Protein utilization in the young steer: digestion and nitrogen retention of ^{15}N-labelled rumen bacterial protein

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(*Received 20 April 1983 – Accepted 20 December 1983*)

1. ^{15}N-labelled mixed rumen bacteria, obtained from a steer that had received [^{15}N]urea in its diet, were disrupted ultrasonically and freed from nucleic acids and their degradation products. Samples were subjected to a simulated abomasal digestion with pepsin.

2. The digests were infused with a non-absorbable marker (polyethylene glycol) into the duodenum of four steers equipped with simple duodenal and re-entrant ileal cannulas and adapted to a diet of barley straw, flaked maize and urea. The outflow from the ileum was collected for 6–7 h. The mean value for the digestibility of ^{15}N bacterial proteins in the small intestine was estimated to be 0·74.

3. [^{14}C]urea was administered intravenously during the infusion of the ^{15}N-labelled protein into the duodenum. Urine and faeces were collected for the next 48 h and the proportion of urea-N produced, that was excreted in the urine, estimated from urine ^{14}C excretion. Total urea ^{15}N production was estimated from this value and the amount of ^{15}N excreted in the urine. The mean proportion of ^{15}N absorbed that was deposited in body protein, 0·70, was calculated by difference. The over-all efficiency of utilization of ^{15}N in the infused rumen bacterial protein was 0·52.

4. An approximate estimate of the mean rate of protein synthesis calculated from the data was 24 g/kg body-weight $(W)^{0·75}$ per d and compared with an estimated net deposition of protein of 1·67 g/kg $W^{0·75}$ per d.

5. The importance of these values in factorial schemes for estimating ruminant N requirements is discussed.

The amino acids required for tissue synthesis in ruminants are supplied both by dietary protein that has escaped degradation in the rumen and by microbial protein synthesized in the rumen, but the latter commonly forms the greater part (Smith, 1975). Information is needed both on the digestion of the protein and the efficiency with which the absorbed products are utilized for tissue synthesis in the animal if factorial systems for protein rationing, such as that suggested by the Agricultural Research Council (1980), are to be put into operation. Some information is available on the digestibility of microbial N compounds (Salter & Smith, 1977; Tas *et al.* 1981) but there is little information on the efficiency of utilization of absorbed amino acids. An efficiency value of 0·75 was adopted by the Agricultural Research Council (1980) but this was based mainly on a single unpublished experiment. The purpose of the present study was to obtain further information on these factors by studying the fate of ^{15}N-labelled bacterial protein subjected to simulated abomasal digestion and infused into the duodenum of the steer.

EXPERIMENTAL

Animals and feeding

Five Friesian bull calves were weaned at 11–13 weeks of age on to a diet of barley straw, flaked maize and urea (diet A, Table 1). At 14–18 weeks, test steers 1–4, subsequently used to study the digestion and utilization of bacterial protein, were equipped with simple duodenal and re-entrant ileal cannulas (Smith & McAllan, 1971), and steer 5, to be used as a donor of bacterial protein, was fitted with a simple rumen cannula. Weights of steers 1–4 ranged from 79 to 113 kg over the experimental period, whilst steer 5 weighed 116 kg when used.

Table 1. *Daily intakes (kg) of major diet components*

Diet ...	A		B
Live weight (kg) ...	68–90	91–113	
Barley straw	1·0	1·16	—
Straw pellets*	—	—	1·10
Flaked maize	1·36	1·50	—
Tapioca flake	—	—	1·30
Decorticated groundnut meal	—	—	0·140
Urea	0·024	0·028	0·038

Vitamins and minerals were added to the diets to meet normal requirements. The diet ration for each day was divided into two equal portions given at 09.00 and 16.00 hours.

* Sodium hydroxide-treated.

Preparation and treatment of ^{15}N-labelled protein and mixed rumen bacteria

The donor steer (5) continued to receive diet A for 6 weeks after surgery but was then transferred to diet B because previous experiments (Salter *et al.* 1983) had shown that greater ^{15}N enrichment was achieved in bacteria provided with most of their N in the form of protein rather than urea. After 10 d on this diet its rumen bacteria were labelled with ^{15}N and harvested as follows. On the morning of the day of the experiment no straw pellets were given and the concentrates mixture with 19 g [^{15}N]urea (32·6 atoms % ^{15}N) dissolved in 500 ml 150 mM-sodium chloride was added directly to the rumen at 09·00 hours. No further feed was given until after the final collection of rumen bacteria. The rumen contents were collected by suction through a tube (approximate i.d. 20 mm) as completely as possible at 2·5 h after the infusion of ^{15}N, by means of a pump. In this way approximately half the rumen contents (about 8 litres) was removed. This amount was replaced by an equal volume of a solution containing (mM) sodium acetate 50, sodium bicarbonate 30, potassium acetate 45 and potassium chloride 150 at 39°. This process was repeated at 5 h after infusion and a final harvest collected 19 h later. Bacteria were prepared from each rumen collection as described previously (Salter & Smith, 1977), resuspended in water, pooled and frozen at −20°. The ^{15}N enrichment of the pooled bacteria was 8·41 atoms % excess.

The frozen suspension of rumen bacteria was thawed and homogenized and the bacteria were disrupted by ultrasonic treatment of 100 ml ice-cooled portions of suspension with 50 ml added glass ballotini (diameter 0·22 mm), using an ultrasonic disintegrator (Soniprobe; Dawe Instruments Ltd, London) at maximum power (5 amps) for 40 min. This treatment caused a gradual rise in temperature to 25–30° and the disruption of approximately 95% of the total bacteria. The product was pooled, filtered through a sintered glass filter (porosity 2) and dialysed at 5° against three changes of 10 litres 150 mM-NaCl solution for 39 h. Analysis of the bacterial suspension before disruption and after the final dialysis of the disrupted bacterial debris showed that RNA and DNA were completely destroyed by this process and 27·4% of the total N and 15·4% of the α-amino N were lost during dialysis. Presumably endogenous nucleases were activated by the ultrasonic treatment and the nucleic acid breakdown products were removed by dialysis. The ^{15}N enrichment of the product was unaffected by ultrasonic treatment but decreased to 7·18 atoms % excess after dialysis. This was consistent with the loss of the more highly enriched ^{15}N-labelled free amino acids, ammonia and nucleic acid bases (Salter *et al.* 1979; Blake *et al.* 1983) during dialysis. The product was used without treatment to remove cell-wall debris. Before an experiment it was incubated with pepsin (*EC* 3.4.23.1) at pH 2 to simulate abomasal digestion as described by Salter & Smith (1977).

Estimation of digestibility and efficiency of utilization of ^{15}N-labelled protein from mixed rumen bacteria

One day before the isotope experiment, a test steer was fitted with a harness and bag for the collection of faeces and put into a metabolism cage, equipped with a collecting tray and funnel for urine collections, to accustom it to experimental conditions. On the day of an experiment, the pepsin digest of the ^{15}N-labelled bacterial protein (7·18 atoms % excess ^{15}N in 600 mg total N) in a solution containing 3·0 g polyethylene glycol (PEG) in 500 ml 150 mM-NaCl was infused at a rate of 20 ml/min into the upper duodenum, starting at the time of a normal morning feed (09.00 hours). The feed was withheld until the infusion was complete. At the same time as the labelled protein was infused into the duodenum, [^{14}C]urea (50 μCi, 61 mCi/mmol; Amersham International plc, Amersham, Bucks) was injected in five equal doses at 5 min intervals into the jugular vein. On completion of the infusions the morning feed was given and the animal was given access to water. The re-entrant ileal cannula was disconnected 40 min after completing the infusion and the outflow collected in successive 50-g fractions for the next 6–7 h. Fractions were analysed for ^{15}N and PEG, and digestibility was calculated as previously described from ^{15}N:PEG values in the fractions collected about 3–6 h after infusion which contained most of the ^{15}N and PEG. The animal subsequently received its normal twice daily feeds of diet A. Urine and faeces were collected at intervals throughout the next 36 h. Acetic acid was added to the urine to give a final concentration of 2 g/100 ml. Urine samples were filtered and faeces samples were homogenized with an equal weight of water before storing at $-20°$ until analysed for ^{15}N and ^{14}C. The efficiency of utilization of absorbed ^{15}N was calculated as the proportion of the ^{15}N absorbed in the small intestine that was retained in the animal. This was calculated by assuming that the proportion of the dose of [^{14}C] urea excreted in the urine in 36 h was the same as the proportion of urea, synthesized with a ^{15}N label, that was similarly excreted. (Evidence relating to this is given on p. 534 and in Fig. 1.) On this basis

$$\frac{^{15}\text{N retained}}{^{15}\text{N absorbed}} = \frac{(^{15}N_{inf} - ^{15}N_{il}) - \left(^{15}N_{ur} \times \frac{^{14}C_{inf}}{^{14}C_{ur}}\right)}{^{15}N_{inf} - ^{15}N_{il}},$$

where $^{15}N_{inf}$ is the ^{15}N dose infused into the duodenum (mg excess), $^{15}N_{il}$ is the flow of ^{15}N at the ileum (mg excess), $^{15}N_{ur}$ is the excretion of ^{15}N in the urine (mg excess), $^{14}C_{inf}$ is the dose of [^{14}C]urea infused into the blood (μCi) and $^{14}C_{ur}$ is the excretion of ^{14}C in the urine (μCi).

Estimation of whole-body protein-synthesis rate

The system set up in the present experiment is similar to the two-pool system proposed by Waterlow *et al.* (1978*b*) to describe the situation in man, in which a single dose of ^{15}N-labelled amino acid was infused into the blood. In interpreting results based on excretion of ^{15}N in an end-product and of total N excreted in that end-product, the assumptions made were: (1) that the proportion of isotope excreted in the end-product was the same as the proportion of the N flux similarly excreted, (2) that there was no recycling of label from degradation of protein and (3) that rates remained constant during the experiment. It was thought reasonable to assume that the ^{15}N excreted was distributed evenly throughout the urinary end-products. If these assumptions are adopted in the present experiments, similar calculations may be made from measurements of amounts (g) of total N excreted in urine/24 h (E_t), cumulative excretion of ^{15}N (mg) in the urinary N compounds ($^{15}N_{ur}$) and amount of ^{15}N (mg) in amino acids absorbed from the alimentary tract ($^{15}N_{abs}$). Then amino N flux, Q (g/24 h), equals $\frac{(E_t \times ^{15}N_{abs})}{^{15}N_{ur}}$, from which the rate of protein synthesis, S (g/24 h),

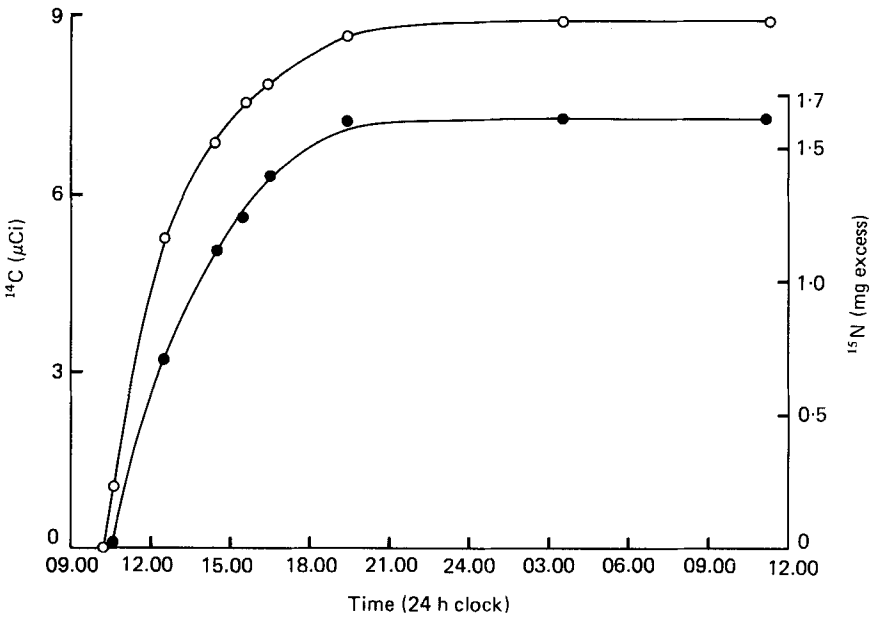

Fig. 1. Cumulative excretion of ^{15}N and ^{14}C in the urine of a steer that had received [^{14}C]urea into the jugular vein simultaneously to an infusion of nucleic acid-free ^{15}N-labelled bacterial protein into the duodenum. (○) ^{14}C, (●) ^{15}N.

may be calculated as $S = Q - E_t$ (Waterlow *et al.* 1978*a*), assuming that E_t gives a maximum estimate of amino acid catabolism. Little error is introduced in using this estimate since E_t is small in comparison with Q.

Analytical methods

Total N, PEG and dry matter were measured and ^{14}C counted as described previously (Salter & Smith, 1977). Total ^{15}N was measured in samples of ileal digesta, faeces and urine and ammonia-^{15}N were measured in samples of ileal digesta by methods described previously (Salter *et al.* 1983) using the NIRD automatic ^{15}N analyser (Goulden & Salter, 1979).

RESULTS

Digestibility of nucleic acid-free ^{15}N-labelled rumen bacterial protein fraction in the small intestine of the steer

The results for the four steers are summarized in Table 2. They show that the mean ^{15}N digestibility of the dialysed rumen bacterial protein fraction in the small intestine was 0·74 and there was very little variation between animals (SEM 0·004).

Efficiency of utilization of absorbed ^{15}N (amino acid ^{15}N)

Fig. 1 shows the patterns of excretion of ^{15}N and ^{14}C in the urine of one of the steers, typical of that observed in all cases. Cumulative excretion of ^{15}N (presumably mainly in the form of urea and derived from the catabolism of the infused labelled protein) was followed closely by that of [^{14}C]urea which had been infused intravenously while the ^{15}N dose was being administered. It is therefore reasonable to assume that the two labelled forms of urea were partitioned similarly between excretion in the urine and elsewhere. From this it may be concluded that, as only approximately 17% of the ^{14}C dose appeared in the urine (Table 2) with the remainder presumably entering the alimentary tract, the same would have

Table 2. *Digestibility and efficiency of utilization of ^{15}N-labelled rumen bacterial protein in the steer*

(Pepsin digested, nucleic acid-free ^{15}N-labelled rumen bacterial protein (533–600 mg N, 7·14 atoms % excess ^{15}N), was infused into the duodenum. At the same time [^{14}C]urea was infused intravenously as described in the text. Ileal digesta were collected and analysed for ^{15}N, and urine for ^{15}N and ^{14}C. The proportions of ^{15}N absorbed and utilized were calculated as described in the text)

Steer no.	Proportion of ^{15}N dose absorbed in small intestine A	Proportion of absorbed ^{15}N recovered in urine B	Proportion of ^{14}C recovered in urine C	Estimated proportion of absorbed ^{15}N converted to urea B/C	Estimated efficiency of utilization of absorbed ^{15}N $1-B/C$	Over-all efficiency of ^{15}N utilization $A(1-B/C)$
1	0·742	0·061	0·156	0·389	0·611	0·454
2	0·737	0·035	0·138	0·256	0·744	0·549
3	0·730	0·047	0·216	0·218	0·782	0·571
4	0·749	0·062	0·179	0·344	0·656	0·491
Mean	0·740	0·051	0·172	0·302	0·698	0·516
SEM	0·004	0·004	0·017	0·039	0·039	0·027

Table 3. *Protein synthesis rate in four steers by end-product ^{15}N analysis*

(Pepsin digested, nucleic acid-free ^{15}N-labelled rumen bacterial protein was infused into the duodenum and samples of ileal digesta were collected to determine absorbed ^{15}N. Urine was collected for 36 h to measure total N excretion/24 h and cumulative ^{15}N excretion to plateau. Protein synthesis rate was determined as described in the Experimental section)

Steer no.	Body-weight (kg)	Urine total N (E_t, g/24 h)	Cumulative ^{15}N excretion at plateau ($^{15}N_{ur}$, mg)	^{15}N in amino acids absorbed ($^{15}N_{abs}$, mg)	Amino acid-N flux (Q, g/24 h)	Amino acid-N synthesis rate ($Q-E_t$, g/24 h)	Protein synthesis rate (g/kg body-weight$^{0·75}$)
1	110	7·49	1·97	32·3	123·1	115·6	21·3
2	113	4·85	1·02	29·2	139·3	134·4	24·2
3	79	3·15	1·03	28·9	85·9	82·7	19·5
4	104	10·87	1·61	26·1	176·5	165·4	31·7
Mean (SEM)	—	—	—	—	—	—	24·2 (2·70)

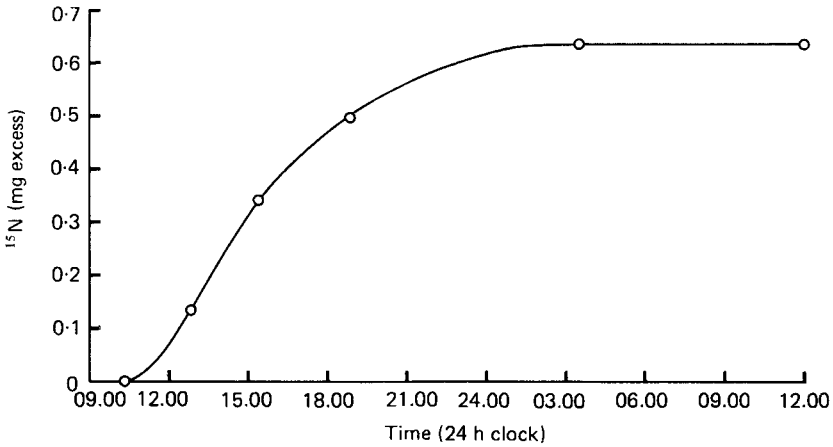

Fig. 2. Cumulative excretion of ^{15}N in the faeces of a steer that had received an infusion of nucleic acid-free ^{15}N-labelled bacterial protein into the duodenum.

been true for [^{15}N]urea. This forms the basis of the equation given on p. 533 for calculating the proportional efficiency of utilization of absorbed ^{15}N. The mean value estimated for this efficiency was 0·70 (Table 2). From this it may be calculated that the mean over-all efficiency of utilization of bacterial protein-N entering the small intestine of the steer was 0·52.

Recycling of ^{15}N to the hind-gut

Fig. 2 shows the recovery pattern of ^{15}N in the faeces of one of the steers, typical of the group. ^{15}N appeared in the faeces within 4 h of the infusion of the labelled bacterial protein in the duodenum, but no further detectable ^{15}N excretion in faeces occurred after 18 h. Since the re-entrant ileal cannula remained open until all the unabsorbed ^{15}N had been collected from the ileum, ^{15}N appearing in the faeces was entirely derived from absorbed ^{15}N recycled to the hind-gut. The mean proportional recovery of the absorbed ^{15}N in he faeces was 0·029 (SEM 0·010 for four steers).

Whole-body protein synthesis rate

Estimates of whole-body protein synthesis rate calculated by the ^{15}N end-product method are shown in Table 3. The mean daily protein synthesis rate for the four steers was 24·2 g/kg body-weight (W)$^{0·75}$ (SEM 2·70). The mean rate of body-weight gain of these steers was 0·32 kg/d.

DISCUSSION

Estimates in the present study of true digestibility of ^{15}N-labelled rumen protein, based upon ^{15}N absorption, were made in the same way as those previously reported for total bacterial ^{15}N (Salter & Smith, 1977). In that paper it was argued that there was little possibility that appreciable amounts of absorbed ^{15}N would be recycled into the small intestine in the short time during which collections were made, and this view was supported by the findings that ^{15}N:PEG values in ileal digesta remained closely similar throughout this period (approximately 3 h). Similar considerations apply in the present study and there seems no reasonable possibility that recycled ^{15}N would affect the results to any appreciable extent. Mean fractional digestibility values of bacterial protein ^{15}N (0·74 (SEM 0·004)) were lower than those found earlier for total bacterial ^{15}N (0·79 (SEM 0·02)) possibly implying that nucleic acid-N (which was removed from the present samples) was more highly digestible than total protein.

This view is supported by earlier findings of apparent digestibility values in the small intestine of the steer of 0·75 for DNA and 0·85 for RNA compared with 0·67 for total N in rumen bacteria (Smith & McAllan, 1971). As RNA forms some 63% of total bacterial nucleic acid (Smith & McAllan, 1974), and as true digestibility would be expected to exceed apparent digestibility, it is reasonable to assume that nucleic acid ^{15}N would have a fractional true digestibility of at least 0·85. The fact that the present result is lower than the digestibility of ^{35}S in labelled bacteria (0·85) (Salter & Smith, 1977) may have been, in part, because methionine was absorbed more efficiently than most other amino acids (Armstrong & Hutton, 1975). No other indices of the true digestibility of bacterial protein in the bovine small intestine appear to exist. Findings that apparent efficiencies of N absorption in the small intestine are usually about 60–70% (Salter & Smith, 1977) are not incompatible with the present results, but it must be recognized that they generally apply to a mixture of protein sources and do not distinguish between protein N and other N.

Estimates of microbial protein digestibility have been made in sheep based upon experiments in which microbial material was harvested from rumen contents, infused in different amounts into the abomasum or duodenum and amino acid-N recoveries determined at the ileum. From regression analysis of these measurements, mean values for true digestibilities of 0·87 (Tas *et al.* 1981) and 0·85 (Storm & Orskov, 1982) were obtained. The reason for these values for sheep being higher than the present estimates for steers is unknown, but it is possible that differences in treatment of microbial samples (including freeze-drying and milling compared with the extraction process used in these experiments) may have been a factor.

The finding that only a small proportion of an intravenous dose of [^{14}C]urea was excreted in the urine was in agreement with earlier observations in the normally functioning ruminant (Cocimano & Leng, 1967; Harmeyer & Martens, 1980). The close correspondence in the pattern of excretion in the urine of ^{15}N-labelled metabolic products derived from bacterial protein (assumed to consist largely of metabolically formed urea) and ^{14}C-labelled urea (Fig. 1) suggests that the proportion of the latter excreted in the urine was similar to the proportion of metabolically produced [^{15}N]urea similarly excreted. This helps to justify the use of the ^{14}C value to calculate the total production of ^{15}N-labelled urea from the amount excreted in the urine (Table 3).

However, there is probably a small error in using this value to assess the initial efficiency of utilization of absorbed amino acids derived from microbial protein. This is because some of the ^{15}N-labelled urea initially produced and recycled into the rumen must be re-incorporated into bacterial protein, absorbed and partially excreted as urea in the urine the second time round. This is not likely to happen to ^{14}C in urea recycled into the rumen, as appreciable proportions of urea C released by urease activity in the rumen wall are not likely to find themselves re-incorporated into metabolites finally giving rise to urea once again. Thus the results for efficiency of utilization of absorbed N shown in Table 3 are minimum values. It is likely that the true figures would be some 5–10% greater than this and a mean fractional efficiency of utilization of 0·75 appears reasonable.

The observation that very little of the ^{15}N dose was excreted in the faeces indicates that this route of ^{15}N disposal was relatively unimportant in the current experiments. As the flow of digesta was diverted through an ileal cannula during the period when most residual ^{15}N was appearing at that site, faecal ^{15}N must have been derived from secretions across the gut wall, presumably mainly as urea. It would be expected that such urea would be rapidly converted to ammonia and, if not captured by micro-organisms, reabsorbed into the blood. Thus, although as suggested by Nolan *et al.* (1976), considerable amounts of urea may have entered the large gut as well as the rumen, it appeared from the results (Fig. 2) that under present conditions very little was captured and, it can be supposed, most was reabsorbed.

It can be argued that as far as protein supply to the animal is concerned, recycling of N to the rumen should be taken into account in assessing the efficiency of use of a given amount of duodenal amino acid. However, in attempting to develop a factorial picture of N transactions within the animal, the role of N recycling to the rumen is a separate issue with which the present experiments were not primarily concerned and on which they throw little light. Within the limitations described above it may be concluded, for the microbial amino acid mixture supplied under the conditions examined, that the results presented in Table 2 provide a satisfactory measure of the true efficiency of utilization of total microbially derived amino acid-N following its absorption from the small intestine. It must be expected that under different dietary or physiological conditions such efficiency values would probably be different.

If an attempt is to be made to assess the net efficiency of utilization it is necessary to bear in mind turnover of protein in the tissues. It has often been demonstrated that in a given period, total protein synthesis in most tissues greatly exceeds the net deposition in those tissues (Waterlow *et al.* 1978*a*; Lobley *et al.* 1980); indeed, it is demonstrated in the present study where the mean estimate of total protein synthesis of 24·2 g/kg $W^{0.75}$ per 24 h was made for four steers (Table 3) using the principle of calculation described by Waterlow *et al.* (1978*b*). This estimate is likely to be an overestimate because in the ruminant there will probably be a substantial production of urea and other urine N-containing end-products from sources other than the amino acid pool labelled by ^{15}N. Nevertheless, with any reasonable assumption about the size of this overestimate (very unlikely to be more than 30%) it is clear that the total protein synthesis greatly exceeds the net deposition of approximately 1·67 g protein/kg $W^{0.75}$ per d achieved in these animals (calculated from the measured mean daily body-weight gain of 0·32 kg, assuming the composition of the gain to be 167 g protein/kg (Agricultural Research Council, 1980)). Most of this total synthetic activity is supported by rapid recycling of amino acids released in the course of protein metabolism. However, there is an inescapable degree of inefficiency in the recapture of amino acids during protein turnover. It is not possible to put a precise figure on the extent of the loss under the conditions of the present experiments but it seems reasonable as a first approximation to regard it as equal to N excretion at zero N intake. This is allowed for (however imperfectly) in the Agricultural Research Council (1980) factorial scheme, by including an estimate of endogenous urine-N excretion and assuming a degree of loss into the alimentary tract by using an estimate of apparent rather than true absorption efficiency. Thus, the efficiency of utilization factor derived from the present study is broadly comparable with that assumed from rather scanty evidence by the Agricultural Research Council (1980). The present mean value (0·70) in fact supports that (0·75) assumed by the Agricultural Research Council (1980) although, as discussed above, it is probably an underestimate of the true value.

In summary, it may be concluded that the present experiments provide information on the efficiencies of absorption of amino acids entering the duodenum of the growing steer and their subsequent utilization. Mean fractional efficiency factors were 0·74 and 0·70 respectively, so that over-all estimated efficiency of utilization under the conditions of the experiment for amino acids entering the duodenum was 0·52. This is close to the over-all efficiency value (0·525) proposed by the Agricultural Research Council (1980) but it should be pointed out that the present absorption efficiency factor is not strictly comparable. The Agricultural Research Council (1980) value of 0·70 is intended to represent a figure for apparent absorption from the small intestine and makes no allowance for N of endogenous origin entering the small intestine, The present value is for true absorption.

The authors would like to thank Dr J. D. Oldham for useful discussions, and Mrs K. Smith and Mrs A. Hudson for technical assistance.

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Printed in Great Britain