	—	11·0	—	39·0	—
				200	150·0	—	10·7	—	39·3	—
	3	*Q*	Same as 2‡							
							Maize grits		Rhodes hay	
3	1	*K*	Control	100	47·8	23·2	—	—	18·0	11·0
		L	17·2 % of the energy content supplied by maize	100	47·8	11·6	11·6	—	18·0	11·0
		M	34·4 % of the energy content supplied by maize	100	47·8	—	23·2	—	18·0	11·0
4	1	*G*	Control	100	50·2§	19·8	—	—	30·0	—
		H	35 % of the energy content supplied by maize	100	50·2§	—	19·8	—	30·0	—
5	1	*A*	See text p. 347	100	70·0	—	—	—	30·0	—
		B		100	49·3	20·7	—	—	30·0	—
		C		100	49·3	—	20·7	—	30·0	—
		D		120·7	70·0	—	20·7	—	30·0	—

* a, diet supplying 50 % of protein requirement; b, supplying 100 %; c, supplying 150 %.

† The protein content of cottonseed hulls was taken to be nil; amounts of it were added in order to satisfy the energy requirements of the sheep.

‡ But with an additional 22·5 % of the energy content supplied by potato starch.

§ Groundnut meal instead of soya-bean meal.

Soluble non-protein N (NPN). TCA was added to centrifuged rumen liquor to a final concentration of 10% (w/v). To remove additional precipitable material this mixture was allowed to stand for 24 h at 2° (Brady, 1960). Kjeldahl determinations were carried out on portions of the filtrate after centrifuging at 3300 *g* for 20 min.

Soluble protein N. The amount was calculated by subtracting values for the soluble NPN from those for the total soluble N; the amount of intermediate products of protein breakdown was calculated by subtracting the NH_3 values from those for the soluble NPN.

Individual amino acids in rumen contents. They were determined by the modification of the dinitrophenylation procedure of de Muelenaere, Chen & Harper (1961). A tenfold amount, by weight, of a mixture of saturated Na_2CO_3 and $NaHCO_3$ solutions (molar ratio 1:9) was added to samples of 100–200 mg freeze-dried dialysate of rumen liquor. Paper chromatography of the ether-soluble DNP-amino acids was done by the method of Pairent & Williamson (1960).

Keto acids in rumen contents. The method developed by Reitman & Frankel (1957) for the colorimetric determination of transaminases in blood was used. Samples of rumen liquor to which equal volumes of 0·4 N-NaOH were added (before addition of dinitrophenyl-hydrazine reagent) served as blanks.

Determination of the dehydrogenase activity in rumen liquor with triphenyltetrazolium chloride (TTC). This method, used for estimating the dehydrogenase activity exerted by soil bacteria (Stevenson, 1959), was applied to rumen micro-organisms. Preliminary experiments showed that maximum extinction of the triphenylformazan formed was obtained when the mixture was incubated for 5 min and contained a final concentration of 0·15% TTC.

A 100 ml flask was filled with CO_2, and 8·5 ml filtered rumen liquor were introduced. After the addition of 1·5 ml 1% TTC the mixture was incubated at 37° for 5 min with continuous shaking. The reaction was then stopped by the addition of 10 ml isopropanol. The mixture was centrifuged at 2400 *g* for 20 min. The clear solution was decanted and diluted with 4–6 vol. of 50% isopropanol. The extinction was read at 485 mμ in a Bausch & Lomb Spectronic colorimeter against a blank which was prepared in the same way, but with isopropanol added before TTC.

Urea. Urea was determined by Conway's (1957) method in 0·3 ml blood withdrawn by syringe. Heparin was the anticoagulant.

Calculation and statistical analysis

To express the variation of concentrations of metabolites with time as a single characteristic number the following calculation was used: the curves giving the concentrations against time were integrated by measuring the areas below them, and the results thus obtained were expressed in m-equiv. N × h/l. rumen liquor or blood.

The three separate measurements obtained (on 3 alternate days) for each sheep could not be regarded as statistically independent observations, at least as far as a between-sheep analysis was concerned; indeed, when the values were plotted in a scatter diagram, the three measurements could easily be seen to be clearly correlated. Therefore all the statistical analyses (including the fitting of regression lines) were

performed on 3-day (individual sheep) averages. All statements about statistical significance refer either to the 5% (significant) or the 1% (highly significant) levels.

For calculating the true digestibility of the nitrogenous constituents, 0·2 or 0·5 g metabolic N was subtracted, for concentrates and roughage respectively, from the amount of undigested nitrogenous constituents expressed as N in every 100 g feed (Mitchell, 1943).

Influence of various levels of protein on its utilization

In vitro Expt 1. Preliminary experiments were conducted in an artificial rumen in order to examine the extent of decomposition of different amounts of proteins subjected to the action of equal volumes of rumen liquor.

Rumen liquor was withdrawn from a 2-year-old Awassi sheep consuming a daily ration of 120 g soya-bean meal and 2 kg cottonseed hulls. All the soya-bean meal and 0·8 kg cottonseed hulls were consumed in the morning. The amounts of substrates incubated with 10 ml rumen liquor were calculated on the assumption that the capacity of the rumen of a sheep is 6 l. (according to our measurements on eight slaughtered Awassi sheep). Hence an incubation mixture of 200 mg soya-bean meal, 1200 mg milled cottonseed hulls and 10 ml rumen liquor may be considered to correspond to a ration supplying the theoretical (i.e. 100%) energy and protein requirements. In five different sets of experiments the respective amounts of both substrates supplying 25, 50, 100, 200 and 300% of the theoretically required amounts of energy and protein were incubated with 10 ml rumen liquor. Levels of free NH_3 and of amino groups liberated in these fixed time intervals are given in Fig. 1. When less than 200% of the theoretically required amount of protein was subjected to in vitro digestion, only a very small amount of NH_2-N could be detected in addition to quite considerable amounts of NH_3. A marked deamination of amino acids primarily resulting from proteolysis may perhaps explain the low concentration of NH_2 groups alongside the high concentration of NH_3. When a large excess of protein was digested in the artificial rumen, the amounts of the amino groups liberated attained their maximum after 4 h. Since the process of deamination continued, although no appreciable amounts of protein were supplied to the micro-organisms, the concentraton of NH_2 groups decreased steadily.

NH_3 concentration curves of the rumen liquor were almost identical during the first 4 h of incubation, irrespective of whether 100, 200 or 300% of the calculated amount of protein was incubated in the artificial rumen, which points to limited decomposing activity of the rumen micro-organisms. A rectilinear and continuous increase in the amount of NH_3 liberated, resulting from a large excess of protein, was observed at later stages of the incubation.

Because of the extent of NH_3 liberation in in vitro incubation of excess protein with rumen liquor, it seemed worth while to study its metabolic pathways in the rumen of the intact animal.

Feeding Expt 1. During the preliminary period (stage 1), four sheep (weight 44–67 kg) were given a ration supplying 50% of their calculated protein requirements. During the three following experimental periods (stage 2), the sheep were subjected to

three treatments as shown in Table 3. They received (*a*) 50%, (*b*) 100%, or (*c*) 150% of the required amount of protein. The sources of the protein contained in the different rations are stated in Table 3.

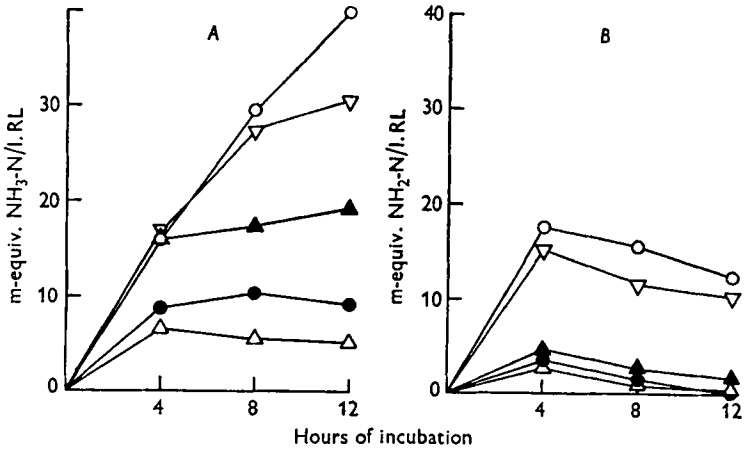

Fig. 1. In vitro Expt 1. Influence of amount of substrate (soya-bean meal and cottonseed hulls) on liberation of NH_3 (*A*) and amino groups (*B*), in an artificial rumen. Amounts of substrates as percentage of the theoretical requirements: △——△, 25%; ●——●, 50%; ▲——▲, 100%; ▽——▽, 200%; ○——○, 300%. RL, rumen liquor.

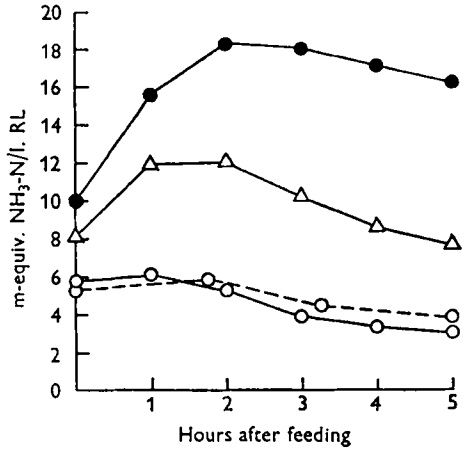

Fig. 2. Feeding Expt 1. Rumen NH_3 concentration of sheep given a ration containing: (stage 1) 50%, ○– – –○, or (stage 2) 50%, ○——○, or 100%, △——△, or 150%, ●——●, of the theoretical requirement of digestible protein. RL, rumen liquor.

It is clear from Fig. 2 that an increase in the protein content of the ration resulted in increased liberation of NH_3. The peaks of the time–concentration curves were delayed with increasing protein content of the ration. The correlation between the protein content of the ration and the amount of NH_3 liberated is clear when the values obtained by integration of the NH_3 accumulation curves are compared (Table 4). The statistical analysis of the integration values showed that the amount of NH_3 liberated in the

rumen was not significantly influenced by individual differences of the sheep or periods, but depended primarily on the nature of the ration.

Feeding Expt 2. Rations supplying 100, 150, 175 and 200% of the theoretical requirements of protein were given to the four sheep used in the preceding experiment. The experimental plan and the amount of protein contributed by the different feeds are given in Table 3 (Expt 2, stage 1, control treatment).

Table 4. *Feeding Expt* 1. *Rumen NH_3 concentrations in sheep given rations supplying* 50 (*a*), 100 (*b*) *and* 150% (*c*) *of the theoretical protein requirement*

(Mean values of the integrations (m-equiv. N × h/l.)* for results obtained on 3 alternate days)

	Stage 1	Stage 2		
Sheep no.	(*a*)	(*a*)	(*b*)	(*c*)
1	28·8	21·1 (3)	51·6 (1)	75·8 (2)
2	23·9	— (3)	45·7 (2)	84·8 (1)
3	19·3	20·1 (2)	58·4 (1)	81·1 (3)
4	25·3	27·2 (1)	46·4 (3)	85·6 (2)
Treatment mean	24·3	22·8	50·5	81·8

Figures in parentheses show experimental period.
The standard error of the treatment mean values was ±·5.
* Measurements of NH_3 concentrations were carried out for 5 h after feeding.

In addition to NH_3, soluble protein N (Fig. 3*A*) and the intermediate products of protein breakdown (soluble NPN less NH_3; see Fig. 3*B*) were determined in rumen liquor, as well as the urea content of the blood (Fig. 3*C*). Fig. 3 shows good correlation between the protein content of the ration and the levels of rumen NH_3 (Fig. 3*B*) and of blood urea (Fig. 3*C*). A comparison of Figs. 3*B* and 3*C* reveals that the highest level of urea in the blood was found 5 h after the peak in rumen NH_3 concentration was reached. This time lag is in accordance with the findings of Lewis (1957) and with earlier results obtained in this laboratory (Tagari *et al.* 1962).

The correlation between the protein content of the ration and rumen NH_3 and blood urea concentration is particularly clear when the excesses of rumen NH_3 and blood urea above the initial levels found at feeding time are compared rather than their absolute amounts (see Fig. 4). The comparison of differences of NH_3 and urea concentrations from their initial concentrations seems to be justified since only these differences can be ascribed to the action of the feed; the usefulness of the comparison of the difference values was evident also in the earlier publication (Tagari *et al.* 1962).

The peak of NH_3 concentration was delayed when increasing amounts of protein were given, as can be seen from the following values (cf. Figs. 2 and 3*A*):

Levels of protein supplied (as % of the required amount)	50	100	150	175	200
Time of peak of NH_3 concentration (h after morning feed)	1·75	1·75	2·00	5·00	5·00

Larger amounts of soluble protein accumulated in the rumen when sheep were given 200% of the required protein quantity than when the 175% protein ration was given. This accumulation of soluble protein in the rumen of sheep given 200% of the required amount of protein was very marked and justifies the assumption of a limited maximum proteolytic, and therefore deaminating, potential of the rumen, which cannot be surpassed even in the presence of a large protein surplus (see Fig. 3). This

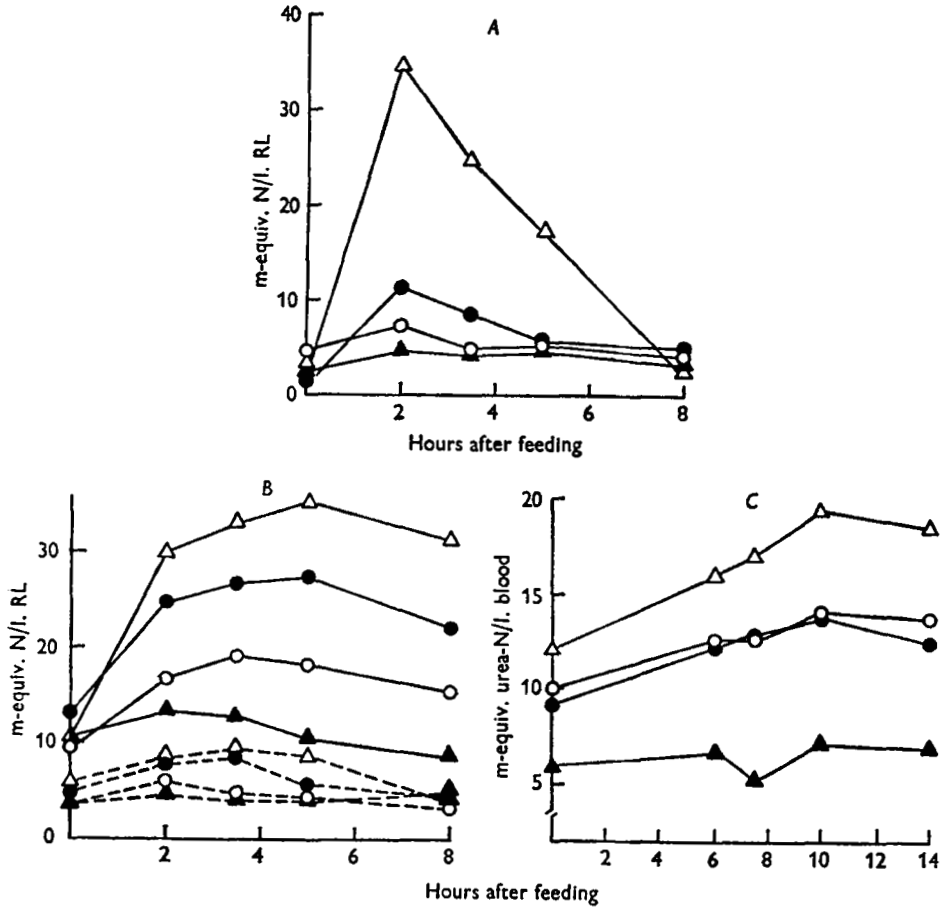

Fig. 3. Feeding Expt 2. Formation of soluble N compounds in the rumen of sheep given a ration supplying 100% (▲), 150% (○), 175% (●) or 200% (△), of the theoretical protein requirement. *A*, soluble protein N; *B*, NH_3-N (——) and intermediate protein breakdown compounds (– – –); *C*, blood urea. RL, rumen liquor.

observation about a limited proteolytic potential of the rumen population is in agreement with results of the in vitro experiments (p. 339). In our work this maximum proteolytic potential appears to have been reached with about 175% of the required amount of protein, particularly during the first hours after feeding. When rations containing 200% of the required amount of protein were given, a rapid increase in soluble protein was found within 2 h after feeding; after this time the rate of

decomposition of soluble protein exceeded that of solution of protein in the rumen liquor and the concentration of soluble protein decreased continuously, at almost rectilinear rate.

The integration values of the time-concentration curves of NH_3 in the rumen and urea in the blood correlated well with the amounts of protein supplied in the rations (Table 5). It seems preferable to correlate the integrated values of the time–concentra-

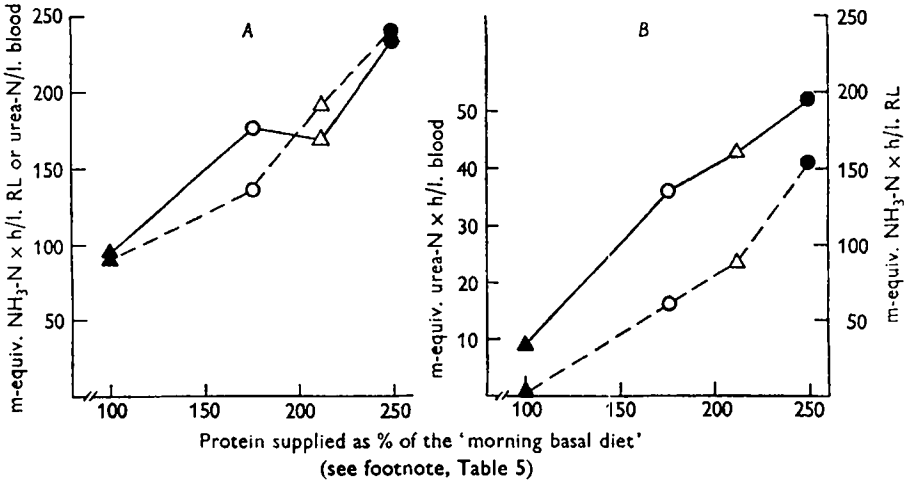

Fig. 4. Feeding Expt 2. Relation between protein content of the 'morning basal ration'* of sheep and integration values of rumen NH_3 (– – –) and blood urea (——). *A*, Total concentrations, *B*, increases above initial concentrations. ▲, Morning basal ration; ○, 176% of the morning basal ration; △, 212% of the morning basal ration; ●, 250% of the morning basal ration. RL, rumen liquor. * The reasons for correlating rumen NH_3 and blood urea values with the protein content of the morning basal ration instead of the whole daily ration are given in the text.

Table 5. *Feeding Expt 2. Influence of different dietary levels of protein on the concentrations of N-containing metabolites in the rumen and blood urea*

(Mean values of integrations (m-equiv. N × h/l.) with their standard errors for results obtained on 3 alternate days)

Protein supplied			Rumen liquor			
As % of theoretical requirement	As % of the morning basal ration*	Sheep no.	Soluble protein	Intermediate products	NH_3	Blood urea
			Total values			
100	100	4	32 ± 9	33 ± 4	90 ± 5	94 ± 5
150	176	1	43 ± 5	36 ± 6	138 ± 10	177 ± 16
175	212	3	53 ± 8	55 ± 8	192 ± 3	170 ± 2
200	250	2	142 ± 51	61 ± 13	241 ± 3	235 ± 8
			Relative values			
100	100	4	100	100	100	100
150	176	1	137	107	152	187
175	212	3	168	165	213	180
200	250	2	450	184	266	248

* Amount of protein contained in the morning portion of the ration supplying the daily theoretical requirement.

tion curves of NH_3 and other N-containing metabolites in the rumen liquor and of urea in the blood with the amount of protein given in the morning ration rather than correlating it with that in the whole daily diet. On this basis the changes in the concentrations of NH_3, N metabolites and urea were followed for the time interval during which only the feed administered in the morning ration affects these metabolites.

The concentrations of other protein decomposition products showed the same trend as those of NH_3, although in a less regular manner, apparently as a result of the competing processes of breakdown and synthesis of protein.

Table 6. *Feeding Expt 2. Influence of replacing roughage carbohydrates by starch on rumen NH_3 and blood urea concentrations*

(Mean values of integrations (m-equiv. N × h/l.) for the results obtained with diets *O* and *Q* on 3 alternate days and with diet *P* on 2 days)

Protein supplied (as % of theoretical requirement)	Sheep no.	*O*. Control		*P*. 12·5% of the energy content supplied by barley		*Q*. Same as *P* but further 22·5% of the energy content supplied by potato starch	
		Rumen NH_3	Blood urea	Rumen NH_3	Blood urea	Rumen NH_3	Blood urea
100	4	90 ± 5	94 ± 5	87 ± 4	80 ± 3	133 ± 11	132 ± 15
150	1	138 ± 10	177 ± 16	160 ± 10	172 ± 2	151 ± 13	193 ± 16
175	3	192 ± 3	170 ± 2	164 ± 13	135 ± 8	192 ± 16	151 ± 6
200	2	241 ± 3	235 ± 8	247 ± 0·1	227 ± 9	251 ± 10	242 ± 30

Effect of easily digestible carbohydrates on protein metabolism of sheep

In this section of the work the possibility of reducing the protein losses resulting from the liberation of NH_3 and excretion of urea was examined. Cottonseed hulls served to complete the energy content of the control ration. In the experimental rations this feed was partly replaced by iso-energetic amounts of starch or grain.

In the second and the third periods of feeding Expt 2, 12·5% and 35% respectively of the energy content of the rations were supplied by barley or starch instead of by cottonseed hulls. The influence of these amounts of starch on protein metabolism was investigated with rations supplying four different levels of protein: 100, 150, 175 and 200% of the theoretical requirements. The experiment was conducted with four sheep. During the previous stage of the experiment (described on p. 340) the sheep received a ration free of starch. During the two consecutive experimental stages the four sheep received the different types of rations presented in Table 3. The rumen NH_3 and blood urea values were integrated and the results are given in Table 6. Supplying from barley 12·5% of the energy contained in the original ration as cottonseed hulls did not cause any marked change in rumen NH_3 or blood urea concentration. A strong deaminative influence, however, was exerted by starch, when this carbohydrate supplied a further 22·5% of the energy content of the ration. This was so only with rations supplying the theoretically required amount of protein, but not with rations containing an excess of protein. The presence of larger amounts of starch in the ration induced a considerable rise in ruminal NH_3 and blood urea concentrations (Table 6, Expt with sheep 4). In order to examine the circumstances

responsible for the deaminative influence of starch, additional experiments were carried out as follows.

Feeding Expt 3. In Expt 2 the influence of starch on the protein metabolism of adult animals was investigated. The purpose of Expt 3 was to establish whether growing animals behave in the same way as adult ones.

Since, in Expt 2, a considerable influence of starch on protein metabolism was found only with rations containing approximately the required amount of protein and not large surpluses of it, in this experiment nine 6-month-old rams, weighing 35–45 kg, received rations supplying their theoretical protein and energy requirements. The animals were divided into three groups of three animals each. The composition of the rations, expressed as percentages of the protein requirement contributed by the different feeds, is given in Table 3.

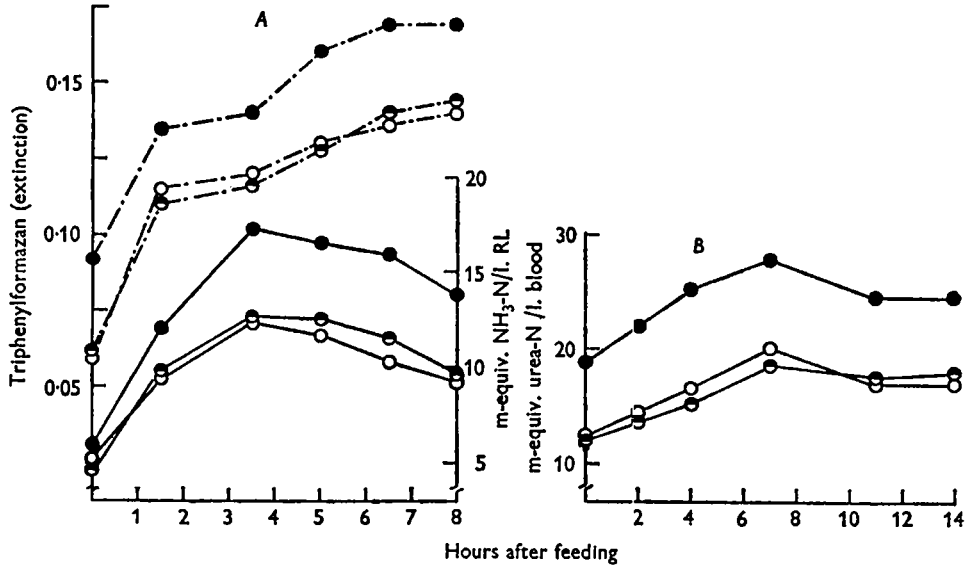

Fig. 5. Feeding Expt 3. Influence of replacing cottonseed hulls by maize in rations of lambs on: (*A*) rumen NH_3 concentrations (——) and dehydrogenase activity (determined with triphenyltetrazolium chloride) (–·–·–) and (*B*) blood urea concentrations. ◓, control rations; ○, 17·2% of the energy content replaced by maize; ●, 34·4% of the energy content replaced by maize. RL, rumen liquor.

Cottonseed hulls were added to the control ration to complete the energy requirements of the animals. In the experimental rations 17·2% and 34·4% of the energy requirements were supplied by partly substituting maize for cottonseed hulls. To ensure an equal protein composition, those rations without maize, or containing only 17% maize, were supplemented with the necessary amounts of maize protein in the form of maize gluten feed. The concentration–time curves of rumen NH_3 and blood urea (Fig. 5) and their integrated values (Table 7) revealed that the lower levels of maize did not have any effect, whereas the substitution of larger amounts of cottonseed hulls by maize induced a marked increase in rumen NH_3 and blood urea con-

centrations, the differences in the rumen NH_3 values being significant and those in the blood urea values highly significant.

The results of both the feeding trials (2 and 3) conducted with adult and growing sheep, are in good agreement. The replacement of moderate amounts of roughage carbohydrates by starch—which represented 17·2% of the energy content of the ration in Expt 3—did not influence ruminal NH_3 and blood urea concentrations. The inclusion of larger quantities of the starch in the ration (34·4%), however, induced a significant rise in ruminal NH_3 and consequently in blood urea concentration, results which agree with those of Expt 2.

Table 7. *Feeding Expt* 3. *Influence of replacing cottonseed hulls by maize on rumen NH_3 and blood urea concentrations*

(Integration values expressed as m-equiv. N × h/l.)

	Control			17·2% of the energy content supplied by maize			34·4% of the energy content supplied by maize		
Lambs nos. ...	4	6	8	2	5	7	1	3	9
				Rumen NH_3					
Mean/lamb	82	103	78	71	85	94	113	121	112
Mean/treatment		88			83			115	
Standard error of the mean 6·1									
				Blood urea					
Mean/lamb	85	113	124	106	104	123	178	163	144
Mean/treatment		107			111			162	
Standard error of the mean 9·4									

Determination of the dehydrogenase activity in rumen liquor of rams receiving rations of different starch content

As an additional criterion for the biochemical activity of rumen liquor from rams receiving different amounts of starch in their ration (feeding Expt 3), the dehydrogenating activity towards triphenyltetrazolium chloride was examined. Fig. 5 shows that there was a good correlation between the dehydrogenase and deaminative activities of samples of rumen liquor as influenced by the presence of starch in the ration. The rumen liquor obtained with a high-starch ration also had the highest dehydrogenating activity, whereas the rumen liquor of sheep fed on the lower-starch ration was only slightly superior in this respect to the rumen liquor of sheep on starch-free rations.

Deaminating activity of rumen liquor of rams receiving rations of different starch content, examined in the artificial rumen

In vitro Expt 2. The deaminating activity of rumen liquor as influenced by the starch content of the ration was also examined in in vitro experiments. A mixture of soya-bean meal and maize gluten feed was incubated with rumen liquor withdrawn from sheep fed on either a starch-free ration (treatment *K* of Expt 3) or rations in which

17·2 or 34·4% of their energy content was supplied as maize (treatments *L* and *M*, respectively), and the NH_3 liberated after 8 h of incubation was measured (Table 8).

The rate of the liberation of NH_3 measured in the three different in vitro incubates (cf. Table 8) was in full agreement with the results of the corresponding in vivo experiments (Table 7 and Fig. 5). The rumen liquor with the strongest deaminative activity was obtained with the high-starch diet, whereas the deaminative activity of the rumen liquor withdrawn from sheep on the low-starch ration only slightly exceeded that of liquor from sheep on the starch-free ration.

Table 8. *In vitro Expt 2. Experiment with artificial rumen. Liberation of NH_3 from proteins incubated with rumen liquor (RL) withdrawn from differently fed sheep, and the influence of carbohydrates added to the incubation mixture on the extent of NH_3 liberation*

Diet of sheep*	Carbohydrates added	Amounts of substrates incubated with 10 ml RL (mg): Soya-bean meal	Maize gluten feed	Cotton-seed hulls	Milled maize	NH_3 liberated† (m-equiv. NH_3-N/l.RL)
K	None	133	53·4	—	—	20·4
L		133	53·4	—	—	22·6
M		133	53·4	—	—	30·7
K	Small amount	133	53·4	333	—	8·5
L		133	26·7	—	133	13·1
M		133	26·7	—	133	19·9
K	Large amount	133	53·4	667	—	3·2
L		133	26·7	333	133	3·8
M		133	—	—	267	12·6

* *K*, starch-free; *L*, 17·2% of the net energy content supplied by starch; *M*, 34·4% of the net energy content supplied by starch.

† Net values after subtraction of blank values.

The deaminating power of the three different kinds of rumen liquor was also examined in additional in vitro experiments in which the protein-rich substrate (a mixture of soya-bean meal and maize gluten feed) was supplemented with carbohydrate feeds, such as cottonseed hulls or maize meal (Table 8). In all these experiments equal quantities of protein were incubated with the same volumes of rumen liquor. The addition of carbohydrate as starch as well as in the form of cottonseed hulls, led to a decrease in the amount of NH_3 liberated. This was true for all rumen liquor samples examined (in in vitro experiments), whether these had been taken from sheep fed on starch-containing diets (*L* and *M*) or from animals on starch-free rations (*K*). The extent of the decrease observed showed good correlation with the amounts of carbohydrate added to the protein substrates. This protein-sparing influence of carbohydrate is in agreement with in vivo experimental results reported by McDonald (1952), Williams *et al.* (1953), Lewis (1957) and Phillipson *et al.* (1962), who studied the effect of adding carbohydrates to different types of rations. In our feeding experiments opposite results were obtained when less-soluble roughage carbohydrates were replaced by starch (in iso-energetic rations).

Feeding Expt 4. An additional experiment was conducted in which four male and four female sheep, all over 4 years of age and weighing 47–83 kg, were used. Groundnut meal replaced soya-bean meal as the main protein source in order to exclude the possibility that the enhanced protein degradation induced by the inclusion of starch in the ration was caused by some specific property of the soya-bean meal. In the previous experiments the effect was evident only in the presence of a high level of starch, so in this trial only the influence of a high level of starch was examined. In the experimental ration cottonseed hulls constituting 35% of the energy content of the control ration were replaced by maize grits. Both the experimental and control rations contained 100% of the required amount of protein. To ensure an equal protein composition in both rations, a quantity of maize gluten feed equivalent to the maize protein present in the experimental ration was included in the control ration (Table 3). By limiting the experiment to two treatments it was possible to examine a larger number of animals. The experiment was divided into two periods, and two male and two female animals were allotted to each group. The animals of each group were interchanged at the end of the first period (change-over design).

Table 9. *Feeding Expt* 4. *Influence of replacing cottonseed hulls (in control ration G) by maize (ration H), to the extent of* 35% *of the energy content, on rumen* NH_3 *and blood urea concentrations*

(Integration values expressed as m-equiv. N × h/l.)

	Sheep no.*								
Ration	1	5	4	8	3	9	2	6	Mean
				Rumen NH_3					
G	70†	58†	32†	42†	47‡	56‡	45‡	31‡	48
H	81‡	79‡	65‡	87‡	61†	63†	86†	53†	72
				Blood urea					
G	96†	65†	40†	45†	61‡	65‡	52‡	40‡	58
H	103‡	94‡	65‡	72‡	81†	94†	80†	57†	81

Standard error of the mean for rumen NH_3 3·6, and for blood urea 2·1.

* Even numbers, male animals; odd, female animals.

† Period A.

‡ Period B.

The shapes of the curves obtained were very similar to those obtained in the preceding experiments. Integration values of concentration–time curves are given in Table 9. They show a highly significant increase in ruminal NH_3 and blood urea concentrations as a result of replacement of cottonseed hulls by maize.

Feeding Expt 5. The purpose of this last experiment was to exclude the possibility that the enhanced degradative activity of the starch-containing rations was influenced by the nature of the particular cereal protein included in them, and to provide additional evidence for the increased deaminative action induced by the replacement of roughage carbohydrates by starch.

The source of the proteins contained in the rations given to the different groups is given in Table 3. Soya-bean meal served as the main protein source in all the rations. Treatments A and B were regarded as controls, since the diets contained considerable

amounts of cottonseed hulls and were similar to the control rations used in the preceding experiments. Of the energy content of rations *C* and *D*, 35% was supplied by maize, which replaced iso-energetic quantities of cottonseed hulls in rations *A* and *B*. In ration *B*, 20·7% of the theoretical protein requirements was supplied as maize gluten feed, in order to equalize the protein composition of rations *B* and *C*.

Ration *D* contained, in addition to the same amount of soya-bean meal as ration *A*, the protein present in maize grain, which served as a source of soluble starch. The protein content of ration *D* was therefore raised to 121% of the required amount. All the rations *A*–*D* were iso-energetic.

Table 10. *Feeding Expt* 5. *Influence of replacing cottonseed hulls by maize on rumen NH_3 and blood urea concentrations in sheep given ration A, B, C, or D, in which the protein source differed in part*

(Mean values* of integrations expressed as m-equiv. N × h/l.)

Period	*A*		*B*		*C*		*D*	
	Rumen NH_3							
1	90·1(1)	101·7(4)	65·1(5)	57·5(8)	73·5(3)	84·8(2)	95·6(9)	101·6(6)
2	50·2(1)	66·1(6)	70·1(5)	49·1(2)	63·4(3)	58·4(8)	69·1(9)	74·2(4)
3	57·4(9)		71·8(3)		78·8(5)		102·4(1)	
Mean	73·1		62·7		71·7		88·5	
	Blood urea†							
1	116·8(1)	95·9(4)	65·1(5)	49·8(8)	95·1(3)	85·5(2)	81·7(9)	129·0(6)
2	63·8(1)	75·8(6)	79·8(5)	57·8(2)	97·2(3)	63·6(8)	86·2(9)	76·5(4)
3	77·0(9)		106·7(3)		98·6(5)		119·1(1)	
Mean	85·8		71·8		88·0		98·5	

Figures in parentheses designate the individual sheep. Even numbers, male animals; odd, female animals.

* Mean standard error for individual sheep: rumen NH_3 6·0; blood urea 7·4.

† Measurements of blood urea were carried out during 10 h from the beginning of the morning feeding. Integrations were calculated for 14 h by extrapolation.

This experiment was carried out with the same four males and four females used in the preceding experiment. The plan of the experiment and the results are given in Tables 3 and 10. Each treatment was tested during three periods; in the first two periods one male and one female animal were used, and in the third period only a female was subjected to each treatment. For technical reasons the male animals were interchanged between the first and the second periods and the female animals between the second and the third periods.

In addition to measurements of the ruminal NH_3 and blood urea concentrations the influence of the starch-free and starch-containing diets *B* and *C* was investigated by determining the changes in concentration of some amino acids in the rumen liquor and the N balance of the male animals on rations *A*, *B*, *C* and *D*.

The time–concentration curves of rumen NH_3 and of blood urea obtained with rations *B* and *C* and the corresponding integration values (Table 10) clearly confirmed the results of the preceding experiments regarding the influence of starch in

increasing the protein-decomposing activity of the rumen. Since rations *B* and *C* contained equal amounts of the same protein, this effect can only have been caused by the presence of starch in ration *C*.

The extent of NH_3 liberation and blood urea formation occurring with ration A exceeded the corresponding values obtained with ration *B*. Since both were starch-free rations, this effect was probably due to the greater susceptibility to protein decomposition of soya-bean meal compared to maize. The same factor was apparently responsible for the similar results obtained with rations *A* and *C*, in spite of the presence of starch in ration *C* and its absence from ration *A*. The greater amounts of readily decomposable soya-bean protein in ration *A*, and starch and a part of the protein as a less decomposable maize protein in ration *C*, induced the liberation of approximately equal amounts of NH_3.

The values of rumen NH_3 and blood urea with ration *D* were high and considerably exceeded those obtained with ration *C*. As both rations contained starch, this effect may be explained again by the presence of an excess of readily decomposable soya-bean protein in ration *D*.

Concentrations of various amino acids in rumen liquor of sheep given rations with and without starch

Subsequently the concentrations of several amino acids in the rumen content of sheep receiving the same protein sources but in the presence of starch (*C*) or its absence (*B*) were assayed (see Table 11). Samples of rumen liquor withdrawn at feeding time from sheep on both kinds of ration contained very similar concentrations of the different amino acids (except glycine). The concentrations of most of the amino acids increased considerably during the first 2 h after feeding. This increase was rather higher in sheep fed on a starch-containing ration (*C*), as compared to those receiving a starch-free ration (*B*). This latter result agrees with other results reported in this study which indicate that the presence of starch in rations induces higher deaminative and dehydrogenase activities, and it is also consistent with observations mentioned by Orth & Kaufmann (1961); these authors refer to the high concentrations of several amino acids in rumen liquor found with rations high in starch compared with those of high cellulose content. As the concentrations of amino acids decreased again between 2 and 8 h after feeding, the absolute concentrations of most of the amino acids measured in the rumen contents on the starch-rich ration (*C*) at 8 h after the beginning of feeding were higher and only a few of them were lower than those present in the rumen contents of sheep on ration *B* at the same time. The total absence of glutamic acid and threonine from rumen contents on ration *C* as analysed 8 h after feeding is remarkable. The disappearance of glutamic acid from the rumen contents of sheep fed on the starch-rich ration may be accounted for by the enhanced activity of dehydrogenases in the rumen contents as observed after feeding on such rations. A widespread occurrence of glutamic acid dehydrogenase has been reported (cf. Cohen & Sallach, 1961).

Further support for the higher enzymic activity prevailing in the rumen contents of sheep fed on a starch-containing diet can be obtained from the determination of keto

acids. It appears from Table 12 that the rumen liquor of sheep fed on a starch-free diet (*B*) contained comparatively low concentrations of keto acids which fluctuated only slightly during the day. In the rumen liquor of sheep fed on a starch-containing ration (*C*) the concentrations of keto acids measured at feeding time were much higher, decreased considerably up to 4 h after feeding and increased again between 4 and 8 h. An increase in the concentration of keto acids was accompanied by an overall decrease in the concentrations of most of the amino acids, except for a marked increase in the concentration of aspartic acid (Table 11, treatment *C*, concentrations found after 8 h). These results indicate a transaminase activity.

Table 11. *Concentrations (m-equiv. N/l.) of various amino acids in rumen liquor of sheep given rations with maize (C) or without maize (B) at 0, 2 and 8 h after feeding*

	B			*C*		
Amino acid	0 h	2 h	8 h	0 h	2 h	8 h
Alanine	0·270	0·395	0·316	0·304	1·293	0·591
Aspartic acid	0·145	0·180	0·214	0·179	0·414	0·591
Glutamic acid	0·142	0·189	0·218	0·106	0·626	0·0
Glycine	0·294	0·337	0·299	0·092	0·593	0·425
Leucine	0·058	0·085	0·031	0·031	0·055	0·059
Lysine	0·043	0·059	0·061	0·046	0·149	0·118
Ornithine	0·043	0·092	0·076	0·042	0·101	0·106
Phenylalanine	0·047	0·102	0·322	0·047	0·142	0·183
Proline	0·072	0·109	0·059	0·087	0·417	0·122
Serine	0·107	0·249	0·225	0·150	0·501	0·272
Threonine	0·065	0·159	0·129	0·045	0·310	0·0
Valine	0·081	0·169	0·126	0·083	0·447	0·151

The analyses were done on mixed samples of rumen liquor, withdrawn from two sheep on each treatment.

Table 12. *Concentrations (m-equiv./l.) of keto acids in rumen liquor of sheep given rations with maize (C) or without maize (B) at 0, 2, 4 and 8 h after feeding*

	0 h	2 h	4 h	8 h
B	0·24	0·30	0·30	0·41
C	0·58	0·30	0·15	0·53

Measurement of N balance

To examine the validity of the conclusions regarding the protein utilization of sheep, the N balance of the male animals used in feeding Expt 5 was determined. The results of measurements of N balance are summarized in Table 13, and they agree with the values for rumen NH_3 and blood urea given above, in that the presence of starch, which enhanced the formation of both these metabolites, caused a decline in the amount of N retained. This is clear from a comparison of the quantities of N retained with ration *B* as compared with ration *C*, and ration *A* as compared with ration *D*. The higher percentage of N present in ration *D* did not counteract the negative action of starch on N retention, as seen from the greater N retention observed with ration *A*, which contained less N than ration *D*.

Protein digestion coefficients of the starch-containing rations *C* and *D* were higher than those of the corresponding starch-free rations *A* and *B* respectively. The digestibility coefficients were comparatively low, since the presence of considerable amounts of cottonseed hulls in the diet caused a decrease in apparent digestibility. The same effect of starch persisted when the true digestion coefficients of the protein contained in the different rations were calculated and compared.

Table 13. *Feeding Expt 5. Mean values for N digestibility and N balance for sheep on diets containing roughage carbohydrates (A and B) or starch (C and D)*

	A	*B*	*C*	*D*
Daily N intake (g/sheep)	24·45	26·36	21·16	22·39
Daily N excretion in faeces (g/sheep)	12·29	14·60	8·38	7·26
Daily N digested (g/sheep)	12·16	11·76	12·78	15·13
Daily N excretion in urine (g/sheep)	7·47	7·04	9·70	12·97
Daily N retention (g/sheep)	4·69	4·72	3·08	2·16
Apparent digestibility of N (%)	49·7	44·6	60·3	67·1
True digestibility of N (%)	84·4	78·5	88·7	89·7
N excretion in urine as % of N digested	61·6	59·9	75·4	86·1
N retained per day (g/kg body-weight$^{0.74}$)	0·197	0·182	0·127	0·093

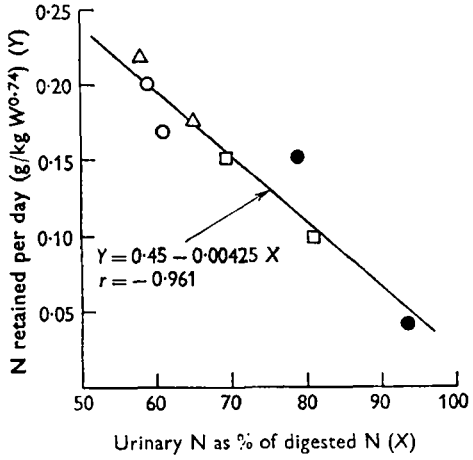

Fig. 6. Feeding Expt 5. Relation between urinary N excretion and N retention of sheep given rations in which the energy source varied in part (maize compared with cottonseed hulls). △, ration A; ○ ration B; □, ration C; ●, ration D (see p. 347). *W*, body-weight.

The presence of starch caused an increase in the protein digestibility coefficient under our experimental conditions. In spite of this increased digestibility of protein in starch-containing rations, the presence of starch was accompanied by a decrease in the N retention, as a result of enhanced deamination and the excretion, as urea in the urine, of the liberated NH_3. An increase in the percentage of the digested N excreted in the urine was followed by a decrease in the amount of N retained by the sheep (see Fig. 6). The values shown in this figure suggest a rectilinear relationship between these factors; calculations show the rectilinear correlation ($r = -0{\cdot}961$) to be highly significant. As a result, the animals did not benefit from the increase in protein digestibility induced by substituting starch for roughage carbohydrates.

DISCUSSION

Balance experiments constitute, apart from the costly and complicated technique of carcass analysis, the most reliable technique for evaluating protein feeds for ruminants. However, this method is also tedious and does not permit sufficient insight into the pathways involved in protein metabolism. Other potential methods for assessing the value of protein feeds depend on the determination after feeding of the changes in urea concentration in blood or in NH_3 or other products of protein decomposition present in the rumen liquor. Considerable attention has been given in the past to finding how far the latter criteria reflect the efficiency of protein feeds in ruminant nutrition (Lewis & McDonald, 1958; Whitelaw, Preston & Dawson, 1961; Chalmers, 1961*a*; Lewis, 1962).

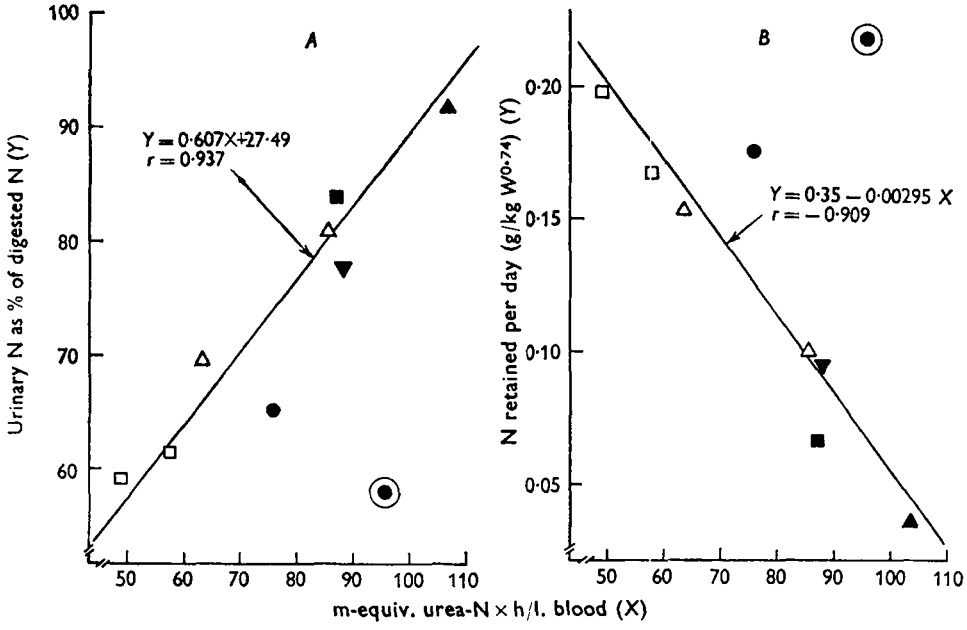

Fig. 7. Relation between blood urea concentrations and (*A*) N excreted in urine and (*B*) N retention, in rams given a ration supplying their theoretical requirement of protein. Values from feeding Expt 5: ●, ration A; □, ration B; △, ration C (see p. 347). Values from Tagari *et al.* (1962): ▼, raw soya-bean meal; ■, commercial untoasted soya-bean meal; ▲, toasted soya-bean meal. The circled point refers to an animal that was ill shortly before the experiment began. *W*, body-weight.

Our findings concerning the relationship between blood urea and N retention are presented in Fig. 7. A clear pattern of increasing urinary N excretion and decreasing N retention with increasing blood urea values can be seen, except for one animal (circled point in the figure) which is known to have been ill shortly before sampling of rumen and blood began. If the results for this animal are excluded, a highly significant correlation (r = 0·937) between the urea concentrations in blood and the quantities of N excreted in urine (as percentages of the digested N) is obtained from the results of feeding experiments carried out with the various rations examined in this and a

previous study (Tagari *et al.* 1962). This relationship seems to be independent of the nature of the protein feeds. There are two main pathways of blood urea in the ruminant body; the larger part of the urea is eliminated in the urine and a smaller portion is recycled into the rumen; therefore blood urea clearances are of decisive influence on the amounts of N excreted in urine.

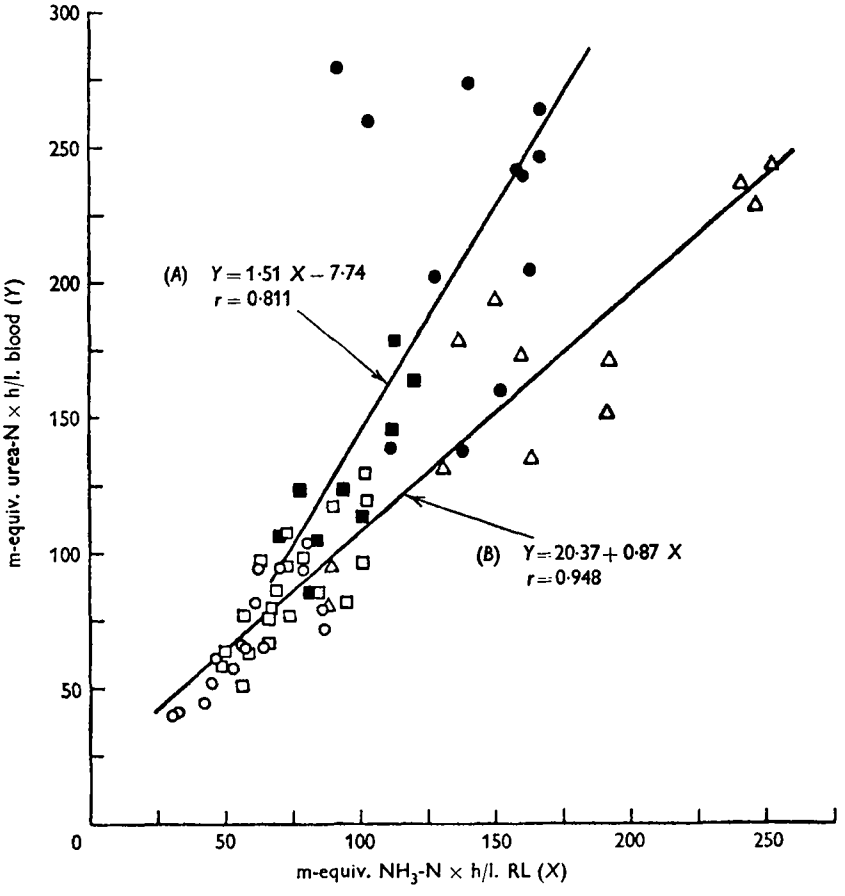

Fig. 8. Relation between rumen NH_3 and blood urea concentrations in (*A*) growing lambs and (*B*) adult sheep. Values from feeding Expt: △, 2; ■, 3; ○, 4; □, 5 described in this paper and ●, from Tagari *et al.* (1962), 200% diet. RL, rumen liquor.

An inverse correlation obviously exists between the amounts of N retained in the body and those excreted in the urine. A relation between blood urea and N retained in the body could therefore be expected. This was observed ($r = -0{\cdot}909$, see Fig. 7).

A relation between rumen NH_3 and blood urea, after the administration of various proteins, was first established by Lewis (1957) and was confirmed in our work; the relation between these factors appears clearly from Fig. 8. Linear regression lines were fitted to the integrated values of rumen NH_3 and blood urea concentration (Fig. 8). The points in this figure represent the experimental results of both this work and that described in the preceding paper (Tagari *et al.* 1962). No significant differ-

ences were found between the regression coefficients obtained with different types of ration at different times, or with male in contrast to female sheep. On the other hand, the slope of the line for adult sheep was significantly different from that for growing sheep; this difference between the two slopes could be due to a difference between the metabolic mechanisms involved in utilizing protein and in differentiating between utilizable and non-utilizable N.

The existence of a correlation between N retention and blood urea and also between blood urea and rumen NH_3 indicates that time curves for blood urea and rumen NH_3 may be useful in assessing protein utilization in the ruminant. The use of blood urea determination, however, seems to be preferable to that of rumen NH_3 assay, owing to the much easier sampling of blood; moreover, the extent of the changes in blood urea concentration as a result of dietary treatments exceeds generally that of the changes in rumen NH_3 concentration. The clear correlation which has been established between blood urea and rumen NH_3 would seem to prove that fluctuations in the volume of rumen contents of individual sheep do not adversely affect the reliability of the rumen metabolite values obtained.

Protein digestion coefficients are generally accepted as an index in the evaluation of feed proteins. This assumption is obviously not quite correct for, according to the results of this work, that part of the protein that is wasted by too rapid ruminal liberation of NH_3 also belongs to the digestible protein fraction. For example, the substitution of roughage carbohydrates by starch caused an increase in the protein digestibility as a result of the enhanced ruminal liberation of NH_3, but it was accompanied by a decrease in the amount of N retained in the body.

Giving excess protein to sheep induced a considerable increase in rumen NH_3 and blood urea concentrations. These findings agree well with observations reported in the literature about the less efficient utilization of excess protein, such as occurs with lush pastures (Head & Rook, 1957). In a search for a way to improve the utilization of protein-rich rations, the possibility of replacing roughage carbohydrates by starch was examined. This change in the composition of the ration often led to considerable increases in ruminal NH_3 and blood urea concentrations and to a decrease in the amounts of N retained in the body. A protein-sparing effect of carbohydrates was by no means revealed, whereas other authors have found a considerable decrease in NH_3 liberation when soluble carbohydrates were added to energy-poor rations. The different trend of action of starch on NH_3 liberation in rations poor or rich in energy is shown by the results of the in vitro experiments described on p. 346. In a recent publication, Lewis (1962), however, found a considerable increase in NH_3 formation when starch was added to rations containing soya-bean protein or arachin as protein source.

The net effect of N utilization in the rumen is determined by the relative rates of the two opposing processes of NH_3 liberation and assimilation, the extent of each process depending on the conditions prevailing in the rumen. An example is given by Reis & Reid (1959), who observed that a decrease in ruminal pH induced by the formation of volatile fatty acids from starch caused a decrease in the rate of NH_3 liberation and an increase in protein biosynthesis.

A marked influence of the nature of the rations on the action of carbohydrate supplement was observed in experiments conducted by Fontenot, Gallup & Nelson (1955). According to these authors, the addition of glucose to rations containing 8% protein resulted in a significant depression in N retention, whereas an increase in N retention occurred when glucose was added to a ration containing 10 or 12% protein. In our work the enhancing effect of starch on NH_3 liberation was much more evident when starch replaced roughage carbohydrates in rations supplying just the protein requirements than when used in rations higher in protein. It has to be concluded that a protein-sparing effect of carbohydrates depends primarily on the nature of the rations, and particularly on the ratio of protein to carbohydrate. It appears from the results of this study that the utilization of proteins may not be improved by replacing roughage carbohydrates by starch. According to observations of earlier authors which agree with those of our in vitro experiments, the utilization of proteins can be increased by addition of carbohydrates to rations supplying the calculated requirements of energy and protein.

SUMMARY

1. When different amounts of protein feeds were subjected to the action of an artificial rumen, the extent of ammonia liberation increased with increasing amount of protein in the incubation mixture.

2. Five feeding experiments have been carried out with four to eight adult or nine growing sheep, given different levels of protein and different quantities and kinds of carbohydrates. The influence of these rations on the metabolic pathways of the proteins was examined by following the changes of concentrations of NH_3 and other nitrogen-containing metabolites in the rumen liquor and of blood urea at different time intervals after feeding.

3. An increase in protein level in the ration administered to sheep was followed by an increase in rumen NH_3 and blood urea concentrations. In order to express the variation in concentrations of metabolites with time as a single characteristic number a special method of calculation was used. A significant correlation was then found between the protein content of the rations and the integration value of rumen NH_3 and blood urea concentrations. In addition to concentration–time curves for rumen ammonia, similar curves have been drawn for other protein metabolites present in the rumen liquor, such as soluble protein-N and other intermediate products. They all showed a similar trend.

4. Whereas it was found by earlier authors that the rate of liberation of NH_3 in the rumen content was decreased by addition of starch to the ration and the utilization of protein thereby improved, it was found in this work that replacement of roughage carbohydrates by starch induced a rise in the rate of liberation of NH_3 and therefore an impairment in protein utilization. It was shown that this was not due to the specific nature of the protein present in the ration.

5. The concentrations of amino acids present in rumen liquor greatly increased when a starch-containing ration was given instead of a starch-free one. The presence of

starch in the ration led to an increased dehydrogenase activity of the rumen content as measured by the triphenyltetrazolium chloride procedure.

6. The substitution of carbohydrate roughages by starch resulted in an increased digestibility of protein owing to enhanced protein decomposition in the rumen and led to an increased rate of NH_3 liberation. This was accompanied by a decrease in the N retained in the body. Therefore an increase in protein digestibility does not in these circumstances indicate an improved protein utilization.

7. A significant correlation was found between blood urea concentration and N retention. This justifies the use of blood urea concentration as an index of protein utilization.

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