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Amino acid metabolism and whole-body protein turnover in lambs fed roughage-based diets: 1. Lysine and leucine metabolism[1]

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The experiments reported in this series were designed to investigate the metabolism of lysine, leucine and methionine in lambs fed roughage diets supplemented with various levels of protein, and to compare these amino acids as tracers of whole-body protein metabolism in ruminants. Amino acid kinetics were estimated using a 12-h intravenous infusion of L-[$U^{14}C$]-lysine, L-[^{35}S]-methionine and L-[4,5-$^{3}H(n)$]-leucine. The kinetics of methionine and a comparison of estimates of whole-body protein turnover are discussed in a following article (Cronjé *et al.*, 1992). The flux, oxidation and incorporation of lysine into protein was increased by protein supplementation ($P < 0.05$). The proportion of flux oxidized was increased ($P = 0.08$) by protein supplementation, and the fraction incorporated into protein was decreased ($P = 0.07$). It is suggested that the supply of lysine was limiting for protein synthesis when the low protein diet was fed. Leucine flux rate was increased by protein supplementation ($P < 0.01$). There was no change in fractional oxidation rate (23% of flux rate) with increasing protein supply ($P > 0.05$), but the total amount of leucine oxidized was increased by protein supplementation ($P < 0.01$). Similarly, the amount of leucine incorporated into protein was increased ($P < 0.01$) but not the proportion of leucine flux ($P > 0.05$). It was concluded that the supply of leucine available from the low-protein diet was lower than the requirement, and that while this restriction was alleviated by further protein supplementation, the efficiency of protein synthesis was still sub-optimal, possibly as a result of an insufficient supply of methionine. It is suggested that considerable scope exists for increasing the efficiency of utilization of existing protein supplements by lambs fed roughage diets via supplementation with specific amino acids protected from rumen degradation.

Hierdie reeks proewe is ontwerp om die metabolisme van lisien, leusien en metionien in lammers wat ruvoerdiëte tesame met verskillende peile van proteïenbyvoeding gevoer is te ondersoek, en om hierdie aminosure ten opsigte van hul geskiktheid as spoorders van heelliggaamproteïenmetabolisme te vergelyk. Aminosuurkinetika is deur middel van 'n 12 h-binneaarse-infusie van L-[$U^{14}C$]-lisien, L-[^{35}S]-metionien en L-[4,5-$^{3}H(n)$]-leusien gemeet. Die metabolisme van metionien sowel as 'n vergelyking van heelliggaamproteïenmetabolisme-beramings word elders bespreek (Cronjé *et al.*, 1992). Die omset, oksidasie en sintese van lisien na proteïen is deur proteïenbyvoeding verhoog ($P < 0.05$). Die proporsie van omset wat geoksideer is, is deur proteïenbyvoeding verhoog ($P = 0.08$), terwyl die proporsie wat in proteïen omgebou is, verlaag is ($P = 0.07$). Dit blyk dat die toevoer van lisien met die lae-proteïendieet beperkend vir proteïensintese was. Leusienomsettempo is deur proteïenbyvoeding verhoog ($P < 0.01$). Daar was geen verandering in die proporsionele oksidasietempo (23% van omsettempo) met proteïenbyvoeding ($P > 0.05$) nie, maar die totale hoeveelheid leusien wat geoksideer is, het verhoog ($P < 0.01$). Insgelyks het die hoeveelheid leusien wat in proteïen omgesit is, verhoog met byvoeding ($P < 0.01$) maar nie die proporsie van omset nie ($P > 0.05$). Daar is tot die gevolgtrekking gekom dat die toevoer van leusien met die lae-proteïendieet minder as die behoefte was. Verder blyk dit dat terwyl hierdie beperking tot 'n mate opgehef is deur verdere proteïenbyvoeding, die lae tempo van heelliggaamproteïensintese, selfs by die hoë vlak van byvoeding, daarop dui dat die doeltreffendheid van proteïensintese deur ander faktore, moontlik metionientoevoer, beperk word. Daar is voorgestel dat daar geleentheid bestaan om die doeltreffendheid van benutting van bestaande proteïensupplemente vir ruvoerdiëte te verbeter deur strategiese byvoeding van sekere aminosure.

Keywords: Amino acids, lysine, leucine, protein turnover, ruminants.

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The metabolic basis of supplementary feeding practices for ruminants grazing forages of low digestibility containing little protein is not well established. While substantial production responses have been achieved by protein supplementation (Preston & Leng, 1987), very little is known of the turnover of amino acids or whole-body protein metabolism in ruminants fed roughage diets. In contrast to this, substantial advances have been made in increasing the understanding of protein malnutrition in man using isotope-based techniques to study amino acid metabolism and whole-body protein turnover (Millward, 1979). In this study, the metabolisms of three amino acids were compared by infusing tracers concurrently into lambs fed a roughage diet supplemented with various levels of protein. The amino acids were labelled with different isotopes in order to obtain simultaneous estimates of flux and oxidation rates. Leucine, lysine and methionine kinetics were estimated from

L-[4,5-^{3}H(n)]-leucine, L-[U^{14}C]-lysine and L-[^{35}S]-methionine using the specific radioactivity (SR) of ^3H_2O, $^{14}CO_2$ and $^{35}SO_4$ to calculate the respective oxidation rates. Lysine and leucine flux, oxidation and incorporation into protein are discussed here; the metabolism of methionine as well as a comparison of estimates of whole-body protein turnover are presented elsewhere (Cronjé *et al.*, 1992).

Materials and Methods

Animals and diets

Twelve Merino cross-bred wethers of live weight of 27 (± 2) kg were allocated at random to four groups of three sheep each and fed one of four diets. The diets were: basal plus 0, 40, 120, or 200 g/d of a protein supplement. The basal diet consisted of wheat straw plus urea (20 g/kg straw) which was sprayed onto the straw as an aqueous solution and dried. The protein supplement was formulated to contain a high proportion of protein resistant to degradation in the rumen, and consisted of (g/kg): cottonseed meal (560), soybean meal (250), meat meal (50), molasses (50), NaCl (5), Ca_2HPO_4 (50), KCl (15), trace minerals plus carrier (20), and contained 62 g N/kg DM. The protein supplement, basal diet and a balanced mineral supplement were fed at hourly intervals using automated feeders. The mineral supplement provided (g/d): Ca (1.6), P (1), S (0.9), Na (3) and trace minerals. The basal diet was fed at a level of *ad libitum* plus 20% (based on a three-day moving average) during a three-week adaptation period. During the measurement period, the amount fed was restricted to the individual *ad libitum* intake recorded over the previous week. Because of the restricted number of animals used, and a large variation in individual voluntary intake (660—980 g/d), there was no statistical difference in total crude protein (CP) intake between the groups fed 0 or 40 g/d protein supplement and between those receiving 120 or 200 g/d supplement. The pooled CP intake of the former two groups did, however, differ significantly from that of the latter two groups; consequently, all data were pooled and treatments were referred to as the low-protein (CP intake: 60 g/d) and high-protein treatments (CP intake: 110 g/d).

The sheep were housed indoors in metabolism crates under continuous illumination. Catheters were inserted into both jugular veins on the day preceding the infusions. An attempt was made to place the infusion catheter in or near the heart, while the sampling catheter was inserted less deeply.

Isotopes and tracer administration

Amino acid flux was estimated using three different tracers which were infused concurrently. A solution containing 80 μCi L-[U^{14}C]-lysine, 50 μCi L-[^{35}S]-methionine, and 125 μCi L-[4,5-^{3}H(n)]-leucine in sterile physiological saline (9 g/l NaCl) was infused into each sheep over 12 h at a rate of 0.25 ml/min. Single injections of $NaH^{14}CO_3$ (50 μCi), Na_2-$^{35}SO_4$ (50 μCi), and ^3H_2O (100 μCi) in saline solution were administered four days after the infusion to determine the irreversible loss rates (ILR) of blood bicarbonate, sulphate, and body water. This data were used in calculating the oxidation rates of ^{14}C-lysine, ^{35}S-methionine, and ^{3}H-leucine respectively.

Sampling and analytical

Blood samples for analysis of amino acid specific radioactivity (SR) were taken into heparinized tubes at the following intervals after commencement of the infusion: 0, 0.5, 1, 2.5, 5, 6, 8, 10, and 12 h. Following centrifugation, the plasma was deproteinized with sulfosalicylic acid (0.5 g/ml plasma), recentrifuged, and the supernatant fraction was decanted and stored at −15 °C. Plasma amino acid concentrations were determined by high-pressure liquid chromatography (Waters Associates; MA, USA). Norleucine was added as internal standard and all samples were corrected to 100% recovery. Chromatography was performed by cation exchange on an amino acid analysis column (Na^+ form) at 62 °C using a pH gradient between sodium citrate buffer (pH 3.3) and sodium borate (pH 9.6), keeping sodium ion concentration constant. Because of difficulties in separating the lysine peak from a peak of similar pK_a, lysine separations were performed using an isocratic system with sodium borate (pH 9.6) as eluent. Amino acids were detected by fluorescence following derivitization using an orthopthalaldehyde-mercaptoethanol reagent at pH 10.6 in 0.5 M potassium borate buffer. Concentrations of amino acids were determined using 50-μl aliquots of deproteinized plasma or less. Samples used for scintillation counting were collected following application of 250 μl aliquots of plasma to the column. Peak separation was reduced by the larger sample volume, making precise collection of specific fractions difficult. However, fractions immediately before and after the peaks contained negligible or no radioactivity for all the amino acids studied. Sampling times were extended to ensure complete collection of all radioactivity. Amino acid isolates were collected as they were eluted from the column, made up to 2 ml with distilled water, and beta emission was measured in 15 ml of a liquid scintillation cocktail. The cocktail consisted of 4 g PPO (2.5-diphenyloxazole) and 0.2 g POPOP (1.4-bis-[2(5-phenyloxazolyl)]-benzene) dissolved in 692 ml toluene and 308 ml Terec X-10 (ICI, Australia).

The fractional contribution of [U^{14}C]-lysine to the ILR of blood HCO_3 was estimated from samples collected at 6, 8, 10, and 12 h after beginning the infusion of [U^{14}C]-lysine. Blood samples for estimation of $NaHCO_3$ ILR were taken at 1, 1.5, 5, 10 and 20 h following injection of $NaH^{14}CO_3$. Bicarbonate in whole-blood samples was isolated by precipitation as barium carbonate (Leng & Leonard, 1965). Specific radioactivity was determined by counting a weighted amount of $BaCO_3$ (± 20 mg) in 10 ml of a scintillation gel. The gel consisted of (g/l xylene): Cab-O-Sil thixotropic powder (Packard Instrument Co., Illinois), 34; PPO, 4; POPOP, 0.2.

The fractional contribution of [^{3}H]-leucine to the irreversible loss rate (ILR) of body water was estimated from the SR of urinary ^3H_2O. Urine voided during the infusion was collected quantitatively into glacial acetic acid (1—2 ml) at intervals corresponding to those for blood sampling. Thereafter urine was collected into 20 ml glacial acetic acid on a daily basis for the following four days. Samples were stored at −15 °C until analysis. The irreversible loss rate (ILR) of body water was determined using a single intravenous injection of ^3H_2O (100 μCi) administered four days after the leucine infusion. Estimates of ^3H_2O ILR were derived from the SR of urine

samples taken at 1, 5, 10, 21, and 24 h after the tracer injection. Urinary ^3H_2O was isolated by vacuum sublimation and counted in the same scintillation cocktail as used for amino acid isolates.

Sample quenching in scintillation mixtures was measured using an external standard. Samples were corrected for counting efficiency using known standards, and special care was taken to standardize counting systems where amino acids and their oxidation products were counted in different media. Residual and background radioactivity levels were determined from samples taken prior to tracer administration.

Calculations

Lysine kinetics

Amino acid flux rate was calculated as follows:

$$\text{Flux } (\mu\text{mol/min}) = \frac{\text{Infusion rate (DPM/min)}}{\text{SR at plateau (DPM/}\mu\text{mol)}}$$

Individual blood samples were used to confirm that plateau SR was reached within 6 h, and the SR of lysine at plateau was determined from a bulk sample derived from samples taken after 6, 8, 10 and 12 h of infusion.

The proportion of blood bicarbonate-C derived from lysine oxidation was estimated as follows:

$$\text{Proportion of } HCO_3\text{-C ILR derived from lysine-C} = \frac{\text{SR } CO_2 \text{ at plateau}}{\text{SR lysine-C at plateau}}$$

Plateau CO_2 SR was calculated as the mean of that observed from 8—12 h after beginning the leucine infusion. The rate of flux of lysine-C through the blood CO_2 pool (i.e. lysine oxidation) was calculated as follows:

$$\text{Lysine oxidation } (\mu\text{mol/min}) = (\text{Proportion of } HCO_3\text{-C ILR derived from lysine-C}) \times (CO_2 \text{ ILR})$$

Lysine incorporated into protein was calculated as the difference between flux and oxidation rates. ILR and recycling of CO_2 were calculated from a second-order exponential function describing the decline in SR with time (White *et al.*, 1969).

Leucine kinetics

Leucine flux rate was calculated as described for lysine from the SR of bulked plasma samples collected between 6 and 12 h of infusion. Leucine oxidation rate was calculated from the proportion of tritium infused as L-[4,5-^{3}H(n)]-leucine which appeared in the body ^3H_2O pool during the leucine infusion. The calculations used were as follows:

H_2O pool size and ILR

H_2O kinetics were estimated from the decline in urinary SR with time following a single dose of ^3H_2O injected four days after the leucine infusion. These values were first corrected for residual background radiation remaining from the leucine infusion: A single exponential function was fitted to the data describing the change in SR with time between the end of the infusion period and the time of injection of the dose. Estimates of baseline radioactivity at sampling times were obtained by extrapolation of this function and subtracted from the actual values. A single exponential function was fitted to the corrected values by linear regression, and water pool size and ILR were calculated as follows:

$$\text{SR at time } t = A.e^{-kt}$$

$$\text{Pool size (g)} = \frac{\text{Dose } ^3H_2O \text{ injected (DPM)}}{A \text{ (DPM/g)}}$$

$$\text{ILR (g/h)} = \text{Pool size (g)} * k \text{ (/h)}$$

Leucine oxidation and incorporation into protein

The amount of tritium in the water pool was calculated as the product of the pool size and the SR of ^3H_2O at the end of the leucine infusion. The latter value was predicted from a single exponential function fitted to the values obtained during the infusion. The amount of tritium that was lost from the pool during the infusion period was also calculated and added to the quantity remaining in the pool to give an estimate of the total amount of tritium which had entered the pool during the infusion period. This was expressed as a proportion of the amount of tritium infused as leucine to give an estimate of leucine oxidation. It was assumed that the fraction of leucine not oxidized represented that incorporated into protein (Reeds *et al*, 1981).

Whole-body protein turnover, synthesis, and amino acid oxidation

Whole-body protein turnover was calculated on the assumption that the fractional contribution of the flux of the tracer amino acid to whole-body protein turnover was proportional to the fractional contribution of that amino acid to whole-body protein content. A value of 0.08 (Waterlow *et al.*, 1978) was used for both amino acids:

$$\text{Whole-body protein turnover (g/d)} = \frac{\text{Leucine/lysine flux (g/d)}}{0.08}$$

Similarly, whole-body amino acid oxidation was calculated on the assumption that the amino acid content of protein deposited was the same as that synthesized (Reeds *et al.*, 1979). Protein synthesis was calculated as the fraction of turnover not oxidized. Whole-body fractional synthesis rate (K_s) was calculated from leucine synthesis rate assuming that whole-body leucine content was 97.88 mmol/kg live mass (Oddy *et al.*, 1987; Williams, 1978):

$$K_s \text{ (/d)} = \frac{\text{Leucine incorporated into protein (mmol/d)}}{\text{Whole-body leucine content (mmol)}}$$

Statistical analysis

Results were analysed for statistical significance by one-way analysis of variance.

Results and Discussion

Lysine concentration in plasma (Table 1) did not differ ($P > 0.05$) between treatments (mean: 12.6 μmol/100 ml), and fell within normal ranges (Nimrick *et al.*, 1970; Faichney, 1974). Lysine flux for the high-protein diet (29 μmol/min) was faster ($P < 0.05$) than for the low-protein diet (19 μmol/min) (Table 1). Using L-[4,5-^{3}H]-lysine, Buttery *et al.* (1975) reported values of 2.8 μmol/min/kg$^{0.75}$ for mature wethers fed an adequate level of lysine (75 mmol/d). The flux rate for the high-protein diet in the present study (2.5 μmol/min/kg$^{0.75}$) is comparable with this value, but that recorded for the

Table 1 Lysine flux, oxidation, and incorporation into protein in sheep fed a diet of wheat straw plus urea and supplemented with two levels of protein (standard errors of the means in parentheses)

	Diet	
	Low protein (60 g/d)	High protein (110 g/d)
Lysine flux		
(μmol/min)	19[a]	29[b]
	(2.7)	(1.3)
(μmol/min/kg$^{0.75}$)	1.69[c]	2.53[d]
	(0.24)	(0.12)
Lysine oxidation		
(μmol/min)	3.2[a]	6.2[b]
	(0.5)	(0.6)
(μmol/min/kg$^{0.75}$)	0.28[a]	0.54[b]
	(0.05)	(0.06)
Lysine incorporated into protein		
(μmol/min)	16[c]	23[d]
	(2.2)	(1.6)
(μmol/min/kg$^{0.75}$)	1.41[c]	1.99[d]
	(0.20)	(0.13)
Plasma lysine concentration		
(μmol/100 ml)	13	12
	(1.2)	(1.1)

Means within the same row with different superscripts differ significantly: [a,b] ($P < 0.01$); [c,d] ($P < 0.05$).

Table 2 Leucine kinetics in sheep fed a diet of wheat straw plus urea and supplemented with a low or high level of protein (standard errors of the means in parentheses)

	Diet	
	Low protein (60 g/d)	High protein (110 g/d)
Leucine flux		
(μmol/min)	23.3[a]	59.5[b]
	(4.9)	(5.1)
(μmol/min/kg$^{0.75}$)	2.06[a]	5.12[b]
	(0.44)	(0.40)
Leucine oxidation		
(μmol/min)	5.6[a]	13.0[b]
	(1.1)	(1.2)
(μmol/min/kg$^{0.75}$)	0.49[a]	1.12[b]
	(0.10)	(0.11)
Leucine incorporated into protein		
(μmol/min)	17.7[a]	46.5[b]
	(3.9)	(4.5)
(μmol/min/kg$^{0.75}$)	1.56[a]	4.00[b]
	(0.35)	(0.34)
Plasma leucine concentration		
(μmol/100 ml)	9.7	11.1
	(0.5)	(0.7)

[a,b] Means within the same row with different superscripts differ significantly ($P < 0.01$).

low-protein diet was much less (1.7 μmol/min/kg$^{0.75}$), which suggests that the supply of lysine to the tissues at this level was low.

Lysine oxidation rates measured in this study (3.2—6.2 μmol/min) (see Table 1) are comparable with the range of published values (Egan & McRae, 1979) of 3.5—4.7 μmol/min in sheep. The mean CO_2 ILR for all treatments was 148 (*SE* 11) mg C/min; there were no differences ($P > 0.05$) between treatments. CO_2 recycling constituted 22% of total flux, and turnover time was approximately 54 min. In animals fed diets deficient in a particular amino acid, supplementation with increasing amounts of the amino acid typically results in a low and relatively constant amino acid oxidation rate until the point of amino acid sufficiency is reached, whereupon there is a dramatic linear increase in oxidation rate (Kim *et al.*, 1983). In the present study, lysine oxidation rate at the highest level of supplementaton was nearly double ($P < 0.05$) that of the low-protein diet (Table 1). In man, lysine oxidation has been shown to increase from 8% of flux rate when lysine was limiting to 20% on an adequate diet (Young *et al.*, 1981). In the present study, lysine oxidation increased from 16% to 21% of flux with increasing amounts of dietary protein ($P = 0.08$). Both the flux and oxidation rates measured in this study suggest that the low-protein diet was deficient in lysine. As lysine is also known to be a first- or second-limiting amino acid for lambs (Schelling *et al.*, 1967; Brookes *et al.*, 1973), it is suggested that there may be considerable scope for improving the efficiency of utilization of protein supplements for similar diets or for lambs grazing dormant natural pastures by supplementation with lysine.

Leucine flux rate (Table 2) for the high-protein diet was 60 μmol/min (5.12 μmol/min/kg$^{0.75}$). This is of the same order as published estimates for adult sheep which vary from 59—67 μmol/min (Riis, 1983; Egan & MacRae, 1979), and corresponds to that for lambs fed ryegrass-clover pasture (6 μmol/min/kg$^{0.75}$; Davis *et al.*, 1981) and that for cattle (5—9 μmol/min/kg$^{0.75}$; Lobley *et al.*, 1980) fed balanced diets. Leucine flux rate decreased from 5.12 μmol/min/kg$^{0.75}$ in lambs fed the high-protein diet to 2.06 μmol/min/kg$^{0.75}$ in lambs fed the low-protein diet. The decrease in flux rate is similar to the trend reported by Hammond *et al.* (1985) for heifers fed high- (9.9 μmol/min/kg$^{0.75}$) vs. low-energy diets (6 μmol/min/kg$^{0.75}$), but the mean flux rate recorded for the low-protein diet is considerably lower than previously published estimates. The leucine content of microbial protein is low relative to body tissue composition (see Lobley *et al.*, 1980). The low rumen microbial protein production rate which is to be expected with diets of this nature may have resulted in a relatively low supply of leucine to the duodenum and hence have contributed to the low flux rate.

Some measure of the adequacy of leucine supply may be estimated from the data of Schaefer *et al.* (1986), who derived a relationship between leucine infusion rate and leucine flux. According to this formula, the leucine supply in this study would be 0.034 g/kg$^{0.75}$/d for the low-protein diet and 0.647 g/kg$^{0.75}$/d for the high-protein diet. The former value is well below estimated leucine requirements for sheep (see Buttery & Foulds, 1988) of 0.272—0.408 g/kg$^{0.75}$/d.

The mean leucine oxidation rate was 23% of the flux rate in this study. This is higher than the values of 15% and 17%

reported for heifers at high and low energy intakes respectively (Hammond *et al.*, 1985), or for mature wethers (12—20%; Egan & MacRae, 1979), but within the limits of 15—30% quoted by Riis (1983). There was no change in the perçentage of leucine oxidized with increasing protein supply ($P > 0.05$), although the total amount of leucine oxidized was increased ($P < 0.01$) by protein supplementation (Table 2). Similarly, although the total amount of leucine incorporated into protein was increased ($P < 0.01$), the proportion of leucine flux diverted to protein synthesis was unaffected ($P > 0.05$). The relatively high proportion of flux oxidized and the fact that additional protein supplementation did not increase the proportion of flux used for protein synthesis may indicate that leucine was not utilized with maximum efficiency.

Whole-body protein turnover, synthesis and amino acid oxidation

Whole-body protein turnover is calculated from the flux of individual amino acids assuming that their contribution to total amino acid flux is in proportion to their concentration in tissue protein (Waterlow *et al.*, 1978). Errors in this assumption may induce large errors in the final estimate of whole-body protein turnover. Unfortunately, considerable uncertainty exists as to which figure to adopt. For instance, for leucine Reeds *et al.* (1980) used 6.6%, Davis *et al.* (1981) used 7%, while others (Waterlow *et al.*, 1978) used 8%. The continued use of the latter figure has been defended on the basis that a large proportion of body protein consists of collagen which contains only 30% as much leucine as other tissue proteins and turns over very slowly, so that 8% may be a more realistic estimate of the leucine content of that protein which is actively turning over during the course of the experiment (Waterlow, 1984). The latter value was used in the current experiment.

Rates of whole-body protein turnover estimated from leucine kinetics decreased from 12.1 g/kg$^{0.75}$/d for sheep fed the high-protein diet (Table 3) to 4.8 g/kg$^{0.75}$/d for sheep fed the low-protein diet. The estimates derived from lysine kinetics decreased from 6.7 to 4.5 g/kg $W^{0.75}$/d (Table 4). This trend is similar to that reported by Bryant & Smith (1982) (using tyrosine as marker amino acid) for wethers fed hay plus concentrates or barley straw. Protein turnover was 15.1 g/kg$^{0.75}$/d for the hay plus concentrate diet (CP intake: 145 g/d), and fell to 10.6 g/kg$^{0.75}$/d for the straw diet which provided 28 g CP/d. A similar trend was reported by Lobley (1986) when intake of a finishing diet for steers was reduced from 1.6 × maintenance to maintenance. In this instance, the rate of protein synthesis estimated from leucine flux fell from 26 g/kg$^{0.75}$/d at the surfeit level to 22 g/kg$^{0.75}$/d at the maintenance level. Although the pattern of slower rates of protein synthesis at lower protein levels observed in this study is in agreement with other reports, the magnitude of the estimates is comparatively low. The inter-species mean for whole-body protein turnover (in animals fed nutritionally adequate diets) is approximately 15 g/kg $W^{0.75}$/d (Waterlow, 1984), although estimates vary from 12.5 to 18.9 (Reeds & Lobley, 1980). Whole-body fractional synthesis rate estimated from leucine kinetics (Table 3) decreased from 0.03 (high-protein diet) to 0.01 (low-protein diet), but was also low in comparison with other estimates (0.03—0.10) derived from young lambs fed balanced diets (Oddy *et al.*, 1987). These low values may be

Table 3 Whole-body protein turnover, synthesis, and amino acid oxidation calculated from leucine kinetics in sheep fed a diet of wheat straw and supplemented with two levels of protein (standard errors of the means in parentheses)

	Diet	
	Low protein (60 g/d)	High protein (110 g/d)
Whole-body protein turnover		
(g/d)	55[a]	140[b]
	(11.6)	(12.0)
(g/kg$^{0.75}$/d)	4.8[a]	12.1[b]
	(1.0)	(0.9)
Protein synthesis		
(g/d)	42[a]	110[b]
	(9.2)	(10.6)
(g/kg$^{0.75}$/d)	3.6[a]	9.4[b]
	(0.8)	(0.8)
Whole-body amino acid oxidation		
(g/d CP)	13[a]	31[b]
	(2.6)	(2.9)
(g/kg$^{0.75}$/d)	1.1[a]	2.6[b]
	(0.2)	(0.3)
Whole-body fractional protein synthesis rate (/d)	0.01[a]	0.026[b]
	(0.002)	(0.002)

[a,b] Means within the same row with different superscripts differ significantly ($P < 0.01$).

Table 4 Whole-body protein turnover, synthesis and amino acid oxidation calculated from lysine kinetics in sheep fed a diet of wheat straw plus urea and supplemented with two levels of protein (standard errors of the means in parentheses)

	Diet	
	Low protein (60 g/d)	High protein (110 g/d)
Whole-body protein turnover		
(g/d)	51[a]	78[b]
	(7.1)	(3.6)
(g/kg$^{0.75}$/d)	4.5[c]	6.7[d]
	(0.6)	(0.3)
Whole-body protein synthesis		
(g/d)	42[c]	61[d]
	(5.8)	(4.2)
(g/kg$^{0.75}$/d)	3.7[c]	5.3[d]
	(0.5)	(0.4)
Whole-body amino acid oxidation		
(g/d)	9[a]	16[b]
	(1.4)	(1.7)
(g/kg$^{0.75}$/d)	0.75[a]	1.4[b]
	(0.12)	(0.15)

Means within the same row with different superscripts differ significantly: [a,b] ($P < 0.01$); [c,d] ($P < 0.05$).

due to the use of leucine *per se* as a tracer of whole-body protein turnover (see Cronjé *et al.*, 1992). Although the use of lysine as tracer may be criticized on the grounds that limiting (indispensable) amino acids may be preferentially retained within the intracellular space, and so give low estimates of whole-body protein turnover, this does not appear to be the case with leucine. On the contrary, the flux of leucine is recommended as a reliable basis for estimation of whole-body protein synthesis because leucine never seems to be limiting (Riis, 1983). If the leucine-based estimate is accepted as being truly representative of whole-body protein synthesis, then it would appear that the extremely low utilization of leucine for protein synthesis, and hence that of most other amino acids, was due to dietary causes.

Despite these uncertainties, relative changes in response to protein supplementation may enable a qualitative estimate of whole-body protein status to be made: Protein deficiency in malnourished animals is generally accompanied by rates of whole-body protein turnover which are lower than that of normal animals (Millward, 1979), and rates of protein synthesis and breakdown were reduced by 40% in malnourished children (Golden *et al.*, 1977). In the present study, protein synthesis was reduced by 31—62% when the low-protein diet was fed, which suggests that the degree of protein deficiency indicated here was relatively severe.

Conclusions

These results suggest that the supply of lysine was limiting or near-limiting for protein synthesis when the low-protein diet was fed. The results of this study show that the supply of leucine from the low-protein diet was below estimated requirements. The comparatively low rate of whole-body protein synthesis, even at the high level of protein supplementation, indicates that the efficiency of protein synthesis was constrained by other factors, possibly the supply of methionine (Cronjé *et al.*, 1992) and the demand for glucogenic precursors for lipogenesis (Cronjé *et al.*, 1991).

The energy costs of a faster, but less efficient rate of protein turnover induced by amino acid imbalances remains to be investigated, but the consequences for animals already in precarious energy balance could be far-reaching because of the high energy cost of protein turnover. Strategic supplementation of roughage-fed animals with amino acids protected from ruminal degradation is a practice which has received little attention to date, but which could substantially improve the efficiency of utilization of commonly used protein supplements.

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