Whole-body protein turnover in Merino sheep selected for wool and body growth

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ABSTRACT

Whole-body protein turnover rate was measured in sheep selected for high (H) fleece production, high fibre diameter, and low (L) live weight (i.e. HHL) and in LLH and LLL genotypes. Whole-body protein turnover was estimated by means of a single intra-abomasal dose of either ^{15}N-glycine or ^{15}N-duckweed (*Spirodela polyrrhiza*), followed by estimation of the recovery of tracer in urine over 48 h. The tracers were administered during a period of N balance measurements. Estimates of whole-body turnover (flux, synthesis and degradation rate) did not differ ($P > 0.05$) between genotypes (duckweed: 0.91, 0.68 and 0.53 *v.* glycine: 0.80, 0.55 and 0.40 g N/kg LW/d, $P > 0.05$). However, protein synthesis and degradation rate (g N/kg LW/d) obtained using the labelled duckweed as a tracer were higher ($P < 0.05$) than when using ^{15}N-glycine as the tracer. The HHL group (high wool producers) partitioned a larger ($P < 0.05$) proportion of absorbed ^{15}N tracer to wool growth than into non-wool body N accretion. We concluded that genotypes selected for high wool production and high fibre diameter used protein more efficiently for wool growth than genotypes selected for high meat (body) growth. Neither tracer method detected any difference ($P > 0.10$) between genotypes in whole-body protein metabolism. It was concluded that both tracers are likely to be adequate for comparative measurements.

Keywords: Merino, wool growth, protein turnover, ^{15}N-glycine, ^{15}N-duckweed.

INTRODUCTION

The non-invasive 'single-dose end-product' tracer method has been widely used to determine protein turnover in human subjects and in farm animals (Waterlow *et al.* 1978; Golden and Jackson 1981; Fern *et al.* 1985). However, the method is considered to have limitations due to tracer type, the route of introduction of tracer and the simplicity of the assumed model of the metabolic pools in the animal. In particular the use of single amino acid, such as ^{15}N-glycine as a tracer for all protein amino acids has been questioned (Wutzke *et al.* 1983; Millward *et al.* 1991).

There is conflicting evidence from previous studies on whether protein turnover differs between Merino genotypes selected for wool growth or meat production (Oddy *et al.* 1995; Adams and Cronjé 2003). In broad terms, patterns of growth are regulated through expression of specific genes, the extent and timing of gene expression being mainly controlled by nutrient supply (Oddy and Sainz 2002). There is a need to determine whether biological mechanisms such as nutrient partitioning and protein turnover in sheep affect the expression of genetic differences in their wool and meat production.

The current study was undertaken to compare a plant protein source (duckweed) containing amino acids uniformly labelled with ^{15}N, or a single amino acid (^{15}N-glycine) as tracers for estimating whole-body protein turnover rate in sheep. The study also determined whether Merino sheep selected for high and low wool production characteristics had different rates of whole-body protein turnover or partitioned different ratios of protein to wool fibre and body tissues.

MATERIALS AND METHODS

Animals and diets

Three groups of four 3-year-old Merino ewes were selected from the Trangie Q-PLU$ flock, each group from selection lines that had been selected for or against fleece or body growth characteristics (Hatcher *et al.* 2004). Each sheep was identified as an 'extreme' individual within its own selection line for combinations of clean fleece weight, fibre diameter and live weight. The three groups (genotypes) exhibited high fleece weight, high fibre diameter and low live weight (HHL), or low fleece weight, low fibre diameter and high live weight (LLH) or low fleece weight, low fibre diameter and low live weight (LLL). Wool production and live weight of the 12 ewes used in this study, measured at 12 months of age having always grazed in the same flock, are given in Table 1.

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TABLE 1: Clean fleece weight (CFW), fibre diameter (FD) and live weight (LW) (mean ± s.e.m.; n = 4) of ewes from three selection lines at Trangie, NSW. These 12 ewes were 12 months old when the measurements below were recorded. They were 3 years old when used in this study.

Category	CFW (kg)	FD (µm)	LW (kg)
HHL	$5.0^{a} \pm 0.15$	$21.4^{a} \pm 0.26$	$41.8^{a} \pm 0.80$
LLH	$3.7^{b} \pm 0.17$	$18.5^{b} \pm 0.30$	$49.3^{b} \pm 0.92$
LLL	$3.2^{b} \pm 0.15$	$17.9^{b} \pm 0.26$	$39.5^{a} \pm 0.80$

[a, b:] Means bearing different superscripts in the same column differ significantly ($P<0.05$).

Chopped lucerne hay was offered daily at 1.7 kg/100 kg of live weight in two equal portions (0800 and 1800 h) throughout the experiment to promote a 'steady-state' metabolism. The lucerne chaff contained 885 g dry matter (DM)/kg, 32.2 g N/kg DM and 10.2 MJ ME/kg DM. Feed supplied and feed refusals were weighed daily to determine DM and N intake. Water was continuously available to the ewes.

Tracer administration

To enable the two tracer methods to be compared in a 'cross-over' experiment, the ewes were randomly assigned to two groups with two ewes of each genotype in each group. At the end of a 21-day adaptation period (start of Period 1), Group 1 was given a single injection into the abomasum of 0.18 g/kg LW of ^{15}N-labelled duckweed (8.68 mol ^{15}N/mol N) in 100 ml of warm distilled water. The duckweed had been grown on water containing ^{15}N-labelled ammonium sulphate according to the method described by Li (2001). At the same time, Group 2 was given a single intra-abomasal injection of 2.5 mg [^{15}N]-glycine/kg LW (99 atom percent excess; Amersham International) in 100 ml warm distilled water. Samples of urine, faeces and wool were taken for 48 h after tracer administration. After 43 d (start of Period 2), the tracer dosing and sampling procedures were repeated for all 12 ewes, with Group 2 receiving the ^{15}N-labelled duckweed and Group 1 receiving the ^{15}N-labelled glycine.

Sample collection

In the 12-h period before each tracer injection, a urine sample and a faecal sample were taken from each ewe and analysed to determine background enrichments in urine and faeces. At the same time as the tracers were administered, a new urine collection was started. The urine was filtered through glass wool into buckets containing about 10 ml of 6 M HCl as a preservative. Urine and faeces were collected separately and removed at 10, 24, 34, and 48 h. Urine weight was recorded at the end of each collection period and the urine was mixed thoroughly before representative aliquots (1 to 2% by weight) were taken and stored at –20 ºC. All faeces for each period were also stored separately at -10°C until analysis.

An area of approximately 100 cm^2 of skin on the left and right mid-side region of each sheep was clipped with small animal clippers three weeks before each of the two tracer injections to create an area to enable the wool grown immediately following the tracer injections to be sampled. Each wool sample was retained for determining the partition of ^{15}N to wool fibre. Ten tufts of wool fibres were plucked from one of the mid-side sample areas at 10, 24, 34 and 48 h post-injection and stored in individual bags. The other mid-side area was sampled following the second injection. Allocation of which side was sampled was balanced across genotypes and tracers.

Laboratory analysis

Dry matter concentration of the food and faeces was determined by drying sub-samples of both materials at 60°C in a forced draught oven to a constant weight. Total N concentration in all samples (feed, urine, faeces and wool) was determined by the Kjeldahl method (AOAC 1980). An automatic N analyser (Carlo Erba Instruments, NA 1500 Nitrogen/Carbon/Sulphur Analyser) interfaced with a mass spectrometer was used to estimate ^{15}N abundance in the sample nitrogen (TRACERMASS, Stable Isotope Analyser; Europa Scientific, Pfeiffer/Balzers). N enrichment values were obtained by subtracting the ^{15}N abundance of samples taken before tracer administration from the ^{15}N abundance of samples collected after tracer administration. For more details of analytical procedures refer to (Li 2001).

The clipped wool samples were washed in warm water to remove extraneous wax, suint and soil in order to obtain a clean sample for nitrogen analysis.

Calculation of whole-body protein turnover

Whole-body protein turnover rate, synthesis rate and degradation rate were calculated using an end-product method (Wessels *et al.* 1997).

Whole-body protein turnover rate was calculated as the amount of ^{15}N (mol) excreted as a percentage of the total ^{15}N dose absorbed at the plateau of the cumulative ^{15}N excretion curve over 48 h according to the following equation.

$$\text{Protein}_{\text{turnover}} = N_{\text{urine}} * 100 / R \qquad (1)$$

where

$Protein_{turnover}$ = Whole-body protein turnover rate (g N/d)
N_{urine} = Excretion rate of urinary N (g N/d)
R = Fractional recovery of ^{15}N in urine (%)

Under steady state conditions where the size of the metabolic N pool remains constant, whole-body protein turnover rate is the sum of the whole-body synthesis rate and the excretion rate for the metabolite in question. Under these conditions the whole-body synthesis rate was calculated from the following relationship.

$$Protein_{synthesis} = Protein_{turnover} - N_{urine} \quad (2)$$

where

$Protein_{synthesis}$ = Whole-body synthesis rate (g N/d)
N_{urine} = Excretion rate of urinary N (g N/d)

Similarly under steady state conditions the whole-body synthesis rate is also the sum of the whole-body degradation rate and the retention rate for the metabolite in question. Whole-body degradation rate was calculated from the following equation.

$$Protein_{degradation} = Protein_{synthesis} - N_{retention} \quad (3)$$

where

$Protein_{synthesis}$ = Whole-body synthesis rate (g N/d)
$Protein_{degradation}$ = Whole-body degradation rate (g N/d)
$N_{retention}$ = Total daily N retention (g N/d)

Total daily N retention was obtained by the expression:

$$N_{retention} = N_{intake} - N_{urine} + N_{faeces} \quad (4)$$

where

$N_{retention}$ = Total daily N retention (g N/d)
N_{intake} = Total daily N intake (g N/d)
N_{urine} = Excretion rate of urinary N (g N/d)
N_{faecal} = Excretion rate of faecal N (g N/d)

Protein turnover (g N/d), protein synthesis (g N/d) and protein degradation rates (g N/d) were each adjusted for body size by dividing each estimate by the respective live weight (LW) of each sheep (g N/kg LW/d) to facilitate comparison with other studies.

Statistical analysis

The multifactorial ANOVA procedure of Statgraphics Plus was used to test the effects of period, treatment and genotype and their interaction. The same animals were used in both experimental periods: the period effect was not significant for any output variable, and consequently the data for each variable for both periods were combined. All values are expressed as the least square mean and the standard error of each mean from the analysis of variance. Multiple comparisons of means were performed using Fisher's least significant difference procedure. The statistical significance level was set at $P<0.05$.

RESULTS

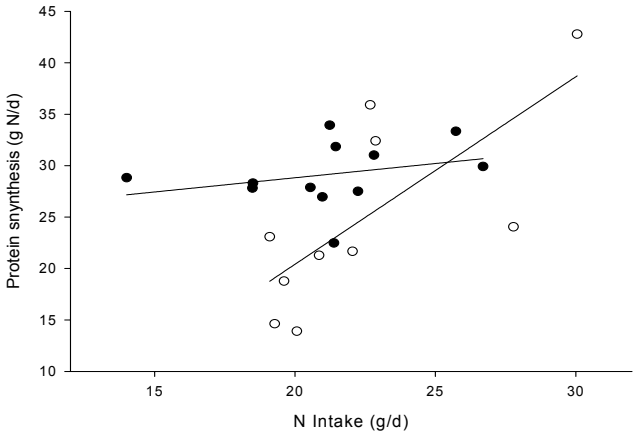
One of the LLH ewes given ^{15}N-duckweed died during Period 1 so no data for this ewe were available in Period 2.

All ewes ingested all feed offered. Average daily DM intakes (g/kg LW/d) did not differ between genotypes ($P > 0.05$) and were 15.3 ± 0.44, 14.4 ± 0.51 and 15.4 ± 0.44 g/kg LW/d for HHL, LLH and LLL, respectively.

Whole-body protein metabolism

When the tracer was ^{15}N-duckweed, the calculated protein synthesis and degradation rates expressed as g N/kg LW/d were higher ($P < 0.05$) than when ^{15}N-glycine was administered. A significant relationship between protein synthesis rate and N intake was evident when ^{15}N-glycine was used as the tracer but not when ^{15}N-duckweed was used (see Fig.1).

FIGURE 1: Relationship between N intake and protein synthesis rate determined by using ^{15}N-labelled glycine (○) and ^{15}N-labelled duckweed (●).



Efficiency of protein synthesis (N retention/N synthesis) was closely related to protein synthesis rate because of the similarity of N retention values and the estimate of efficiency was

therefore higher with the glycine tracer than with the duckweed tracer. However, there were no significant differences between genotypes in the whole-body protein turnover were detected by either tracer method when results were expressed as g N/kg LW/d. (Table 2).

Partitioning of absorbed ^{15}N

When given a ration calculated to provide 6.5-7.2 MJ/d and a dietary CP intake of 128-142 g/d, there were no differences ($P > 0.05$) between genotypes in the fraction of the ^{15}N dose that was apparently absorbed from the intestines, or in the proportions of apparently absorbed ^{15}N excreted in urine. However, the higher wool producing ewes (HHL) partitioned a greater ($P < 0.05$) fraction of absorbed ^{15}N to wool than the lower producers (LLH and LLL) (Table 3).

DISCUSSION

Comparison of uniformly labelled protein and single amino acid

This study indicated that estimates of whole-body protein metabolism differed significantly between the two tracer methods, with whole-body protein synthesis/degradation rates determined using ^{15}N-labelled duckweed being significantly higher than when using ^{15}N-labelled glycine (Table 2). One possible explanation is that the single amino acid (glycine) does not truly represent the mixture of all amino acids needed for tissue protein synthesis. Moreover, some glycine enters pathways that do not result in protein synthesis.

The lower fraction of administered ^{15}N that was excreted in urine from ^{15}N-labelled duckweed compared to ^{15}N-labelled glycine (Table 3), suggested that a lower fraction of the mixture of amino acid N entering the free amino acid pool was oxidized or used in reactions that did not result in protein synthesis than was the case for glycine. Differences in the pathways by which glycine is metabolised relative to the mixture of all amino acids might also explain why protein synthesis rate determined by the glycine tracer was apparently significantly related to N intake but there was no such relationship when a mixture of uniformly labelled amino acids from duckweed was used as the tracer.

TABLE 2: Estimates (means ± s.e.m.) of whole-body protein metabolism (g N/kg LW/d) derived using either ^{15}N-labelled duckweed or ^{15}N-labelled glycine in three genotypes of Merino sheep selected for high (H) or low (L) fleece weight, mean fibre diameter or live weight respectively.

	^{15}N-duckweed			**^{15}N-glycine**			**s.e.m.**	**P values**		
Genotype	HHL	LLH	LLL	HHL	LLH	LLL		Genotype	Treatment	Genotype × Treatment
Live weight (kg)	45.0	47.3	41.4	45.9	49.3	41.0	3.02	NS	NS	NS
Protein intake (g N/kg LW/d)	0.49	0.44	0.50	0.50	0.50	0.49	0.020	NS	NS	NS
Protein synthesis (g N/kg LW/d)	0.64	0.64	0.71	0.47	0.63	0.54	0.063	NS	<0.05	NS
Protein retention (g N/kg LW/d)	0.13	0.14	0.14	0.16	0.15	0.13	0.023	NS	NS	NS
Protein degradation (g N/kg LW/d)	0.51	0.50	0.57	0.32	0.48	0.40	0.072	NS	<0.05	NS
Protein turnover (g N/kg LW/d)	0.92	0.84	0.97	0.71	0.88	0.81	0.067	NS	<0.05	NS
Efficiency of protein deposition (%)[1]	19.3	26.3	19.6	36.4	23.2	25.2	5.82	NS	NS	NS

[1] Protein retention/protein synthesis × 100

TABLE 3: Percentage recovery in urine and wool after 48 h (means ± s.e.m.) of absorbed ^{15}N following the injection of ^{15}N-labelled duckweed or ^{15}N-labelled glycine into the abomasum of three genotypes of Merino sheep selected for different of high (H) or low (L) combinations of, respectively, fleece weight, mean fibre diameter or live weight.

	^{15}N-Duckweed			**^{15}N-Glycine**			**s.e.m.**	**P values**		
	HHL	LLH	LLL	HHL	LLH	LLL		Genotype	Treatment	Genotype × Treatment
Absorption of ^{15}N (% dose injected)	90.0	91.5	90.6	96.3	97.5	97.1	1.08	NS	<0.01	NS
Urine (%)	30.2	23.8	26.6	33.8	27.7	34.6	3.17	NS	<0.05	NS
Wool (%)	0.15	0.03	0.1	0.10	0.10	0.08	0.02	0.04	NS	NS

Comparison between different genotypes

No differences were detected between genotypes in protein intake or protein retention so it is not surprising that neither tracer method detected any differences in whole-body protein turnover rate (Table 2). The estimates of whole-body protein turnover were based on the excretion of all N in urine, Methods based on total N excretion rather than on urea or ammonia N may be less imprecise because some N-containing compounds in urine such as creatinine, methylhistidine and hippuric acid arise from metabolic processes unrelated to amino acid oxidation, and do not reflect the N pool generated from oxidation of amino acids (Wessels *et al.* 1997).

Our estimates of whole-body protein synthesis and degradation rates by both methods (duckweed: 29.4 and 24.5 g N/d v. glycine: 24.1 and 18.2 g N/d, respectively) are considerably lower than those reported by Liu *et al.* (1995) and Adams *et al.* (2000) in younger sheep. Liu *et al.* (1995) measured whole-body protein turnover by using [1-^{13}C]-leucine in 7-month-old castrated lambs (Suffolk × Greyface; live-weight about 37 kg) intra-gastrically infused with casein and volatile fatty acids. They reported that the average protein synthesis and degradation rates were 35.1 and 39.5 g N/d, respectively, for sheep with a low N intake (0.5 mg/kg $LW^{0.75}$/d). Adams *et al.* (2000) also determined whole-body protein turnover by using L-[ring-d_5]phenylalanine in 20-month-old Merino wethers (live-weight about 41 kg) selected for or against staple strength, when fed at 1.1 × maintenance and found that the protein synthesis rate was 49.3 g N/d and degradation rate was 48.3 g N/d. Difference in age and sex of the sheep used may have accounted for the differences in whole-body protein turnover rate between the studies.

Differences in the protein: energy ratio in the absorbed nutrients may also have affected the amino acid partitioning. Even though the animals were given a maintenance intake of metabolisable energy (ME), their protein supply (0.44-0.50 g/kg LW/d or 20-25 g N/d) was very high relative to minimum requirements (according to (SCA 1990), the net protein requirement is about 27 g/d for 50 kg Merino ewes) which might affect the whole-body protein synthesis and degradation rates. If the amount of absorbed amino acids exceeds the requirements for protein synthesis as determined by ME intake (or by the genetic potential for growth), the excess is mainly subject to oxidation, releasing the keto acids that can enable fat to be stored and ammonia that is converted to urea. A further study of energy expenditure and protein turnover in different genotypes is needed to determine whether there are penalties in relation to the animal's survival ability on poor quality feed when animals are selected for wool production characteristics (Adams and Cronjé 2003).

In summary, the present study of mature Merino ewes suggests that genotypes selected for high fleece yield and high fibre diameter partitioned a greater fraction of absorbed ^{15}N to wool production and therefore used absorbed protein more efficiently for wool growth than genotypes selected for high live weight. The fraction of absorbed ^{15}N excreted in urine was significantly higher when ^{15}N-glycine was the tracer rather than ^{15}N-labelled duckweed whereas differences in the fractions recovered in wool were not detectable by either method. The technique involving uniformly labelled duckweed gave significantly higher estimates of whole-body protein turnover, synthesis and degradation rate than the single amino acid-glycine technique but both tracers are likely to be adequate for comparative measurements.

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