Lysine requirements and whole-body protein turnover in growing pigs

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The influence on protein accretion and whole-body protein turnover of changing dietary protein quality while maintaining constant energy intake was studied by varying the degree of lysine supplementation of a lysine-deficient barley-based diet given to growing pigs. Measurements of nitrogen metabolism and whole-body protein turnover, using both classical and ^{15}N end-product methods following a single dose of [^{15}N]glycine, were made in 49-kg male pigs given diets containing 109 g lysine-deficient protein/kg supplemented to make them (1) 'deficient', (2) 'adequate' and (3) 'in excess' with respect to lysine. The ^{15}N dose and protein intake values used to calculate amino N flux from the cumulative urinary excretion of ^{15}N in urea and ammonia were corrected respectively for apparent digestibilities of [^{15}N]glycine and total N determined in a separate experiment in pigs fitted with simple ileal cannulas. N retention and biological value were significantly increased by lysine supplementation of the deficient diet to the 'adequate' level, but were not further increased by the higher level of supplementation. Rates of growth paralleled these changes. The poorer biological value of the unsupplemented diet 1 was shown also in a significantly higher excretion of urea N compared with diets 2 and 3. N digestibility was not markedly influenced by the level of lysine supplementation. Both whole-body protein synthesis and degradation increased markedly on 'adequate' supplementation of the diet with lysine, but did not increase further with an excess of lysine. It is concluded that the increase in protein accretion rate observed on supplementation of the diet with lysine was due to a greater increase in the rate of protein synthesis than of degradation, rather than a decrease in degradation rate.

Growth: Lysine requirement: Protein turnover: Pig

Rates of protein turnover in man are known to be sensitive to short-term changes in protein and energy supply such as occur in the absorptive and postabsorptive states (Clugston & Garlick, 1982). There is also evidence that whole-body protein synthesis increases in response to an increase in protein intake, in both the fed and fasted states (Young *et al.* 1983), but amino acid oxidation only increases with the highest protein intakes, and then only in the fed state. This indicates that a priority for storage of protein may be met by increasing protein synthesis and that oxidation is only enhanced when this process becomes saturated (Garlick *et al.* 1986).

The longer-term influences of diet composition and nutritional state on protein turnover are less well understood. Effects of medium- or long-term feeding with constant high- or low-protein diets indicate a threshold level of protein intake above which protein synthesis changes little, but below which the rate falls (Waterlow & Jackson, 1981). In experiments with growing pigs, however, when the protein quality of a lysine-deficient diet was improved by supplementation with lysine, nitrogen retention and protein accretion increased but no consistent or significant changes in protein synthesis or degradation could be demonstrated (Fuller *et al.* 1987*b*). To investigate this anomaly further we carried out experiments in growing pigs in which an increase in protein accretion was produced without

Table 1. *Composition of the diets*

Diet no....	1	2	3
Ingredients (g/kg)			
Barley meal	972	967	964
Limestone flour	0·5	0·5	0·5
Dicalcium phosphate	15·0	15·0	15·0
L-Lysine monohydrochloride	0	5·4	7·9
Mineral + vitamin supplement*	12·5	12·5	12·5
Analysis			
Dry matter (g/kg)	887	890	891
Crude protein (nitrogen × 6·25) (g/kg)	109·4	113·8	113·1
Heat of combustion (kJ/g)	16·1	15·9	15·9
Metabolizable energy (MJ/kg)	12·8	12·7	12·7
Acid-detergent fibre (g/kg)	43·5	45·9	46·8
Total lysine (g/kg)	3·5	7·8	9·8
Lysine (g/kg crude protein)	33	69	87

* NIRD Growing Pig 051-700.

increasing protein and energy intakes, by giving diets in which lysine content was varied from deficient through adequate (Agricultural Research Council, 1981) to excess by making graded additions of lysine. Diets were given for 8 d, and whole-body protein turnover and N balance measured towards the end of the treatment period. A brief account of some preliminary results has been given elsewhere (Salter *et al.* 1988).

MATERIALS AND METHODS

Animals

For the main experiment six uncannulated Large White × (Large White × Landrace) male pigs were used, with a mean weight of 49 kg during the experiment. During the experimental period animals were kept in metabolism cages adapted for separate collection of faeces and urine. In the preliminary experiment to determine the ileal digestibilities of [^{15}N]glycine and total N, two Large White × (Large White × Landrace) male pigs of 28–32 kg were each provided with a simple infusion tube of PVC (3·0 mm internal diameter and 5·0 mm external diameter) inserted into the duodenum so that its tip was approximately 300 mm from the pylorus, for the infusion of ^{15}N-labelled amino acids and polyethylene glycol. They were also provided with a simple ileal cannula approximately 400 mm anterior to the ileo-caeco-colic junction to facilitate continuous sampling of ileal digesta during the course of digestion of each test meal. The animals were allowed 21 d to recover from surgery before infusions and sampling were started.

Diets and feeding

Pigs were raised on the standard Shinfield diet until they reached 35–40 kg. The composition of this diet (g/kg) was as follows: barley 580·5, soya-bean meal 150, fishmeal 50, wheat 200, vitamin–mineral mixture 12·5, dicalcium phosphate 2, sodium chloride 5 (analysis: crude protein (N × 6·25) 172 g/kg, lysine 9·5 g/kg, digestible energy (DE) 12·8 MJ/kg). Three experimental diets were then used (Table 1). Diet 1 (lysine-deficient) was a simple mixture of barley meal with minerals and vitamins added, containing 3·5 g lysine/kg diet. Diets 2 and 3 were essentially the same as diet 1 but had 5·4 and 7·9 g L-lysine monohydrochloride (Eurolysine; Colborne Dawes Nutrition Ltd, Derby) per kg added

respectively to make them adequate or in excess with respect to the calculated lysine requirement (Agricultural Research Council, 1981). Apart from lysine the essential amino acids content of the diets was reasonably well-balanced, except that levels of cystein + methionine and threonine were marginally low. The diets were given at a daily rate of 91 g/kg metabolic body-weight ($W^{0.75}$). To ensure a reasonably steady-state of amino acid absorption the diets were given by means of automatic feeders in twelve equal amounts at two-hourly intervals. Animals were conditioned to metabolism crates and automatic feeding for a minimum of 7 d before the first test period began. There were no feed refusals.

Preparation of infusion mixtures for preliminary measurements of ileal digestibilities

A portion (1/24th) of the daily allowance of the appropriate diet, ground to pass a 0·5 mm screen, was homogenized with three times its weight of distilled water for 15 min using a Silverson laboratory homogenizer (Silverson Machines Ltd, Chesham, Bucks). To this suspension was added porcine pepsin (*EC* 3.4.23.1; 3200–4500 units/mg protein; Sigma, Poole, Dorset) at the rate of 12 mg pepsin-N/g diet N and the pH adjusted to 4·0 with 1 M-hydrochloric acid. The mixture was then incubated at 37° for 3 h in a water-bath, shaking at 120 strokes/min. After incubation, 250 mg [^{15}N]glycine, 95 atoms % (VEB Berlin Chemie), and polyethylene glycol 4000 (British Drug Houses, Poole, Dorset; 20 g/kg infusion mixture; PEG) were added.

Experimental design

The main experiment with six non-surgically modified pigs was carried out in two 3 × 3 Latin square designs performed consecutively, animals being of the same mean weight and age for each Latin square and assigned to the three diets so that each pig received each diet in random order. Complete separate collections of faeces and urine were made daily on days 4 and 5 and immediately on voiding on days 6–8 inclusive. Faeces were stored deep-frozen at −20° and urine was acidified with 20 ml 4·5 M-sulphuric acid/l and stored at 4° until analysed. However, on the morning of day 6, [^{15}N]glycine (250 mg, 95 atoms %; VEB Berlin Chemie) was added as a solution to a half-portion of the 09.00 hours feed in the trough. This was consumed rapidly and followed by the other half of the feed delivered by the auto-feeder into the trough. By this method losses of ^{15}N were negligible, and stresses to the animal due to surgery and handling were avoided. Collection of urine and faeces continued until the end of day 8 when the diet was changed and the sequence repeated. Samples of urine and faeces collected on days 4–8 inclusive were used for the determination of daily N balance and metabolizable energy. Those collected on days 6–8 were used to measure whole-body protein turnover by the end-product method (Waterlow *et al.* 1978*a*). The sequence of treatments and sampling periods are shown for one pig in Fig. 2 (see p. 509).

For the preliminary ileal digestibility experiment, two pigs were given each of the three experimental diets for periods of 8 d, in random order. On the morning of day 6, half of the 09.00 hours feed was omitted from the auto-feeder and was given to the animal as a pepsin digest (see above) containing [^{15}N]glycine. This was infused directly into the duodenum using a peristaltic pump (Model 502S/R; Watson Marlow Ltd, Falmouth, Cornwall) which delivered the infusion mixture over a 12 min period. The other half of the feed was then delivered in the normal way by the autofeeder. The ileal cannula was then opened and connected to a 500 mm length of colostomy tubing (Thames Valley Medical Supply Co., Reading, Berks) leading into a 50 ml polythene collecting jar. The cannula remained open for 10 h. As each collecting jar became filled it was removed, screw-capped,

stored at −20° for subsequent analysis, and a fresh jar put in its place, so that continuous sampling was achieved. Ileal samples were used for the measurement of the ileal digestibility of ^{15}N.

^{15}N analysis

^{15}N analysis of urinary ammonium and urea were carried out using the batch cation-exchange resin method of Read *et al.* (1982). Briefly, this involved adsorption of free ammonium-N from 2 ml neutralized urine on to Dowex 50W-X8 resin (100–200 mesh) in the Na–K form and separating the resin from the supernatant fraction. The supernatant fraction was then treated with Jack bean urease (*EC* 3.5.1.5; type III; Sigma) to convert urea to ammonium which was bound to a fresh sample of resin. After thorough washing and drying, 8-ml glass vials containing the resin were attached directly to an isotope-ratio mass spectrometer (VG Micromass MM622; VG Micromass, Middlewich, Cheshire) fitted with a computer-controlled dual-inlet system (Europa Scientific, Crewe), evacuated and N_2 gas liberated by treatment with lithium hypobromite.

For ^{15}N analysis of ileal digesta, samples were subjected to Kjeldahl digestion and ammonia was distilled into 0·025 M-HCl using a Kjeltec automatic distillation unit (no. 1004; Tecator Ltd, Thornbury, Bristol). The ammonium chloride solutions produced were transferred to glass vials, dried, attached to the mass spectometer, and N_2 gas liberated as described previously. The repeatability of analysis (1 SD) was 0·0005 atom % ^{15}N for urine samples and 0·0015 atom % for ileal digesta.

Energy measurements

Gross energy contents of diets, faeces and urine were determined by bomb calorimetry using a Gallenkamp adiabatic bomb calorimeter. DE was calculated as the difference between energy consumed and faecal energy; metabolizable energy as the difference between energy consumed and the sum of energy excreted in faeces and urine.

Other analytical methods

Total N was determined in samples (1 g) of digesta, faeces, diets and infusion mixtures with a semi-micro-Kjeldahl technique using 3 ml concentrated sulphuric acid, 100 mg mercuric oxide and 2·4 g potassium sulphate for digestion. The ammonium sulphate formed was estimated using an AutoAnalyzer (Technicon Instruments Co. Ltd, Basingstoke, Hants) by the procedure of Ferrari (1960). PEG was determined in supernatant fractions prepared by centrifugation of digesta samples at 30000 *g* for 10 min at 1° as described by Smith (1962), except that 20 min were allowed for the development of turbidity. From estimates of dry matter content, PEG in total digesta was calculated. Acid-detergent fibre (ADF) was determined by the method of Van Soest (1973).

Total urea N was determined in samples of 10-fold-diluted urine with a Baker Clinical Analyser (Encore Special Chemistry System; Baker Instruments Corp., Allentown, PA, 18103, USA) using an automated urease and glutamate dehydrogenase (*EC* 1.4.1.2) enzymic procedure. Total NH_3-N was measured by the method of Conway (1957).

Calculation of protein turnover

Whole-body protein turnover was estimated by means of the single-dose ^{15}N end-product method (Waterlow *et al.* 1978*a*, *b*). The rationale for this is based on a simplified two-pool model, in which the label is introduced into a single homogeneous 'free amino acid pool' from which it may either be incorporated into protein (the whole-body 'protein pool') or be oxidized to produce carbon dioxide and urinary N. The assumption is made that the

proportion of isotope excreted in the chosen end-product is the same as the proportion of the amino N flux (Q) excreted in the end-product. It is also assumed that recycling of label from protein breakdown is negligible and that rates of flow in and out of the amino N pool remain constant during measurement of Q.

Amino N flux (Q). This was calculated as:

$$Q = E_{EP} \times \frac{d}{e_{EP}},$$

where E_{EP} is the rate of excretion of N in the urinary end-product (ammonia or urea), d is the ^{15}N dose absorbed and e_{EP} is the total amount of ^{15}N excreted in the end-product when the cumulative ^{15}N excretion has reached a plateau. This was achieved in 12–24 h for ammonia and 24–72 h for urea. The ^{15}N dose absorbed (d) was calculated from the total amount of ^{15}N added to the feed ($^{15}N_{in}$) and the ileal digestibility (δ_{il}) of free [^{15}N]glycine determined in the preliminary experiment as follows:

$$d = {}^{15}N_{in} \times \delta_{il}.$$

In the present work it was observed that N fluxes calculated from NH_3 were higher than those from urea (Table 2), presumably due to compartmentation of the free amino N pool. The effect of this discrepancy was reduced by using an 'end-product average' for the calculation of protein synthesis. We found only small differences between results calculated from the harmonic average, Q_{ha}, or the arithmetic average, Q_{aa} (Fern *et al.* 1985).

Protein synthesis and degradation. Under conditions in which the amino acid pool remains constant its turnover rate or flux is given by the expression:

$$Q = S + E = B + I$$

where S, E, B and I represent rates of whole-body protein synthesis, excretion of amino-N, protein breakdown and protein intake respectively. Hence protein synthesis was calculated as the difference between the determined values of the flux and the rates of total urinary N excretion. Protein breakdown was calculated as the difference between the flux and protein intake. For this purpose protein intake (I) was assumed to be the amount of protein absorbed, as calculated from the N content of the feed consumed (N_{in}) and the apparent ileal digestibility (Δ_{il}) of total N determined in the preliminary experiment, as follows:

$$I = N_{in} \times \Delta_{il}.$$

Protein accretion. Whole-body protein accretion was estimated from the difference between the rates of whole-body protein synthesis and degradation. An approximation was also obtained on the basis of N retention × 6·25.

Ileal digestibility of ^{15}N

The proportions of total N and [^{15}N]glycine absorbed from the small intestine were determined in the preliminary experiment by measuring the ratio of total N and ^{15}N:PEG in successive ileal digesta samples and in infusion mixtures. The method has been described previously (Salter & Smith, 1977).

Statistical analysis

Results for the main experiment were subjected to an analysis of variance in which effects of animal, period and diet were estimated and responses to dietary lysine calculated. All animals completed the experiment successfully. For the preliminary experiments, results were calculated as treatment means for each pair of pigs.

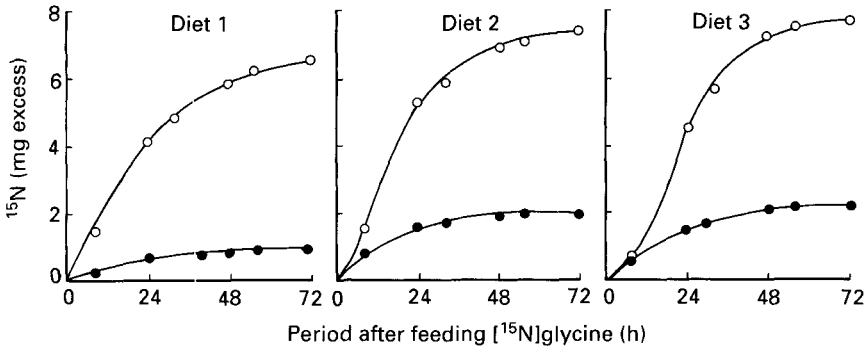

Fig. 1. Cumulative ^{15}N excretion in urinary end-products ((○) urea, (●) ammonia) after feeding barley-based diets deficient (diet 1), adequate (diet 2) or in excess (diet 3) with respect to lysine content. Diet compositions are shown in Table 1.

Table 2. *Main experiment: whole-body nitrogen fluxes (g/kg body-weight$^{0.75}$ per d) calculated from different end-products*

(Means of six values)

End-product...	Urea	Ammonia	Difference
Diet 1	3·25	3·66	0·41
Diet 2	3·67	4·30	0·63
Diet 3	3·82	4·28	0·46
Mean	3·58	4·08	0·50* (SED 0·145) (5 df)

SED, standard error of the difference of urea *v.* ammonia.
* $P < 0·05$.

RESULTS

Preliminary experiment

The ileal digestibility of [^{15}N]glycine determined in the preliminary experiment was 0·99. It is clear that the free amino acid was absorbed with very high efficiency, and only small corrections to the values assigned to the ^{15}N dose absorbed, *d*, in the calculation of N flux were required. Ileal digestibilities of total N did not differ markedly between diets and the mean ileal digestibility was 0·61. N intake values used in turnover calculations were, therefore, corrected as indicated previously (p. 507).

Main experiment

Excretion of ^{15}N in urea and ammonia declined to very low levels in 32–56 h so that cumulative ^{15}N excesses reached plateau values that increased little after this time (Fig. 1). Whole-body N fluxes calculated from the ^{15}N enrichment in urinary urea or ammonia respectively are reported in Table 2. Although N fluxes calculated from NH_3 were higher than those from urea, the effects of dietary changes followed the same direction with each end-product.

Table 3 shows the N balance data, DE and metabolizable energy, mean rates of growth, mean body-weights, urea-N excretion and urinary energy for the main experiment, and Fig. 2 shows a typical growth curve with the corresponding dietary treatments and measurements. Despite the problems of measuring growth rate over short periods, there

Table 3. *Main experiment: nitrogen metabolism and growth of pigs given barley diets deficient in lysine (1), supplemented with lysine to meet requirements (2) or to provide a 25% excess of lysine (3)*

(Mean values for six pigs in a double 3 × 3 Latin square design)

Diet no....	1	2	3		
Lysine (g/kg crude protein)...	33	69	87	SED (8df)	Significance of linear effect*
Mean body-wt (kg)	48·7	48·5	48·5		
Growth (g/d)	296	500	514	76·5	$P < 0·05$
N digestibility (g N/g N intake)	0·73	0·75	0·77	0·015	$P < 0·05$
N retention (g N/g N intake)	0·37	0·50	0·54	0·018	$P < 0·001$
Biological value	0·51	0·67	0·70	0·015	$P < 0·001$
Urinary urea N (g/d)	8·52	5·71	5·50	0·38	$P < 0·001$
Digestible energy (MJ/MJ intake)	0·82	0·81	0·82	0·028	NS
Metabolizable energy (MJ/MJ intake)	0·80	0·79	0·81	0·007	NS
Urinary energy (MJ/d)	0·50	0·44	0·37	0·032	$P < 0·01$

SED, standard error of the difference; NS, not significant.

* There was also a significant curvilinear effect of lysine on biological value and urinary urea N ($P < 0·05$).

was a significant increase ($P < 0·05$) as lysine content was increased from 3·3 to 6·9 g/kg. Apparent N digestibility increased ($P < 0·05$) by 5% as lysine content increased and urea-N excretion decreased by 33% ($P < 0·001$) from diet 1 to diet 2. Consequently, the N retention and the apparent biological value of the diets increased ($P < 0·001$) with increasing dietary lysine content, most of the effect being between diet 1 (low) and diet 2 (adequate).

There were no significant changes in the DE or metabolizable energy of the diets as the lysine content increased. However, when urinary energy was considered alone there was a significant decrease in energy excretion with diet 3 compared with diet 1, and a nearly significant decrease ($P < 0·10$) with diet 2.

Whole-body protein turnover values are shown in Table 4. Protein synthesis rate, protein breakdown and protein accretion increased significantly ($P < 0·01$, $P < 0·05$ and $P < 0·001$ respectively) as dietary lysine was increased from 33 to 69 g/kg crude protein, but the values for diets 2 and 3 were similar. The increases in protein accretion on diets 2 and 3 compared with diet 1 showed a similar trend to the observed increases in N retention and growth.

DISCUSSION

The diets used in these experiments contained 8·3–8·8 g protein/MJ DE, lower with respect to ideal protein than that recommended by the Agricultural Research Council (1981) for 15–50 kg pigs. Presumably, therefore, N was somewhat limiting for growth from 30 to 50 kg. For subsequent growth, protein:energy ratios could be regarded as close to optimal. The lysine contents of the diets (33, 69 and 87 g/kg crude protein) corresponded to 0·45, 1·0 and 1·26 of the recommended requirements (Agricultural Research Council, 1981). The addition of sufficient lysine to meet the requirements (diet 2) resulted in significant increases in N retention and protein accretion, but excess lysine (diet 3) produced no further significant increase (Tables 3 and 4). Changes in growth followed a similar pattern. The

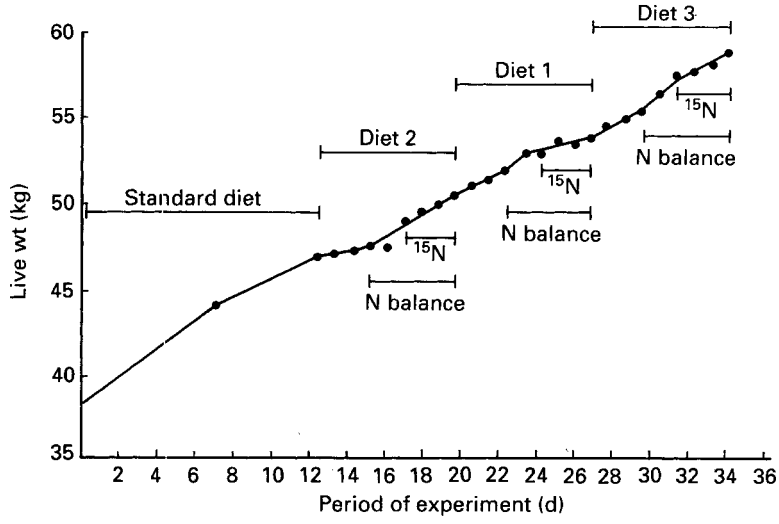

Fig. 2. Typical growth curve and sequence of dietary treatments for one of the pigs used in the main protein-turnover experiment which started in June. Diets were barley-based and were deficient (diet 1), adequate (diet 2) or in excess (diet 3) with respect to lysine content. Periods over which nitrogen balance and ^{15}N end-products were measured are also indicated by horizontal bars.

Table 4. *Main experiment: whole-body protein turnover in growing pigs given barley diets deficient (1), supplemented with lysine to meet requirements (2) or to provide a 25% excess of lysine (3)*

Mean values for six pigs in a double 3 × 3 Latin square design)

Diet no....	1	2	3		
Lysine (g/kg crude protein)...	33	69	87	SED (8df)	Significance of linear effect
N flux (g/kg body-wt $(W)^{0.75}$ per d)	3·46	3·98	4·05	0·221	$P < 0.05$
Protein synthesis (g/kg $W^{0.75}$ per d)	17·9	22·2	23·0	1·37	$P < 0.01$
Protein breakdown g/kg $W^{0.75}$ per d)	15·5	18·5	19·1	1·30	$P < 0.05$
Protein accretion (g/kg $W^{0.75}$ per d)	2·39	3·73	3·88	0·24	$P < 0.001$

SED, standard error of the difference.

objective of producing increases in protein accretion by improving protein quality while maintaining the same energy level (1·53 × maintenance requirement) was, therefore, achieved. This is confirmed by the urea N excretion results (Table 3) which were consistent with the effects of lysine supplementation on protein quality: higher urea N excretion with diet 1 compared with diets 2 and 3 indicated that a higher proportion of amino N entering the free amino acid pool from tissue protein degradation or from feed was oxidized due to the diet's less than ideal amino acid balance.

Since the threonine and cysteine + methionine contents of the diets were marginally low it is possible that these amino acids could have become limiting on addition of lysine. If so,

even diet 2 may have contained excess lysine. The probable effect of this would have been to lessen the increases in rates of protein synthesis and degradation in response to lysine addition. However, the tendencies for growth and N retention to show further small increases and for urinary urea to decrease on diet 3 suggest otherwise.

The values for protein synthesis obtained in the present investigation are in general accord with previously reported values for growing pigs (Reeds & Fuller, 1983). The results (Table 4) indicate clearly that the increase in protein accretion produced by supplementing the diet with sufficient lysine to meet the requirement (diet 2) was accompanied by significant increases in both protein synthesis and degradation, and resulted from an increase in the difference between these two rates. Also, increasing the lysine supplement in excess of the requirement led to no further significant increases in either growth, protein synthesis or protein degradation and, therefore, no further increase in protein deposition. The values for protein breakdown calculated alternatively from the difference between protein synthesis and protein accretion measured as N retained were 14·2, 17·0 and 17·5 g/kg $W^{0.75}$ per d for diets 1, 2 and 3 respectively. These results differ by only 8% from those in Table 4, confirming an increase in degradation with improved protein quality. Present results, relating to an increase in effective protein intake without increasing energy intake, are consistent with the conclusions of Reeds & Fuller (1983) in a review of findings from several laboratories on the influence of longer-term changes in the whole diet (protein and energy changing in concert). These authors showed that, as intake increased, protein synthesis increased proportionately more than degradation. They concluded that the effects of protein and energy on N retention were additive, the effect of protein on synthesis being large, whilst that of energy was small and accompanied by a small decrease in degradation. The results are consistent with the observation of Nishizawa *et al.* (1977) that urinary methylhistidine excretion of rats on restricted food intake was enhanced by increasing the protein content of the diet from 100 to 600 g/kg, indicating raised skeletal muscle protein catabolism. They also accord with the results of Omstedt *et al.* (1978) who showed that urinary 3-methylhistidine excretion increased as the nutritive quality of dietary protein improved.

Reduced whole-body protein synthesis due to amino acid deficiency is consistent with in vitro effects of amino acid deprivation in a number of cell types (Jagus *et al.* 1981). With rat hepatocytes, for example, deprivation of total amino acids or single essential amino acids including lysine resulted in a rapid decrease in the rate of protein synthesis, accompanied by inhibition of 43S initiation complex formation, which was reversed by re-addition of the deficient amino acids (Everson *et al.* 1989).

A different conclusion was reached by Fuller *et al.* (1987*b*) who were unable to find any consistent or significant changes in protein turnover rates in growing pigs when protein accretion was varied by altering the level of the first limiting amino acid, lysine, in isoenergetic diets of either 150 or 290 g protein/kg diet. These authors used constant rate infusions of [4,5-^{3}H]leucine and [^{14}C]urea, or fed ^{15}N-labelled yeast protein, to measure fluxes of leucine and N. The only effect of lysine supplementation was a decrease in the rate of leucine breakdown, whilst neither body protein synthesis nor protein degradation deduced from the leucine flux showed any significant change. Although they demonstrated a substantial increase in N retention on supplementing the low-protein diet with lysine, they concluded that this did not cause any large perturbation in the rate of protein turnover. It is difficult to reconcile this conclusion with the implied increase in protein accretion, which must have resulted from changes in rates of protein synthesis or degradation, or both.

The assumptions required and the problems inherent in the measurement of protein turnover with ^{15}N-labelled amino acids have been examined in some detail (Waterlow *et al.* 1978*a*; Fern *et al.* 1985). The accuracy of the 'end-product' method used in the present

work is dependent on the assumption that metabolically active N and tracer ^{15}N in the body are contained in a homogeneous pool that is supplied with N from the food and tissue protein breakdown, and which supplies N for protein synthesis, for amino acid oxidation and for the formation of excretory products. This has been shown to be an oversimplification, since estimates of whole-body flux or protein synthesis from ammonia or urea generally give different values with the same tracer amino acid (as was indeed found in the present results, Table 2), whilst estimates also varied according to the amino acid used (Fern *et al.* 1985). Fern *et al.* (1985), however, found that the use of end-product averages, as in the present study, lessened the effect of physical compartmentation. They also showed that of the ^{15}N-labelled amino acids tested, [^{15}N]glycine gave estimates nearest to those obtained with ^{14}C- or ^{13}C-labelled leucine.

It is recognized that during the time taken to establish plateau ^{15}N abundance in urea, recycling of ^{15}N from labelled protein will have occurred. A measure of the impact of recycling on plateau values is given by the slope of the pseudo-plateau (Waterlow *et al.* 1978*a*). The approximate mean rates of increase at plateau in these experiments were 8, 3 and 4%/d for diets 1, 2 and 3 respectively. This indicates an error of not more than 4% on the plateau value, which would result in a small underestimate of protein synthesis rate.

The factors influencing the enrichment of ^{15}N in ammonia and urea, including the balance of precursor amino acids, have been discussed by Jackson & Golden (1981). Overall differences in amino acid composition of the three diets used were relatively small, hence changes in the origin of N compounds contributing to the amino acid pool were also probably small. The perturbation of the pool due to lysine deficiency resulting in catabolism of other amino acids can be estimated from the decrease in lysine relative to that in the adequate diet as causing an approximately 4% increase in the N pool. Similarly, with lysine excess, the increased catabolism of lysine would contribute an increase of less than 2% of unlabelled N entering the pool. The effect of these factors on the calculated N flux is likely to be small. Again, since glycine N was not limiting, the flow of glycine N as a proportion of urea N should not have changed markedly between the three diets. Thus, changes in the measured flux should properly reflect the effect of lysine supplementation on protein synthesis and degradation.

It has been estimated (Millward *et al.* 1976) that the energy required to synthesize 1 g protein is approximately 3·45 kJ, whereas the apparent energy cost of protein deposition is about 20·3 kJ/g (Fuller *et al.* 1987*a*), a ratio of 6:1. Fuller *et al.* (1987*a*) suggested that the discrepancy could be explained if for every 1 g protein deposited, 6 g protein were synthesized. Interestingly, the results presented here (Table 4) show that the mean ratio of protein synthesis:protein accretion for the three diets was 6·45, and for diets 2 and 3 it was 5·94. However, Reeds *et al.* (1980) pointed out that the more important relationship was the change in synthesis associated with the change in accretion, and both these authors and Fuller *et al.* (1987*b*) found a ratio of approximately 2·2 g protein synthesized:1 g additional protein deposited, insufficient to account for the energetic difference. They concluded that the relationship between protein synthesis and deposition is not fixed but is influenced by the nature of the diet. Results of the experiments presented here confirm this, the mean ratio of the increment of protein synthesis to protein deposition being approximately 3·3.

We conclude from our results that improvement of the protein quality of a barley diet by supplementation with lysine resulted in increases in both protein synthesis and protein degradation rates, there being a threshold around the point at which the lysine requirement was satisfied above which neither variable increased further. This finding is consistent with known mechanisms of protein synthesis that require that all the amino acids needed are present simultaneously at the sites of protein synthesis.

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