Amino acid metabolism and whole-body protein turnover in lambs fed roughage-based diets: 2. Methionine metabolism and a comparison of estimates of whole-body protein turnover derived from lysine, leucine and methionine kinetics[1]

P.B. Cronjé*

Irene Animal Production Institute, Private Bag X2, Irene, 1675 Republic of South Africa

J.V. Nolan and R.A. Leng

Department of Microbiology, Biochemistry and Nutrition, University of New England, Armidale, NSW 2351, Australia

Received 28 May 1991; revised 31 January 1992; accepted 26 August 1992

The effect of protein supplementation of a wheat straw diet on the metabolism of lysine, leucine, methionine and urea, and on whole-body protein turnover rate was investigated in lambs. The metabolism of lysine and leucine is reported elsewhere (Cronjé *et al.*, 1992); in this paper methionine metabolism is discussed, and estimates of whole-body protein turnover derived from all three amino acids are compared. Methionine flux rate was determined from a 12-h intravenous infusion of L-[^{35}S] -methionine. Methionine flux, oxidation and incorporation into protein were increased ($P < 0.01$) by protein supplementation, but the proportion of flux oxidized or incorporated into protein was not significantly increased ($P > 0.05$). Methionine flux was used with a high efficiency for protein synthesis (92%). The results indicate that the supply of methionine was limiting for protein synthesis – even with the high-protein diet. Estimates of whole-body protein turnover derived from the various tracers did not differ significantly for the low-protein diet ($P > 0.05$), but all estimates for the high-protein diet were significantly different ($P < 0.06$). The same trend was apparent in the case of whole-body protein synthesis, but leucine and methionine provided similar estimates for the high-protein diet. It was suggested that, although much stands to be gained from studies of the metabolism of specific amino acids under such dietary conditions, the use of such data to calculate whole-body protein metabolism should be approached with caution. The protein metabolic status of the animals in this experiment was characterized as one of primary protein deficiency, exacerbated by amino acid specific deficiencies (methionine and lysine). It was suggested that substantial potential exists for improvement of the efficiency of utilization of existing protein supplements for roughage diets by supplementation with specific amino acids protected from degradation in the rumen, particularly in young growing lambs.

Die invloed van proteïenbyvoeding tot 'n koringstrooidieet op die metabolisme van lisien, leusien en metionien, ureum en heelliggaamproteïenomsettempo in lammers is ondersoek. Die metabolisme van lisien en leusien word elders bespreek (Cronjé *et al.*, 1992); hier word die metabolisme van metionien bespreek, en bepalings van heelliggaamproteïenomsettempo vergelyk. Metionienomsettempo is met behulp van 'n 12 h-binneaarse-infusie van L-[^{35}S] -metionien bepaal. Metionienomsettempo, oksidasie en omset na proteïen is deur proteïenbyvoeding verhoog ($P < 0.01$), maar die proporsie van omsettempo wat geoksideer of in proteïen omgebou is, is nie beïnvloed nie ($P > 0.05$). Metionienomset is met 'n hoë doeltreffendheid vir proteïensintese benut (92%). Hierdie resultate dui aan dat die toevoer van metionien met albei diëte beperkend was vir proteïensintese. Daar was geen verskille tussen beramings van heelliggaamproteïenomset vir die lae-proteïendieet wat vanaf leusien, lisien of metionien gemaak is ($P > 0.05$) nie; alle beramings het egter verskil ($P < 0.06$) ten opsigte van die hoë-proteïendieet. Dieselfde tendens is vir heelliggaamproteïensintese waargeneem, behalwe dat vergelykbare beramings vir die hoë-proteïendieet vanaf metionien en leusien verkry is. Daar is tot die gevolgtrekking gekom dat, alhoewel die studie van die metabolisme van spesifieke aminosure onder hierdie voedingstoestande van groot waarde kan wees, die gebruik van hierdie data om heelliggaamproteïenmetabolisme te bereken versigtig benader behoort te word. Die proteïen-metaboliese status van die diere in hierdie proef is gekenmerk deur 'n primêre, algemene gebrek aan proteïen wat vererger is deur tekorte aan beperkende anminosure (metionien en lisien). Daar word voorgestel dat daar ruim geleentheid bestaan om die benuttingsdoeltreffendheid van bestaande proteïensupplemente noemenswaardig te verhoog, veral in die geval van groeiende lammers, deur byvoeding met aminosure wat teen rumenafbraak beskerm is.

Keywords: Amino acids, methionine, protein turnover, ruminant.

[1] Extract from a PhD thesis submitted by PBC to the University of New England.

* To whom correspondence should be addressed.

Supplementation of roughage diets of low digestibility and nitrogen content with a source of protein which is resistant to degradation in the rumen has been shown to increase ruminant animal production considerably (see Preston & Leng, 1987). Responses to protein supplementation include increased growth rate, voluntary intake, calving percentage, and improved feed conversion ratios. Although several theories have been proposed to account for these effects including slow release of amino acids, sulphur and energy in the rumen (Kellaway & Leibholz, 1983) and provision of glucose and amino acids at the tissue level (Leng *et al.*, 1977), direct evidence at a biochemical level is lacking. In particular, there is little information on the amino acid metabolism of sheep under these conditions. Although techniques for estimating amino acid and whole-body protein turnover are well established for use in humans (Waterlow *et al.*, 1978), and have been success-

fully applied to the study of human protein malnutrition (Millward, 1979), there are few comparisons of tracers for determining protein turnover in ruminants fed roughage diets.

The experiments reported in this series were designed to compare three amino acids as tracers of whole-body protein metabolism in ruminants. Three amino acid tracers, labelled with different isotopes, were infused simultaneously into each animal to ensure that identical metabolic conditions prevailed. Comparisons were also carried out at different levels of protein supplementation in order to evaluate the consistency of ranking under different dietary regimes. Results pertaining to the metabolism of lysine and leucine are presented elsewhere (Cronjé *et al.*, 1992); in this paper the metabolism of methionine is discussed and estimates of whole-body protein turnover derived from all three tracers are compared.

Materials and Methods

Four groups of Merino cross-bred wethers (20—25.5 kg live mass) consisting of three animals per treatment were fed a diet of wheat straw supplemented with either 0, 40, 120, or 200 g/d of a protein mixture designed to provide a high proportion of bypass protein. Details of diet composition are given elsewhere (Cronjé *et al.*, 1992). The amount of basal diet fed was held constant during the infusion period and was equal to the mean voluntary intake recorded over the previous week. Because of the restricted number of experimental animals used here, and a large variation in individual voluntary intake (660—980 g/d), there were no differences in total crude protein (CP) intake between sheep 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 referred to as the low-protein (CP intake: 60 g/d) and high-protein treatments (CP intake: 110 g/d).

Catheters were inserted into both jugular veins on the day before the infusions. 50 μCi L-[^{35}S]-methionine in a solution of sterile physiological saline (9 g/l NaCl) was infused intravenously into each sheep over 12 h at a rate of 0.25 ml/min. Blood samples for analysis of methionine specific radioactivity (SR) were taken into heparinized tubes at the following intervals after beginning the infusion: 0, 0.5, 1, 2.5, 5, 6, 10, 12 h.

The fractional contribution of L-[^{35}S]-methionine to the flux of urinary SO_4^{-2} was estimated from the SR of urine samples collected during the infusion period. 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 on a daily basis into 20 ml glacial acetic acid for the following 4 days. Urine samples were stored at −15 °C until further analysis.

Four days after the methionine infusion, a single dose of 100 μCi $Na_2{}^{35}SO_4$ in 5 ml saline solution was administered intravenously. The irreversible loss rate (ILR) of sulphate was estimated from the SR of urine samples taken at 1, 5, 10, 21, and 24 h following injection of tracer.

Details of sample preparation and determination of methionine SR by HPLC are given elsewhere (Cronjé *et al.*, 1992). Urinary sulphate sulphur was isolated following reduction to sulphide by the method of Bird & Fountain (1970) and mixed with a 2:1 toluene / Triton-X scintillation cocktail (Patterson & Greene, 1965) for measurement of beta emission.

Urea ILR was determined using a single injection of [$^{15}N_2$]-urea (1.5 mg atoms ^{15}N) in 5 ml saline. Urine was collected quantitatively into glacial acetic acid (2 ml) at 1, 5, 10, 21 and 24 h after the injection and stored at −15 °C. Urea concentration was determined colorimetrically using the diacetyl-monoxime method (Marsh *et al.*, 1957). Urea-N was isolated enzymatically and its enrichment with ^{15}N was measured using a mass spectrometer (GEC-AEI Ltd, Manchester, UK) according to the procedures of Nolan & Leng (1974).

Nitrogen (N) balance and organic matter (OM) digestibility were measured over a period of 7 days commencing on the day of infusion. N and OM concentrations were analysed according to standard (AOAC, 1980) methods.

Calculations

Methionine SR in plasma reached a plateau within 3—4 h after commencing the infusion. Methionine flux rate was estimated from the SR of a bulk sample derived from aliquots collected every 2 h between the 6th and 12th hours of infusion as follows:

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

Methionine oxidation rate was calculated on the assumption that plasma $^{35}SO_4^{-2}$ represents the oxidation product of ^{35}S-methionine, and that the SR of the urinary SO_4^{-2}-sulphur pool reflects that of plateau SR in plasma (Kennedy *et al.*, 1975). The SR of urinary SO_4^{-2} from methionine oxidation did not reach plateau within the 12-h methionine infusion in all cases and was estimated by extrapolation. The proportion of urinary SO_4^{-2}-sulphur arising from methionine (transfer quotient) was calculated from the ratio of the estimated urinary SO_4^{-2} SR plateau and the plasma methionine SR plateau. The rate of urinary methionine-sulphate excretion was derived from the product of the transfer quotient and the ILR of urinary SO_4^{-2}.This figure was corrected using the proportional contribution of urinary sulphate loss to total ILR to give an estimate of the total amount of methionine-S passing through the plasma SO_4^{-2} pool (methionine oxidation rate). It was assumed that the proportion of flux not oxidized represented that incorporated into protein (Reeds *et al.*, 1981).

The ILR of SO_4^{-2} was calculated as:

$$SO_4^{-2}\ \text{ILR} = \frac{\text{Dose of } {}^{35}SO_4^{-2}\ \text{(DPM)}}{(S/k)}$$

where S and k represent the intercept and slope respectively of the single exponential function describing the disappearance curve of urinary SO_4^{-2} with time.

Whole-body protein turnover was calculated assuming that whole-body protein contains 2.5% methionine (Mathers & Miller, 1979), and that methionine flux represents 2.5% of whole-body protein turnover:

$$\text{Whole-body protein turnover (g/d)} = \frac{\text{Methionine flux (g/d)}}{0.025}$$

Whole-body amino acid oxidation was calculated as above,

and protein synthesis was calculated as the difference between turnover and oxidation.

Urea ILR was calculated from the cumulative excretion of ^{15}N-urea in urine over a 24-h period (Nolan & Stachiw, 1979) where:

$$\text{Urea ILR (g/d)} = \frac{\text{Urea-N excreted (g/d)}}{\text{Proportion of injected } ^{15}\text{N-urea excreted as urea in the urine}}$$

Urea degraded in the gut was estimated as the difference between ILR and urinary excretion rate.

Statistical analysis

Data were analysed for statistical significance by analysis of variance.

Results and Discussion

Methionine and sulphate-S kinetics

The ILR of SO_4^{-2} from plasma (0.49—0.61 g/d) found in this study (Table 1) is intermediate between that of sheep fed a diet of spear grass (0.13—0.2 g/d) and that of sheep fed a lucerne diet (1.26–1.54 g/d) as reported by Kennedy *et al.*, (1975). Urinary SO_4^{-2} excretion rate (Table 1) was also intermediate between the values of 0.02 g/d (spear grass) and 0.66 g/d (lucerne) reported by Kennedy *et al.* (1975).

Table 1 Sulphate 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)
Sulphate-S ILR (g/d)	0.49	0.61
	(0.04)	(0.07)
Sulphate-S excretion in urine (g/d)	0.21	0.33
	(0.03)	(0.05)
(Proportion of ILR)	0.42[a]	0.53[b]
	(0.03)	(0.03)

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

Methionine flux rate (Table 2) was increased from 7 to 13 μmol/min ($P < 0.01$) by protein supplementation. These rates are similar to those summarized by Egan *et al.* (1984) for sheep fed wheaten chaff diets (8—10 μmol/min). In their study, abomasal infusion of methionine (1.5 mmol/h) increased flux rate to 40—46 μmol/h. A similar response was reported by Gill & Ulyatt (1979): methionine flux rate of sheep fed silage (9 μmol/min) was increased to 13 μmol/min by an intraperitoneal methionine infusion (1 g/d). In that experiment, a flux rate of 9 μmol/min corresponded to an estimated methionine absorption rate of 0.9 g/d, which only just falls within the range of estimated minimum dietary requirements (0.7—2.2 g/d; Buttery & Foulds, 1988) for a 25-kg sheep. Compared with a flux rate of 7 μmol/min for the low-protein diet in the present experiment, this would suggest that the methionine supply was below requirements. The flux rate measured for the high-protein diet corresponds to that reported by Gill & Ulyatt (1979) for a diet which supplied 2.1 g methionine/d, which corresponds to the upper estimate of minimum requirements.

The percentage of methionine flux incorporated into protein has been used as a measure of the efficiency of utilization of methionine (Mathers & Miller, 1979). Typically, the oxidation of an amino acid rises sharply once requirements are exceeded (Armstrong & Annison, 1973); conversely, a limiting amino acid will be used with high efficiency until it is supplied in excess, when efficiency will decrease to a level determined by the supply of the next limiting amino acid. In this study, 93% of methionine flux was incorporated into protein, and there were no differences ($P > 0.05$) between treatments. In other experiments with sheep (Gill & Ulyatt, 1979), this proportion decreased from 85% to 81% when a diet deficient in methionine (0.9 g/d methionine available for absorption) was supplemented with methionine (2.1 g/d methionine available for absorption), and in experiments with calves also decreased from 82% to 75% with methionine infusion (Mathers & Miller, 1979). In unpublished experiments with sheep, quoted by Egan *et al.* (1984), supplementation of a diet providing an estimated methionine absorption of 0.9 g/d with excess levels of methionine (6.2 g/d methionine absorbed) decreased the proportion of flux incorporated into protein from 70% to 20%. The high proportion of flux incorporated into protein, and the lack of response to protein supplementation in the present experiment suggest that methionine supply was limiting for protein synthesis – even at the high level of protein supplementation. Although this interpretation is open to criticism, it does provide a measure of the relative amino acid status on these diets without recourse to incremental additions of a particular amino acid; a procedure which is in any case open to criticism (see Millward, 1985).

Whole-body protein turnover

Using L-[1-^{14}C]-methionine, Mathers & Miller (1979) estimated whole-body protein synthesis in calves to be 10 g/kg$^{0.75}$/d. This is similar to that observed for the high-protein diet in this study (9 g/kg$^{0.75}$/d; Table 2), but is substantially higher than that measured for the low-protein diet (5 g/kg$^{0.75}$/d). In the former study, infusion of methionine (6 g/d) increased whole-body protein synthesis to 16 g/kg$^{0.75}$/d.

Comparison of estimates of whole-body protein turnover derived from lysine, leucine, and methionine kinetics

Techniques for estimating whole-body protein turnover from the total amount of amino acids passing through the free amino acid pool of the blood are well established for use in humans and have been particularly useful in studies of human protein malnutrition, but have not been applied extensively to investigate consequences of low-protein diets fed to ruminants. Whole-body protein turnover may also be estimated as the sum of the protein synthesis rates of some major tissues. This involves slaughter of the animal immediately after the infusion of tracer. Both methods have theoretical limitations (Reeds *et al.*, 1979). Recently, a method has been described where whole-body turnover was derived from direct measurements in all tissues (Attaix *et al.*, 1988) following a large flooding dose

Table 2 Methionine flux, oxidation, and incorporation into protein, and whole-body protein 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)
Methionine flux		
(μmol/min)	6.8[a]	13.1[b]
	(1.3)	(1.2)
(μmol/min/kg$^{0.75}$)	0.59[a]	1.13[b]
	(0.11)	(0.10)
Methionine oxidation		
(μmol/min)	0.43[a]	0.97[b]
	(0.08)	(0.09)
(μmol/min/kg$^{0.75}$)	0.038[a]	0.084[b]
	(0.007)	(0.007)
Methionine incorporated into protein		
(μmol/min)	6.3[a]	12.1[b]
	(1.2)	(1.2)
(μmol/min/kg$^{0.75}$)	0.56[a]	1.05[b]
	(0.1)	(0.1)
Plasma methionine concentration		
(μmol/100 ml)	2.4	2.8
Whole-body protein turnover		
(g/d)	58[a]	113[b]
	(11)	(10)
(g/kg$^{0.75}$/d)	5.1[a]	9.7[b]
	(1.0)	(0.8)
Whole-body protein synthesis		
(g/d)	54[a]	104[b]
	(10)	(10)
(g/kg$^{0.75}$/d)	4.8[a]	9.0[b]
	(0.9)	(0.8)
Whole-body amino acid oxidation		
(g/d)	3.7[a]	8.3[b]
	(0.7)	(0.8)
(g/kg$^{0.75}$/d)	0.33[a]	0.72[b]
	(0.06)	(0.06)

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

Table 3 Comparison of estimates of whole-body protein turnover, protein synthesis, and amino acid oxidation derived from lysine, leucine (Cronjé *et al.*, 1992), and methionine in lambs fed wheat straw plus urea and supplemented with two levels of protein (standard errors of the means in parentheses)

	Lysine	Leucine	Methionine
Whole-body protein turnover (g/d)			
(Low-protein diet)	51	55	58
	(7)	(12)	(11)
(High-protein diet)	78[a]	140[b]	113[c]
	(4)	(12)	(10)
Whole-body protein synthesis (g/d)			
(Low-protein diet)	43	42	54
	(6)	(9)	(10)
(High-protein diet)	61[x]	110[y]	104[y]
	(4)	(11)	(10)
Whole-body amino acid oxidation (g CP/d)			
(Low-protein diet)	8.5[x]	13.3[x]	3.7[y]
	(1.4)	(2.6)	(0.7)
(High-protein diet)	16.4[x]	30.5[y]	8.4[z]
	(1.7)	(2.9)	(0.8)

Means within the same row with different superscripts differ significantly: ([a,b,c] $P < 0.06$; [x,y,z] $P < 0.01$).

of tracer amino acid. Although this technique is attractive on theoretical grounds, the large amount of tracer required for animals of the size used in this experiment would make costs prohibitive. The plasma amino acid method used in this study was chosen because it is less expensive and quicker than other methods, and is suitable for studies of diet changes as it does not involve slaughter of the animal. This technique is based on the assumption that the metabolism of the particular amino acid tracer used is representative of that of all amino acids in the body. Estimates of whole-body protein turnover derived from the various tracers did not differ ($P > 0.06$) for the low-protein diet (Table 3), but all estimates differed ($P < 0.06$) for the high-protein diet. The same trend was apparent in the case of whole-body protein synthesis, but leucine and methionine provided similar estimates for the high-protein diet. This trend is similar to the findings of Young *et al.* (1981) who observed that the fluxes of leucine and lysine were similar, but diverged at higher levels of protein supplementation.

Differences between estimates of whole-body protein synthesis may be related to inherent differences between amino acids because of differences in partitioning between the extracellular and intracellular free and bound amino acids (Garlick & Clugston, 1981; Simon *et al.*, 1978; Lobley *et al.*, 1980), or they may arise as a consequence of the amino acid content of the diet. It is often difficult to distinguish between these two factors, as indispensable amino acids may be preferentially retained within the intracellular space (Riis, 1983), particularly when dietary conditions are such that they become limiting. The fact that the supply of all three amino acids with the low-protein diet was shown to be below estimated minimum requirements and indications that methionine and lysine were limiting, may explain the similarity of estimates of whole-body protein turnover and synthesis at that level. Both methionine and lysine are known to be potentially limiting for growth in sheep (Schelling *et al.*, 1967), and for the reasons discussed above cannot therefore be regarded as reliable tracers for estimation of whole-body protein kinetics. Both these amino acids gave lower estimates of whole-body protein turnover, synthesis and oxidation for the high-protein diet than leucine, which is probably never limiting in ruminants (Riis, 1983), and is regarded as a reliable tracer for this purpose (Riis, 1983). If reliance is to be placed on estimates obtained using leucine, the comparatively low values measured in this experiment (Cronjé *et al.*, 1992) indicate that whole-body protein metabolism was sub-optimal, probably because efficiency was constrained by an inadequate supply of the limiting amino acids, methionine and lysine. This interpretation is supported by the other indices

of nitrogen metabolism that were measured. Although the amount of crude protein retained at the high level of protein supplementation was double ($P < 0.05$) that for the low-protein diet (Table 4), the efficiency of CP retention (retention as a proportion of intake) was not increased ($P > 0.05$). The proportion of urea ILR excreted in the urine was 42% with the high-protein diet, and decreased to 27% ($P < 0.01$) with the low-protein diet (Table 4). This pattern is in agreement with comparable studies (Allen & Miller, 1976), and does suggest that some metabolic adjustment was made to contain non-amino-acid-nitrogen losses. However, the percentage of urea ILR lost by urinary excretion (27%) for the low-protein diet is higher than the estimate of 20% recorded by Allen & Miller (1976) for animals of comparable live mass fed a diet which also supplied 60 g/d CP.

Table 4 Urea metabolism, feed intake, digestibility and nitrogen retention 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)
Urea ILR (g N/d)	11.1	14
	(1.7)	(0.6)
Urinary urea excretion rate (g N/d)	2.6[a]	5.9[b]
	(0.24)	(0.29)
(proportion of urea ILR)	0.27[a]	0.42[b]
	(0.05)	(0.02)
Urea degraded in the gut (g N/d)	8.4	8.1
	(1.8)	(0.5)
(proportion of urea ILR)	0.73[c]	0.58[d]
	(0.05)	(0.02)
Retention of crude protein (g/d)	11[c]	25[d]
	(4)	(4)
(proportion of intake)	0.17	0.22
	(0.05)	(0.03)
Organic matter intake (g/d)	509[c]	599[d]
	(9)	(36)
Organic matter digestibility (g/kg DM)	470[c]	517[d]
	(11)	(14)
Straw intake (g DM/d)	616	619
	(13)	(49)

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

Conclusions

It is suggested that the techniques used in this study for quantifying amino acid flux, oxidation and incorporation into protein could be applied to good effect to improve supplementary feeding strategies, but caution should be exercised in calculating whole-body protein turnover rates from such data. Leucine appeared to give reasonable estimates of whole-body protein kinetics, but in view of the large differences between estimates given by the amino acids used, it is suggested that more than one tracer be used in such studies.

It appears reasonable to characterize the protein metabolic status of the animals used in this experiment as one of primary protein deficiency, exacerbated by amino acid specific deficiencies, notably methionine and lysine. Although there can be little doubt that supplementation of roughage-fed ruminants with bypass protein will increase growth rates, further improvements may be attainable with supplements balanced to alleviate specific amino acid deficiencies. Indeed, because of the high energy costs associated with increased protein turnover, protein supplementation may have a negative effect on the precarious energy balance of roughage-fed ruminants if this is not offset by an increase in efficiency. Supplementation with protein alone has been shown in practice to decrease the fat reserves of roughage-fed sheep (Ørskov & Hovell, 1986). In this study, only 26% of protein synthesized in sheep fed the low-protein diet was retained in the tissues, and this fell to 22% for the high-protein diet.

The addition of amino acids protected against rumen degradation to protein supplements for low-quality roughages is a field which has received little attention to date, but which may yield promising results under grazing conditions.

Acknowledgements

The authors thank Messrs S. Stachiw and F. Ball, and Mrs M. Goosem for their excellent technical assistance and advice. This work was conducted at the University of New England while P.B.C. was on study leave from the Animal and Dairy Science Research Institute, South Africa.

References

AOAC, 1984. Official methods of analysis (14 edn.). Association of Official Analytical Chemists, Inc. Arlington, Virginia.

ALLEN, S.A. & MILLER, E.L., 1976. Determination of nitrogen requirement for microbial growth from the effect of urea supplementation of a low N diet on abomasal N flow and N recycling in wethers and lambs. *Br. J. Nutr.* 36, 353.

ARMSTRONG, D.G. & ANNISON, E.F., 1973. Amino acid requirements and amino acid supply in the sheep. *Proc. Nutr. Soc.* 32, 107.

ATTAIX, D., AUROSSEAU, E., MANGHEBATI, A. & ARNAL, M., 1988. Contribution of liver, skin and skeletal muscle to whole-body protein synthesis in the young lamb. *Br. J. Nutr.* 60, 77.

BIRD, P.R. & FOUNTAIN, R.D., 1970. A method for determination of sulphur in some biological materials. *Analyst* 95, 98.

BUTTERY, P.J. & FOULDS, A.N., 1988. Amino acid requirements of ruminants. In: Recent developments in ruminant nutrition. 2. Eds. Haresign, W. & Cole, D.J.A., Butterworths, London. p. 19.

CRONJÉ, P.B., NOLAN, J.V. & LENG, R.A., 1992. Amino acid metabolism and whole-body protein turnover in lambs fed roughage-based diets. 1. Lysine and leucine metabolism. *S. Afr. J. Anim. Sci.* 22, 194.

EGAN, A.R., BENEVENGA, N.J. & RADCLIFFE, B.C., 1984. The metabolism of S-amino acids in sheep. In: Ruminant physiology – concepts and consequences. Eds. Baker, S.K., Gowthorne, J.M., Mackintosh, J.B. & Purser, D.B., University of Western Australia, Perth. p. 241.

GARLICK, P.J. & CLUGSTON, G.A., 1981. Measurement of whole-body protein turnover by constant infusion of carboxyl-labelled leucine. In: Nitrogen metabolism in man. Eds. Waterlow, J.C. & Stephen, J.M.L., Applied Science Publishers, London. p. 303.

GILL, M. & ULYATT, M.J., 1979. The metabolism of methionine in silage-fed sheep. *Br. J. Nutr.* 41, 605.

KELLAWAY, R.C. & LEIBHOLZ, J., 1983. Effects of initrogen supplements on intake and utilization of low-quality forages. *Wld. Anim. Rev.* 48, 33.

KENNEDY, P.M., WILLIAMS, E.R. & SIEBERT, B.D., 1975. Sulphate recycling and metabolism in sheep and cattle. *Aust. J. Biol. Sci.* 28, 31.

LENG, R.A., KEMPTON, T.J. & NOLAN, J.V., 1977. Non-protein nitrogen and bypass proteins in ruminant diets. Australian Meat Research Committee, Review No. 33.

LOBLEY, G.E., MILNE, V., LOVIE, J., REEDS, P.J. & PENNIE, K., 1980. Whole body and tissue protein synthesis in cattle. *Br. J. Nutr.* 43, 491.

MARSH, W.H., FINGERHUT, B. & KIRSCH, E., 1957. Determination of urea nitrogen with the diacetyl method and an automatic dialyzing apparatus. *Am. J. Clin. Pathol.* 28, 681.

MATHERS, J.C. & MILLER, E.L., 1979. The determination of amino acid requirements. In: Protein metabolism in the ruminant. Ed. Buttery, P.J., Agricultural Research Council, London. p. 3.1.

MILLWARD, D.J., 1979. Protein deficiency, starvation and protein metabolism. *Proc. Nutr. Soc.* 38, 77.

MILLWARD, D.J., 1985. Human protein requirements: The physiological significance of changes in the rate of whole-body protein turnover. In: Substrate and energy metabolism. Eds. Garrow, J.S. & Halliday, D., John Libbey & Co., London. p. 135.

NOLAN, J.V. & LENG, R.A., 1974. Isotope techniques for studying the dynamics of nitrogen metabolism in ruminants. *Proc. Nutr. Soc.* 33, 1.

NOLAN, J.V. & STACHIW, S., 1979. Fermentation and nitrogen dynamics in Merino sheep given a low-quality roughage diet. *Br. J. Nutr.* 42, 63.

ØRSKOV, E.R. & HOVELL, F.D.DeB., 1986. Protein metabolism and utilization during undernutrition in ruminants. In: Nuclear and related techniques in animal production. Proceedings of a symposium, Vienna, 17—21 March 1986. International Atomic Energy Agency, Vienna. p. 429.

PATTERSON, M.S. & GREENE, R.C., 1965. Measurement of low energy beta-emitters in aqueous solution by liquid scintillation counting of emulsions. *Analytical Chemistry* 37, 854.

PRESTON, T.R. & LENG, R.A., 1987. Matching ruminant production systems with available resources in the Tropics and Sub-tropics. Penambul Books, Armidale, Australia.

REEDS, P.J., FULLER, M.F., LOBLEY, G.E. & McDONALD, J.D., 1981. Effects of changes in the intakes of protein and non-protein energy on whole-body protein turnover in growing pigs. *Br. J. Nutr.* 45, 539.

REEDS, P.J., LOBLEY, G.E., HARRIS, C.I. & FULLER, M.F., 1979. Methods of studying protein metabolism in large animals. In: Protein metabolism in the ruminant. Ed. Buttery, P.J. Agricultural Research Council, London. p. 8.1.

RIIS, P.M., 1983. The pools of cellular nutrients. Amino acids. In: Dynamic biochemistry of animal production. Ed. Reis, P.M., Elsevier, Amsterdam. p. 151.

SCHELLING, G.T., HINDS, F.C. & HATFIELD, E.E., 1967. Effect of dietary protein levels, amino acid supplementation and nitrogen source upon the plasma free amino acid concentrations of growing lambs. *J. Nutr.* 92, 339.

SIMON, O., MUNCHMEYER, R., BERGNER, H., ZEBROWSKA, T. & BURACZEWSKA, L., 1978. Estimation of the rate of protein synthesis by constant infusion of labelled amino acids in pigs. *Br. J. Nutr.* 40, 243.

WATERLOW, J.C., GARLICK, P.J. & MILLWARD, D.J., 1978. Protein turnover in mammalian tissues and in the whole body. North Holland Publ. Co., Amsterdam.

YOUNG, V.R., ROBERT, J.J., MOTIL, K.J., MATTHEWS, D.E. & BIER, D.M., 1981. Protein and energy intake in relation to protein turnover in man. In: Nitrogen metabolism in man. Eds. Waterlow, J.C. & Stephen, J.M.L., Applied Science Publishers Ltd., London. p. 419.