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# Whole-body valine and cysteine kinetics and tissue fractional protein synthesis rates in lambs fed Sulla (*Hedysarum coronarium*) and infected or not infected with adult *Trichostrongylus colubriformis*

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Poor growth during parasitic infection may be due to a redistribution of amino acids away from skeletal muscle protein synthesis to the intestinal site of infection. The effect of a *Trichostrongylus colubriformis* infection on whole-body amino acid kinetics and tissue fractional protein synthesis rates were determined in lambs fed fresh Sulla (*Hedysarum coronarium*; 800 g DM/d). Lambs were dosed with 6000 L3 *Trichostrongylus colubriformis* larvae daily for 6 d ($n$ 6) or kept as parasite-free controls ($n$ 6). On day 45 post-infection, the lambs received an intravenous injection of $^2H_2O$ and infusions (8 h) of [$^{35}$S]sulphate to measure the size of the whole-body water and sulphate pools, respectively. On day 48, the lambs were continuously infused for 8 h with [3,4-$^3$H]valine into the jugular vein as well as with [1-$^{13}$C]valine and [$^{35}$S]cysteine into the abomasum. After the 8 h infusions, the lambs were killed and tissue samples collected from the duodenum, ileum, mesenteric lymph nodes, liver, spleen, thymus, muscle and skin. Feed intake (769 $v$. 689 (SD 47) g DM/d) was not affected by infection, whereas liveweight gains (50 $v$. − 50 (SD 70) g/d) were lower and intestinal worm burdens (240 $v$. 18 000 (SD 7000) worms) higher in the infected lambs. Parasitic infection increased the fractional protein synthesis rates in the small intestine, mesenteric lymph nodes and liver but did not affect skin and skeletal muscle fractional protein synthesis rates during the established parasitic infection.

**Parasite infection: Whole-body amino acid kinetics: Tissue protein synthesis: Sheep**

The negative impacts of a parasitic infection on ruminant productivity have been well documented and include decreased liveweight gain (Sykes & Coop, 1976; van Houtert *et al.* 1995). This change has been attributed to a decrease in skeletal muscle protein synthesis and an increase in skeletal muscle protein degradation (Symons & Jones, 1971, 1972, 1975, 1978). The latter suggests that more amino acid are mobilised from skeletal muscle to sustain the higher metabolic rate of the intestine and liver observed during parasitic infection (Symons & Jones, 1971, 1978, 1983; Jones & Symons, 1982; Yu *et al.* 2000) and consequently to support the higher whole-body fractional protein synthesis observed during other types of infection (Breuillé *et al.* 1994). The increased amino acid requirements of the intestinal, liver and immune tissues may be further exacerbated as dietary intake may also reduce during infection (Sykes *et al.* 1988).

Parasitic infection in the small intestine is, however, likely to cause more than a redistribution of amino acids between these tissues because the composition in terms of specific amino acids of the intestinal and liver proteins is different from that of skeletal muscle proteins (MacRae *et al.* 1993). Therefore, to meet the increased requirements for specific amino acids in the affected tissues, proportionally more skeletal muscle amino acids are likely to be mobilised than required (MacRae & Lobley, 1991; MacRae, 1993). Excess amino acids above these demands will be catabolised, mainly in the liver, resulting in a net loss of amino acids from the body and reduced amino acid availability for muscle protein synthesis and other metabolic purposes.

Therefore, the hypotheses of this study were (1) that an established *Trichostrongylus colubriformis* infection would increase the repartitioning of amino acids from skeletal muscle protein by increasing the degradation of skeletal muscle protein to sustain higher protein synthesis in the intestinal, liver and immune tissues and in the whole body, and (2) that these alterations in amino acid utilisation within the whole body would reduce amino acid availability to peripheral tissues and hence decrease skeletal muscle protein synthesis. This was investigated by determining the effects of an established parasitic infection on whole-body protein turnover and

**Abbreviations:** FSR, fractional protein synthesis rate; $FSR_I$, fractional protein synthesis rate based on intracellular pool; $FSR_P$, fractional protein synthesis rate based on plasma pool; ILR, irreversible loss rate; SRA, specific radioactivity; $SRA_B$, specific radioactivity in protein-bound pool; $SRA_I$, specific radioactivity in tissue intracellular pool; $SRA_{MAX}$, plateau SRA in plasma; $SRA_P$, specific radioactivity in plasma pool.

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fractional protein synthesis rate (FSR) in the small intestine (duodenum, ileum), liver, lymphoid tissues (spleen, mesenteric lymph nodes, thymus), muscle and skin of lambs fed fresh forage.

## Materials and methods

### *Animals and feed*

The experimental procedures for this trial were reviewed and approved by the Crown Research Institute Animal Ethics Committee in Palmerston North, New Zealand, according to the Animals Protection Act (1960) and Animals Protection Regulations (1987) and amendments.

Wether lambs (33 kg) were weaned from their dams and transported to AgResearch Ltd, Grasslands Research Centre, Palmerston North, New Zealand. The lambs were shorn, drenched twice with ivermectin (Ivomec, Merial New Zealand Ltd, Manukan City, New Zealand) and treated for external parasites using Wipeout (Coopers, Schering-Plough Animal Health Ltd, Upper Hutt, New Zealand). The lambs were fed lucerne pellets (800 g DM/d) and chaffed lucerne hay (200 g DM/d) for approximately 3 weeks. One week before surgery, the lambs were brought indoors and housed in individual metabolism crates, and were maintained on the same diet until 3 d after surgery.

Twelve lambs were fasted for 24 h before surgery. Anaesthesia was initiated with ketamine (Phoenix, Pham Distributors Ltd, Auckland, New Zealand; 100 mg/ml; 4 mg/kg intravenous) and diazepam (Diazepam DBL, Mayne Pharma Pty Ltd, Karori, Wellington, New Zealand; 5 mg/ml; 1 mg/kg intravenous) and maintained with isoflurane (1·5 %) administered through an endotracheal tube. Permanent indwelling catheters were placed in the mesenteric artery, and the mesenteric, portal and hepatic veins (Huntington *et al.* 1989), vena cava and abdominal aorta (Ortigues & Durand, 1995) for blood sampling and infusions. These arteriovenous catheters were used in a larger study to determine tissue amino acid fluxes on day 48 post-infection (EN Bermingham, NC Roy, IA Sutherland, BR Sinclair, BP Treloar and WC McNabb unpublished results). Additionally, a permanent Teflon cannula was fitted into the abomasum in preparation for the infusion of labelled amino acids on day 48 post-infection. The lambs received a daily intramuscular injection of procaine penicillin (3 ml) for 4 d post-surgery. Catheters were maintained as described by Huntington *et al.* (1989).

When feed intake had recovered after surgery, the lambs were offered approximately 800 g DM/d fresh Sulla (*Hedysarum coronarium*) until the conclusion of the trial. Fresh Sulla was selected instead of the usual ryegrass/white-clover-dominant New Zealand pasture because the proportion of ryegrass and white clover from harvested pasture is variable and it is difficult to maintain feed quality over time. Sulla also promotes a high intake and high liveweight gain because it contains a high level of soluble carbohydrate (18–25 g carbohydrate/kg DM) and contains condensed tannin, which reduces rumen proteolysis and increases the flow of amino acids to the small intestine (Bermingham *et al*. 2001; Burke *et al.* 2002). The Sulla was harvested every 2 d with a sickle-bar mower by 10.00 h and stored at 4°C. The DM content of the Sulla was determined daily in order to adjust the amount of wet forage given to maintain the level of DM offered at approximately 800 g/d. The intake of 800 g/d was chosen to be below intake *ad libitum* (at about 0·6–0·8 of maintenance) to ensure that the control and parasitised animals ate similar amounts. The lambs were fed at hourly intervals from overhead feeders, and water was available *ad libitum*. The lambs were weighed weekly to monitor liveweight change during the experimental period.

### *Treatment and parasitology*

One week after surgery (day 1 of the experimental period), six sheep were given 6000 *T. colubriformis* L3 larvae/d orally for 6 d (parasite treatment), while the remaining six sheep were drenched once with ivermectin (Ivomec, Merial New Zealand Ltd) to serve as controls (control treatment).

This infection protocol was essentially a single dose, with the larval dose split across 6 d to reduce the possibility of rumen bypass and therefore maximise the establishment of larvae in the small intestine. The choice of this method for larval dosing resulted mainly from the use of a complex surgical model involving the catheterisation of seven visceral and peripheral blood vessels and abomasal cannulae combined with an infection protocol. To our knowledge, this was the first attempt to achieve this complex *in vivo* model to quantify the metabolic cost of parasitic infection, and how multicatheterised lambs would respond to the consequences of trickle dosing (i.e. a continual onslaught of larvae as well as the demands caused by the presence of the adult parasite population) after having undergone such an invasive surgical procedure was unknown. Therefore, in order to reduce the ethical cost of the experiment, a single-dose infection method was selected. Additionally, by utilising this single-dose method as opposed to the trickle dosing more frequently utilised, the effects of an adult population of parasite on whole body amino acid kinetics and tissue FSR were quantified without any confounding effects of new larval challenge.

The lambs were assigned to either the control or the parasite group according to a completely randomised block design. Owing to the time-consuming nature of the surgical procedure, a maximum of four lambs were surgically prepared with catheters in any 1 week. Therefore, the lambs were blocked according to the week that they underwent surgery, referred to as the group effect in the statistical model.

Faecal egg counts from individual sheep were determined every second day from day 20 to day 45 of infection using the modified McMaster method in which one egg counted equates to 50 eggs/g wet faeces (Whitlock, 1948). Total intestinal worm burdens in the proximal 10 m of the small intestine were determined after slaughter (Sutherland *et al.* 1999*b*).

### *Infusions and blood sampling*

A temporary catheter was inserted into the jugular vein on day 43 in preparation for isotope administration on days 45 and 48. On day 48, whole-body valine and cysteine kinetics and tissue FSR were measured by performing a continuous 8 h infusion of [3,4-$^{3}$H]valine (5·8 MBq/h) into the jugular vein. Concurrently, [1-$^{13}$C]valine (99 atom percent; 101 mg/h) and [$^{35}$S]cysteine (2.4 MBq/h) were continuously infused into the abomasum. Two routes of infusion were used for valine iso-

topes to simulate the luminal and arterial entry sites of valine into the intestinal cells for measuring intestinal valine kinetics (data not shown). Isotopes of valine were selected because valine is present in high concentration in intestinal endogenous secretions (MacRae & Lobley, 1991). [$^{35}$S]Cysteine was used because of its role in immune responses and the repair of the gastrointestinal tract (MacRae & Lobley, 1991).

Three days prior to the labelled amino acid infusion, administration of other isotopes was performed for measuring parameters that are necessary to calculate the whole-body oxidation of valine and cysteine from [$^{3}$H]valine, [$^{13}$C]valine and [$^{35}$S]cysteine infusions. The lambs received a bolus injection of $^{2}H_2O$ (0·45 ml/kg body weight) into the jugular vein to measure the size of the whole-body water pool. The lambs also received an 8 h continuous infusion of [$^{13}$C]$Na_2SO_4$ bicarbonate (99 atom percent, 205 mg/h) and [$^{35}$S]sulphate (4·56 MBq/h) into the jugular vein to measure the whole-body production of $CO_2$ (data not reported because $CO_2$ isotopic enrichment has yet to be analysed) and sulphate, respectively. This could not be done on day 48 because the same isotopes (i.e. $^{13}$C, $^{35}$S) were used. The residual isotopic activities had returned to background levels at the start of the infusions on day 48.

To prevent blood clotting during the continuous sampling, 2000 IU ovine heparin/h was infused into the jugular vein of the lambs over the 8 h infusion periods on both days (Lobley *et al.* 1995). Sampling lines and syringes were kept in an ice-water bath to minimise the degradation of blood constituents (Lobley *et al.* 1995). On day 45, 20 ml blood was withdrawn continuously from the mesenteric artery over the 8 h infusion period and bulked over 2 h periods. The blood was centrifuged (3270 **g** for 15 min at 4°C), and the plasma harvested and stored at −85°C in order to determine total count, sulphate specific radioactivity (SRA) and concentration, DM content and $^{2}H_2O$ isotopic enrichment. On day 48 and as part of a larger study, 30 ml blood was withdrawn continuously every 2 h from the mesenteric artery, the mesenteric, portal and hepatic veins, and the vena cava over the infusion period. After each 2 h collection period, whole blood was centrifuged (3270 **g** for 15 min at 4°C), and the plasma was harvested and either processed or stored at −85°C for further analysis, as described later.

The plasma data presented in this paper for both days represent the average of samples taken from the last two sampling periods from the mesenteric artery (times 4–6 and 6–8 h). Only the whole-body kinetics relating to the infusion of [3,4-$^{3}$H]valine, [$^{13}$C]valine and [$^{35}$S]cysteine, as well as the tissue FSR calculated from the [3,4-$^{3}$H]valine dilution by the intracellular free valine and incorporation into protein-bound pool, will be presented in this paper.

Radioactive and stable isotopes were purchased from Amersham Life Science (Little Chalfont, Bucks., UK) and Mass Trace, Inc. (Woburn, MA, USA), respectively.

### *Slaughter*

After the completion of blood sampling, but while the isotopic infusions were still being administered, the sheep were killed by an intravenous overdose of sodium pentobarbitone (300 mg/ml, 0·5 ml/kg liveweight). Tissue samples were rapidly collected from the sheep in the following order: skin, muscle (biceps femoris), liver, duodenum, ileum, spleen, mesenteric lymph nodes and thymus. The tissues were washed in 0·9 % NaCl to remove traces of digesta or blood, and quickly frozen in liquid N. A section of whole duodenum and ileum was kept and frozen as described above, with a further section of each tissue separated into the smooth muscle component of duodenal or ileal tissue by gently passing a glass slide over the tissue to remove the mucosal layer. Once frozen, the tissue samples were stored at −85°C until analysed. Time from death until storage of all the tissues in liquid N was less than 20 min.

### *Sample-processing and chemical analysis*

*Plasma parameters*. To determine the concentration of valine in plasma, 0·5 ml plasma was treated with 80 mM dithiothreitol and 3 mM-norleucine (in 1g/litre phenol) as an internal standard and stored at −85°C. The samples were thawed and transferred to a Centrisart filter (10 000 molecular weight cut off; Sartorius AG, Goeffingen, Germany) and then centrifuged at approximately 28 000 ***g*** for 60 min, with the filtrate removed and stored at −85°C until analysis. Quantification of plasma valine concentration was determined after the reverse-phase HPLC separation of phenylisothiocyanate derivatives, using a Waters Pico-Tag column (3·9 × 300 mm; Waters Corporation, Milford, MA, USA) as modified from Bidlingmeyer *et al.* (1984) on a Shimadzu HPLC-10/A HPLC system (Shimadzu Scientific Instruments Ltd, Columbia, MD, USA).

In order to determine plasma cysteine concentration, 2 ml plasma was mixed with 1 ml of a solution containing 75 g/litre w/v SDS, 9 mM-EDTA and 200 μl dithiothreitol (80 mM). Norleucine 100 μl (3 mM in 1g/litre phenol) was added as an internal standard. The samples were mixed and re-weighed after each addition and then left at room temperature for 15 min before adding 1 ml TCA (300 g/litre w/w) to precipitate plasma protein. The tubes were reweighed and centrifuged (3270 **g** for 15 min at 4°C), the resulting supernatant being filtered (0·45 μm) and stored at −85°C. The supernatant was reacted with acid ninhydrin after reduction using dithiothreitol, and the cysteine concentration was determined spectrophotometrically at 570 nm using a continuous flow analyser (Technicon Autoanalyser II; Technicon Corporation, Ardsley, NY, USA) as described by Gaitonde (1967).

Plasma samples from days 45 and 48 were thawed, and 0·5 ml was transferred to a Vivaspin (cut-off 10 000 molecular weight; Viviascience Ltd, Auckland, New Zealand) in order to measure the concentration of sulphate. The samples were spun at approximately 12 000 **g** for 45 min, and 50 μl of the resulting filtrate was removed, placed into an autosampler vial and stored at −20°C until analysed for sulphate concentration. The plasma concentration of sulphate was obtained by correcting back to a sulphate standard. The standard was made using $K_2SO_4$ stock solution (1 mM in buffer), with both plasma samples and standards analysed on an HPLC (Shimadzu HPLC-10/A VP system; Shimadzu Scientific Instruments Ltd). The temperature of the column was 35°C. Analysis of the samples was carried out on a 4·1 mm × 250 mm column with guard column (Wescan; Alltech Associates Inc., Deerfield, IL, USA), with a conductivity detector without suppression. Injections of 5 μl were made with a mobile phase

buffer of *p*-hydoxybenzoic acid (5 mM, pH 8·4) with a flow rate of 1·6 ml/min.

In order to measure the partitioning of tritiated label between valine and water, and of $^{35}S$ label between cysteine and sulphate, total [$^{3}H$] and [$^{35}S$] radioactivity in the infusates and plasma were quantified by mixing 50 μl sample in 2 ml scintillation mixture (Starcint; IN/US Systems Inc., Farifield, NJ, USA) and counting for 10 min (Packard Tricarb Model 1500 Scintillation counter) as described by Lee *et al.* (1999). The proportion of total [$^{3}H$] or [$^{35}S$] radioactivity attributed to valine or cysteine and its breakdown product (water or sulphate, respectively) in these sampled pools was then determined by inline flow on an online detector through a liquid scintillation counter (Model 2, β-ram, IN/US Systems Inc.) coupled to an HPLC (HPLC4/A; Shimadzu Corporation, Kyoto, Japan) as described in Lee *et al.* (1999).

The isotopic enrichment of water in plasma was determined using a method adapted from Yang *et al.* (1998). The samples were analysed by GC (GC QP; Shimadzu) equipped with an MS detector (QP2010; Shimadzu). The temperature of the GC injector and detector was 250°C. Analysis of [$^{13}C_3$-]acetone was carried out on a 30 m × 0·25 mm internal diameter × 0·25 μm ZB-Wax capillary column (Phenomenex Ltd, New Zealand) with the target ions eluting between 1·52 and 1·72 min. The column temperature programme was as follows: initial temperature 70°C, increased to 105°C at 20°C/min, held for 4 min, increased to 250°C at 35°C/min and held for 10 min (source temperature 200°C). Sample injections of 1 μl were made in split mode with a 1:30 split. The carrier gas was He at a flow rate of 40·9 $cm^3$/min. The MS detector was operated using electron impact and selected ion monitoring m/z 61, 62 and 63 for the M, M + 1, M + 2 ions respectively.

*Tissue parameters*. Subsamples (4–5 g) of frozen tissue were pulverised in liquid N using a modified French Cell press as described by Lee *et al.* (1993). The pulverised tissue was then stored at −85°C until further processing. Approximately 1 g smashed tissue was homogenised in an extraction buffer (20 mM Tris pH 7·8, 2·5 mM EDTA, 3g/litre SDS). The homogenate was centrifuged at approximately 28 000 ***g*** for 30 min at 4°C. The supernatant, containing intracellular peptides, amino acids and soluble protein-bound amino acids, was removed, and the resulting pellet (protein-bound fraction) was rewashed in extraction buffer and centrifuged for a further 30 min in order to determine the total [$^{3}H$] counts associated with valine in the protein-bound pool as described earlier for plasma valine. The pellet was freeze-dried and stored at room temperature until analysed for the concentration of amino acids.

Valine concentration in the protein-bound fraction was determined by ion exchange chromatography (Shimadzu Scientific Instruments Ltd, Columbia, MD, USA) with a post-column reaction system using ninhydrin as the derivatising agent after acid hydrolysis of a 50 mg pellet with 6·0 M HCl at 110°C for 22 h. The hydrolysates were filtered and rotary-evaporated to near dryness, washed in deionised water and rotary-evaporated again before being taken up into 0·2 M-sodium citrate buffer (pH 2·2). The total radioactivity of the hydrolysate and the proportion of radioactivity attributed to valine and its breakdown product ($H_2O$) was determined as described earlier for plasma valine.

Free pool supernatant (2 ml) was mixed with 1 ml 7·5g/litre (9 mM) SDS/EDTA to break up the protein structures in the supernatant, 200 μl of 80 mM dithiothreitol (pH 8·0) as an antioxidant and 100 μl 3 mM norleucine as an internal standard. The samples were mixed and left to stand at room temperature for 15 min and then deproteinised with 1 ml 300 g/litre and centrifuged at 4°C at 3270 ***g*** for 15 min. The resulting supernatant containing free pool amino acids was stored at −85°C until analysed for amino acid concentration, total radioactivity and the proportion of radioactivity attributed to valine, as described earlier for plasma valine.

*Calculations*

The SRA (dpm/nmol) of valine and cysteine in plasma ($SRA_P$), tissue free pool ($SRA_I$) and tissue-bound proteins ($SRA_B$) was calculated by dividing the total radioactivity associated with either valine or cysteine in the sampled pool (dpm/ml) by the valine or cysteine concentration in the sampled pool (nmol/ml). The rise in plasma valine or cysteine SRA to plateau was described using the following exponential model (Equation 1). This equation is shown only for valine SRA:

$$\text{Valine SRA}_{\text{A or X}} = \text{valine SRA}_{\text{MAX}}\,\text{X}\,(1 - e^{-kt}) \qquad (1)$$

The valine $SRA_{MAX}$ in Equation 1 is the plateau value of valine SRA assuming the radioactivity increases at a rate $k$ over time $t$ (Waterlow *et al.* 1978). The whole-body model for amino acid kinetics described by Waterlow *et al.* (1978) was used to estimate whole-body valine kinetics. The whole-body irreversible loss rate (ILR) of valine estimated from the [$^{3}H$]valine infusion was determined according to Equation 2 (Harris *et al.* 1992):

$$\text{Valine ILR (mmol/h)} = \frac{[^{3}\text{H}]\text{-valine infusion rate (dpm/h)}}{\text{plateaued valine SRA (dpm/mmol)}} \qquad (2)$$

In order to calculate the whole-body valine oxidation from the infusion of [$^{3}H$]valine, the total amount of [$^{3}H$]water produced from valine over the period of infusion was quantified using the equations outlined by Beckett *et al.* (1992). Following the injection of $^{2}H_2O$, the isotopic enrichment of water follows an exponential decay curve. Therefore, to determine the actual body water volume using a $^{2}H_2O$ bolus, an exponential decay curve was plotted with the plasma water isotopic enrichments on the *y*-axis and time of sampling (0–2 h, 2–4 h, 4–6 h and 6–8 h) in relation to the administration of the $^{2}H_2O$ on the *x*-axis. The value extrapolated back to time zero is the isotopic enrichment of water at the time of the injection. The latter is used to calculate the volume of body water. Valine used for whole-body protein synthesis was calculated as the difference between ILR and oxidation, and was converted to protein synthesis (g/d) assuming a valine concentration of 36 mg/g tissue (MacRae *et al.* 1993). Similarly, whole-body valine ILR calculated from the infusion of [$^{13}C$]valine into the abomasum was also determined using Equation 2. As the analysis of blood [$^{13}C$]$NaHCO_3$ has yet to be completed, breakdown of this calculation into oxidation and protein synthesis is not yet possible.

Whole-body cysteine ILR was calculated according to Equation 2, replacing the infusion rate of [$^3$H]valine and the SRA of valine with the appropriate values for the infusion rate of [$^{35}$S]cysteine and the SRA of cysteine, respectively. The transfer quotient (TQ) of cysteine to sulphate was measured at plateau and is defined as the proportion of radioactivity in the secondary pool (sulphate) that originated from the infusion into a primary pool (cysteine) as described by McNabb *et al.* (1993) and shown in Equation 3. The whole-body cysteine oxidation was calculated using Equation 4.

$$\text{TQ cysteine to sulphate} = \frac{\text{sulphate SRA (dpm/nmol)}}{\text{cysteine SRA (dpm/nmol)}} \quad (3)$$

$$\text{Cysteine oxidation (mmol/h)} = \text{TQ cysteine to sulphate} \times \text{sulphate ILR (mmol/h)} \quad (4)$$

Cysteine leaving the plasma pool for productive purposes was calculated as the difference between ILR and oxidation and then converted to protein synthesis (g/d) assuming a cysteine concentration of 5 mg/g tissue (MacRae *et al.* 1993).

The traditional equation for the estimation of tissue FSR was not used in this study as this equation requires the estimation of the rate-constant describing the rise to plateau of free valine SRA in plasma. This can be achieved by performing frequent intermittent blood samples during the course of a labelled amino acid infusion. Therefore, the accurate rise to plateau of valine SRA in plasma could not be estimated. Instead, the FSR in whole duodenum and ileum, duodenal and ileal smooth muscles, mesenteric lymph nodes, spleen, liver, thymus, skeletal muscle and skin samples was determined according to Equation 5 (Wykes *et al.* 1996):

$$\text{FSR (\%/d)} = \frac{\text{valine SRA}_{(\textit{protein bound})}}{\text{valine SRA}_{(\textit{precursor pool})} * \text{period of infusion (d)}} \times 100 \quad (5)$$

In Equation 5, the length of the infusion period equals 8 h, or 0·33 d. The SRA of valine in the free pool of each tissue was assumed to reflect the steady-state SRA of the true precursor pool, valine-tRNA, for protein synthesis ($FSR_I$). These FSR estimates were also compared with the FSR obtained using the SRA of free valine in arterial plasma (mesenteric artery) as a precursor pool ($FSR_P$).

As isotopic steady state was achieved in arterial plasma between 6 and 8 h of infusion, the FSR estimates calculated using Equation 5 will underestimate the actual rate of FSR. This equation assumes that the SRA of plasma valine is at plateau from the start of the labelled amino acid infusion (which can be achieved by giving a priming dose of the same amino acids).

### *Statistical analysis*

Statistical analysis for all variables was performed using a General Linear Model (SAS Software version 8, 1999-2001, SAS Institute Inc., Cary, NC, USA), with treatment and group (the week that the lamb underwent surgery) used as sources of variation in the model. Additionally, feed intake, faecal egg counts and liveweight were analysed using Proc Mix repeated measures. The data was checked for normality and the presence of outliers by plotting residuals *v.* the predicted residuals. Faecal egg counts were transformed by *ln* $(x + 1)$ before analysis in order to ensure symmetry in the data and to standardise variances across the treatments (Sutherland *et al.* 1999*a*).

Probability values lower than 0·05 were considered to indicate a significant difference, and values between 0·05 and 0·10 to indicate a trend. Results are presented as least squares means and associated pooled standard deviations (SD).

## Results

Faecal egg production peaked at day 26 post-infection (approximately 1800 eggs/g wet faeces) and was significantly higher ($P<0·001$) in the parasitised lambs on day 48 of infection (0 *v.* 600 eggs/g wet faeces in the control and parasitised lambs, respectively). Intestinal worm burdens were significantly higher ($P<0·001$) in the parasitised lambs on day 48 post-infection (240 *v.* 18 000 (SD 7000) worms in the control and parasitised lambs, respectively). The presence of parasites in the small intestine did not change DM intake over the course of the experiment (769 *v.* 689 (SD 47) g DM/d in the control and parasitised lambs, respectively). Whereas liveweight gain over the 48 d period was not affected by parasite infection (75 *v.* −17 (SD 50) g/d in the control and parasitised lambs, respectively) there was a trend ($P=0·06$) for liveweight gain to be reduced in the infected lambs over the last 20 d of the experiment (50 *v.* −50 (SD 70) g/d in the control and parasitised lambs, respectively).

### *Plasma amino acid concentration and isotopic activity*

The concentrations and isotopic activities of cysteine (day 48), valine (day 48) and sulphate (day 45) in the mesenteric artery are presented in Table 1. Parasite infection had no effect on the concentration or SRA of sulphate in the mesenteric artery (Table 1). Cysteine concentration and SRA in the mesenteric artery were similar between the control and parasitised lambs (Table 1). Similarly the concentration, SRA and isotopic enrichment of valine in arterial plasma were similar between control and parasitised lambs (Table 1).

### *Whole-body amino acid kinetics*

Whole-body cysteine kinetics are presented in Table 2. Whole-body sulphate ILR tended to decrease ($P<0·10$) during infection (6·8 *v.* 4·5 (SD 2·1) mmol/h in the control and parasitised lambs, respectively). The whole-body ILR of cysteine was similar between control and parasitised lambs (Table 2). Cysteine oxidation in the whole body did not change during infection (Table 2). Whole-body protein synthesis estimates based on cysteine kinetics were unaffected by parasitic infection (Table 2). The proportion of cysteine ILR used for either protein synthesis or oxidised to sulphate averaged 50 % and was similar between treatments (Table 2).

The valine kinetics in the whole body based on the intravenous infusion of [$^3$H]valine are described in Table 2. Parasitic infection decreased ($P<0·10$) the size of the whole-body water pool (21·7 *v.* 19·4 (SD 1·9) l in the control and parasitised lambs, respectively). The total amount of $^3H_2O$ produced from valine was similar between the control and parasitised

**Table 1.** Cysteine, sulphate and valine level and specific radioactivity (SRA) and/or isotopic enrichment in the mesenteric artery plasma of lambs fed fresh Sulla (*Hedysarum coronarium*) and infected with *Trichostrongylus colubriformis* or kept as parasite-free controls

(Least squares means and associated pooled standard deviations)

| | Treatment | | | |
|---|---|---|---|---|
| | Control (*n* 6) | Parasite (*n* 6) | SD | Statistical significance of effect: *P* |
| Cysteine | | | | |
| Level (mmol/l) | 31·0 | 26·0 | 6·0 | 0·20 |
| SRA (dpm/nmol) | 110·7 | 117·7 | 63·2 | 0·85 |
| Sulphate | | | | |
| Level (mmol/l) | 1·4 | 1·2 | 0·4 | 0·37 |
| SRA (dpm/nmol) | 10·7 | 17·6 | 10·9 | 0·30 |
| Valine | | | | |
| Level (μmol/l) | 202·0 | 235·0 | 64·0 | 0·41 |
| SRA* (dpm/nmol) | 68·9 | 74·6 | 23·3 | 0·68 |
| Enrichment† (APE%) | 4·6 | 4·1 | 3·6 | 0·82 |

APE, atom percent excess.
* Based on the infusion of [$^{3}$H]valine into the jugular vein.
† Based on the infusion of [$^{13}$C]valine into the abomasum.

lambs (39·2 *v.* 39·5 (SD 2·9) dpm × $10^{7}$ in the control and parasitised lambs, respectively). There was no effect of parasitic infection on whole-body valine ILR, oxidation or protein synthesis estimated from the intravenous [$^{3}$H]valine infusion (Table 2). The proportion of valine ILR used for either protein synthesis (approximately 84 %) or oxidised (approximately 16 %) was similar between treatments (Table 2). Whole-body valine ILR, based on the infusion of [$^{13}$C]valine into the abomasum, tended to decrease ($P<0·10$) in the infected lambs (18·2 *v.* 12·3 (SD 3·4) mmol/h in the control and parasitised lambs, respectively).

### *Fractional protein synthesis rates*

The concentration of valine in the protein-bound fraction of duodenal smooth muscle was increased ($P<0·05$) by parasitic infection (Table 3). This was not the case for the intracellular concentration of valine in this tissue (Table 3). The $SRA_P$ and $SRA_I$ of the duodenal smooth muscle was also not affected by the treatments (Table 3). Parasite infection doubled ($P<0·05$) the $SRA_B$ of valine in the duodenal smooth muscle and consequently the $FSR_P$ in this tissue (Table 3). In whole duodenal tissue, the concentration of valine in the protein-bound fraction, but not in the intracellular pool, was increased ($P<0·10$) by parasitic infection (Table 3). There was no effect on $SRA_I$, $SRA_B$, $FSR_I$ or $FSR_P$ in the whole duodenal tissue (Table 3).

Parasitic infection did not affect the concentration of valine in the protein-bound fraction and the intracellular pool but did increase ($P<0·05$) the $SRA_B$ of valine and the $FSR_P$ of the ileal smooth muscle in the parasitised lambs (Table 3). Both the $SRA_I$ and $FSR_I$ in this tissue were unaffected by the treatment. In the whole ileal tissue of

**Table 2.** Whole-body irreversible loss rate (ILR), oxidation and protein synthesis based on the infusion of labelled cysteine (abomasum) and valine (jugular) in lambs fed fresh Sulla (*Hedysarum coronarium*) with or without a *Trichostrongylus colubriformis* infection

(Least squares means and associated pooled standard deviations)

| | Treatment | | | |
|---|---|---|---|---|
| | Control (*n* 6) | Parasite (*n* 6) | SD | Statistical significance of effect: *P* |
| Cysteine | | | | |
| ILR (mmol/h) | 1·4 | 1·1 | 0·2 | 0·17 |
| Oxidation (mmol/h) | 0·7 | 0·5 | 0·2 | 0·13 |
| Synthesis | | | | |
| (mmol/h) | 0·7 | 0·7 | 0·3 | 0·92 |
| (g/d) | 46·8 | 35·5 | 12·7 | 0·32 |
| Synthesis: ILR (%) | 47·9 | 55·5 | 19·2 | 0·48 |
| Oxidation: ILR (%) | 53·1 | 44·5 | 19·2 | 0·48 |
| Valine | | | | |
| ILR (mmol/h) | 4·9 | 4·4 | 1·3 | 0·62 |
| Oxidation (%) | 15·9 | 15·6 | 2·4 | 0·82 |
| (mmol/h) | 0·8 | 0·7 | 0·2 | 0·48 |
| Synthesis | | | | |
| (mmol/h) | 4·1 | 3·8 | 1·1 | 0·66 |
| (g/d) | 320·6 | 297·4 | 88·5 | 0·66 |
| Synthesis: ILR (%) | 84·0 | 85·0 | 1·9 | 0·82 |
| Oxidation: ILR (%) | 16·2 | 15·3 | 1·5 | 0·73 |

**Table 3.** Valine concentration and specific radioactivity (SRA) of the tissue free pool and protein-bound pool and fractional protein synthesis rates (FSR) in the small intestine in lambs infected with or without *Trichostrongylus colubriformis* and fed fresh Sulla (*Hedysarum coronarium*) based on the infusion of [$^3$H]valine into the jugular vein

(Least squares means and their associated pooled standard deviations)

| | Treatment | | | |
|---|---|---|---|---|
| | Control (*n* 6) | Parasite (*n* 6) | SD | Statistical significance of effect: *P* |
| Duodenal smooth muscle | | | | |
| $Valine_I$ (μmol/l) | 28·9 | 35·0 | 8·2 | 0·24 |
| $Valine_B$ (μmol/l) | 19·8 | 22·8 | 1·2 | 0·003 |
| $SRA_I$ (dpm/nmol) | 38·3 | 36·2 | 25·2 | 0·89 |
| $SRA_B$ (dpm/nmol) | 0·8 | 2·0 | 0·8 | 0·03 |
| $FSR_P$ (%/d) | 3·4 | 7·4 | 2·5 | 0·03 |
| $FSR_I$ (%/d) | 8·3 | 26·8 | 22·9 | 0·20 |
| Whole duodenum | | | | |
| $Valine_I$ (μmol/l) | 97·9 | 96·1 | 35·1 | 0·93 |
| $Valine_B$ (μmol/l) | 21·1 | 22·3 | 1·2 | 0·10 |
| $SRA_I$ (dpm/nmol) | 21·6 | 31·5 | 11·6 | 0·18 |
| $SRA_B$ (dpm/nmol) | 5·2 | 5·6 | 1·8 | 0·74 |
| $FSR_P$ (%/d) | 23·9 | 23·8 | 9·0 | 0·98 |
| $FSR_I$ (%/d) | 87·3 | 54·1 | 39·1 | 0·21 |
| Ileal smooth muscle | | | | |
| $Valine_I$ (μmol/l) | 36·7 | 35·0 | 11·8 | 0·81 |
| $Valine_B$ (μmol/l) | 22·5 | 22·9 | 0·9 | 0·44 |
| $SRA_I$ (dpm/nmol) | 23·7 | 18·0 | 15·3 | 0·54 |
| $SRA_B$ (dpm/nmol) | 0·5 | 1·0 | 0·3 | 0·03 |
| $FSR_P$ (%/d) | 2·4 | 4·0 | 1·0 | 0·03 |
| $FSR_I$ (%/d) | 15·8 | 19·3 | 16·2 | 0·72 |
| Whole ileum | | | | |
| $Valine_I$ (μmol/l) | 105·1 | 89·9 | 19·9 | 0·23 |
| $Valine_B$ (μmol/l) | 24·3 | 23·9 | 1·7 | 0·74 |
| $SRA_I$ (dpm/nmol) | 11·1 | 34·3 | 9·9 | 0·01 |
| $SRA_B$ (dpm/nmol) | 2·5 | 3·1 | 1·4 | 0·45 |
| $FSR_P$ (%/d) | 11·6 | 12·0 | 5·8 | 0·92 |
| $FSR_I$ (%/d) | 65·9 | 28·9 | 35·1 | 0·16 |

Subscripts I and P, intracellular and plasma precursor pools, respectively; subscript B, the SRA of the protein-bound fraction.

parasitised lambs, the $SRA_I$ of valine was increased ($P<0·05$), but there was no effect of infection on any of the other tissue parameters (Table 3).

Parasitic infection tended to increase ($P<0·10$) the intracellular concentration of valine in the liver (Table 4). Parasite infection also increased the hepatic $FSR_I$ ($P<0·05$; Table 4). The concentration of valine in the protein-bound fraction, $SRA_I$ and $FSR_P$ in liver were not affected by the treatments (Table 4). Parasite infection increased ($P<0·05$) the $SRA_B$ of valine in the mesenteric lymph nodes, and as a result the $FSR_P$ in this tissue was increased (Table 4). None of the other parameters for the mesenteric lymph nodes were affected by the parasitic infection (Table 4). No parameters calculated for the spleen and thymus showed any change owing to the presence of parasites in the small intestine.

Infected lambs had similar estimates of skeletal muscle $FSR_I$ and $FSR_P$ to those of control lambs, and all the other parameters related to skeletal muscle were unaffected (Table 5). Parasitic infection had no effect on skin parameters including $FSR_I$ or $FSR_P$ estimates despite a lower concentration of valine in the protein-bound fraction (Table 5).

## Discussion

This study shows for the first time that the presence of an established parasitic infection in the small intestine of lambs increased the $FSR_P$ in the duodenal and ileal smooth muscle and mesenteric lymph nodes. The $FSR_I$ in the duodenal smooth muscle was also increased, although this did not achieve statistical significance. Increases in FSR of the intestinal tissues indicate that more of their constitutive proteins are being resynthesised per d. The changes in FSR of the intestinal tissues contradict data in the literature relating to parasitic infection, which suggest that parasitic infection increases the absolute rates of protein synthesis in the small intestine, with no effect on FSR (Symons & Jones, 1983). However, absolute protein synthesis was not measured in the current study, as the weight and N content of the tissues were not measured. Increased ileal smooth muscle FSR has been observed in septic rats, but the authors noted that different models of infection would be likely to have different effects on tissue FSR (Breuillé *et al.* 1994).

The parasite *T. colubriformis* inhabits the proximal section of the small intestine (Vlassoff & McKenna, 1994) and may cause damage to the intestinal tissues either by direct action in the duodenal tissue as the parasite burrows into the epithelial layers (Coop & Angus, 1975), or indirectly owing to the initiation of a general immune response that results in altered functional integrity of subsequent sections of the gastrointestinal tract (Symons, 1978). In the current study, re-establishment of duodenal tissue integrity might account for the increased FSR in the duodenal smooth muscle, whereas the initiation of a general immune response may account for the increased FSR in the ileal smooth muscle.

**Table 4.** Valine concentration and specific radioactivity (SRA) of the tissue free pool and protein-bound pool and fractional synthesis rates (FSR) in the liver and lymphoid tissues in lambs infected with or without *Trichostrongylus colubriformis* and fed fresh Sulla (*Hedysarum coronarium*) based on the infusion of [$^3$H]valine into the jugular vein

(Least squares means and their associated pooled standard deviation (SD))

| | Treatment | | | |
|---|---|---|---|---|
| | Control (*n* 6) | Parasite (*n* 6) | SD | Statistical significance of effect: *P* |
| Liver | | | | |
| $Valine_I$ (μmol/l) | 105·9 | 129·3 | 18·2 | 0·06 |
| $Valine_B$ (μmol/l) | 21·7 | 23·1 | 3·2 | 0·45 |
| $SRA_I$ (dpm/nmol) | 22·6 | 28·5 | 14·7 | 0·97 |
| $SRA_B$ (dpm/nmol) | 2·3 | 3·8 | 1·5 | 0·12 |
| $FSR_P$ (%/d) | 11·4 | 14·7 | 6·3 | 0·38 |
| $FSR_I$ (%/d) | 21·3 | 54·1 | 11·5 | 0·01 |
| Mesenteric lymph nodes | | | | |
| $Valine_I$ (μmol/l) | 118·9 | 111·8 | 37·4 | 0·75 |
| $Valine_B$ (μmol/l) | 21·2 | 20·1 | 2·1 | 0·42 |
| $SRA_I$ (dpm/nmol) | 13·6 | 17·8 | 6·9 | 0·32 |
| $SRA_B$ (dpm/nmol) | 2·3 | 5·1 | 1·3 | 0·01 |
| $FSR_P$ (%/d) | 10·9 | 20·8 | 5·6 | 0·001 |
| $FSR_I$ (%/d) | 67·0 | 92·0 | 39·2 | 0·30 |
| Spleen | | | | |
| $Valine_I$ (μmol/l) | 61·9 | 74·8 | 21·6 | 0·33 |
| $Valine_B$ (μmol/l) | 31·3 | 32·9 | 6·6 | 0·68 |
| $SRA_I$ (dpm/nmol) | 14·9 | 30·7 | 16·6 | 0·19 |
| $SRA_B$ (dpm/nmol) | 1·1 | 1·5 | 0·6 | 0·30 |
| $FSR_P$ (%/d) | 5·1 | 5·6 | 2·4 | 0·95 |
| $FSR_I$ (%/d) | 27·4 | 17·5 | 17·6 | 0·39 |
| Thymus | | | | |
| $Valine_I$ (μmol/l) | 29·2 | 34·3 | 9·5 | 0·38 |
| $Valine_B$ (μmol/l) | 21·3 | 21·2 | 1·1 | 0·88 |
| $SRA_I$ (dpm/nmol) | 19·6 | 24·7 | 15·3 | 0·61 |
| $SRA_B$ (dpm/nmol) | 3·7 | 3·9 | 1·8 | 0·84 |
| $FSR_P$ (%/d) | 15·9 | 15·6 | 5·9 | 0·94 |
| $FSR_I$ (%/d) | 52·8 | 49·7 | 28·8 | 0·86 |

Subscripts I and P, intracellular and plasma precursor pools, respectively; subscript B, the SRA of the protein-bound fraction.

Values for intestinal FSR in the current study were comparable to those in the literature for ruminants under similar infusion protocols (20–107 %/d; Baracos *et al.* 1991), although observations for the intestinal smooth muscle were lower in the current study (8–16 %/d) than those obtained from a flooding dose of valine (31–42 %/d; Lobley *et al.* 1994). Typically, the flooding dose method involves a relatively rapid injection of a large dose of trace amino acids along with the tracer

**Table 5.** Valine concentration and specific radioactivity (SRA) of the tissue free pool and protein-bound pool and fractional synthesis rates (FSR) in the muscle and skin in lambs infected with or without *Trichostrongylus colubriformis* and fed fresh Sulla (*Hedysarum coronarium*) based on the infusion of [$^3$H]valine into the jugular vein

(Least squares means and their associated pooled standard deviations)

| | Treatment | | | |
|---|---|---|---|---|
| | Control (*n* 6) | Parasite (*n* 6) | SD | Statistical significance of effect: *P* |
| Muscle | | | | |
| $Valine_I$ (μmol/l) | 30·9 | 38·1 | 11·0 | 0·29 |
| $Valine_B$ (μmol/l) | 29·4 | 27·8 | 3·5 | 0·44 |
| $SRA_I$ (dpm/nmol) | 37·8 | 38·8 | 17·5 | 0·93 |
| $SRA_B$ (dpm/nmol) | 0·1 | 0·2 | 0·1 | 0·37 |
| $FSR_P$ (%/d) | 0·5 | 0·6 | 0·6 | 0·84 |
| $FSR_I$ (%/d) | 0·3 | 1·4 | 0·9 | 0·11 |
| Skin | | | | |
| $Valine_I$ (μmol/l) | 40·5 | 40·7 | 12·2 | 0·98 |
| $Valine_B$ (μmol/l) | 25·8 | 21·4 | 2·7 | 0·02 |
| $SRA_I$ (dpm/nmol) | 52·5 | 43·8 | 41·2 | 0·76 |
| $SRA_B$ (dpm/nmol) | 0·8 | 0·8 | 0·3 | 0·82 |
| $FSR_P$ (%/d) | 3·8 | 3·3 | 1·5 | 0·57 |
| $FSR_I$ (%/d) | 7·9 | 4·4 | 5·0 | 0·27 |

Subscripts I and P, intracellular and plasma precursor pools, respectively; subscript B, the SRA of the protein-bound fraction.

amino acids to minimise the differences between extracellular and intracellular free amino acids isotopic activity and the isotopic activity of the amino acids acylated to tRNA. The flooding dose is usually performed over a shorter period (5–30 min) than the constant infusion (approximately 8 h) and thus reduces the extent of tracer recycling, which is especially important for tissues with a high turnover rate and with a large secretion component, such as the small intestine and the liver (Davis *et al.* 1999). Estimates obtained using a continuous intravenous approach, such as the current study, are likely to underestimate true intestinal FSR values.

Similarly, estimates of $FSR_I$ were increased in the liver of the parasitised lambs. In the literature, liver FSR estimates were increased in sheep (Jones & Symons, 1982) and guinea-pigs (Symons & Jones, 1971, 1978) during parasitic infection, which is in agreement with the results presented in this study. The increase in liver $FSR_P$ in the current study suggests that there was an increase in the amount of constitutive proteins synthesised by the liver. Some of the constitutive proteins might be required to support the hepatic synthesis and secretion of acute-phase proteins used for the immune response during parasitic infection. Such an increase would be in agreement with the increase in $FSR_P$ in the mesenteric lymph nodes observed in parasitised lambs. The continuous infusion method that we used will result in an underestimation of liver FSR because this method does not measure the FSR of acute-phase proteins synthesised and secreted by the liver.

The increase in FSR in the intestinal tissues (including the mesenteric lymph nodes) and liver might indicate that there was an increase in the amino acid requirement of the intestinal tissues and the tissues involved in the immune response (liver, mesenteric lymph nodes). Although these tissues represent as much as 28 % of whole-body protein turnover (Lobley, 1994), estimates of whole body protein synthesis based on both valine ($^3$H) and cysteine tracers were not increased by parasite infection. A higher contribution of the liver to whole-body protein synthesis has been reported with other types of infection (Breuillé *et al.* 1994) and appears to be in agreement with our data.

A lack of effect of parasitic infection on whole-body amino acid ILR is consistent with that reported by Yu *et al.* (2000) with an intravenous infusion of labelled leucine. However, whole-body valine ILR based on abomasal [$^{13}$C]valine infusion was lower in parasitised lambs. The discrepancies between these two isotopes is not clear, but it may be caused by differences in the metabolism of the [$^{13}$C]- or [$^3$H]valine in the splanchnic tissues, an observation previously noted by Krempf *et al.* (1990). It is also possible that some of the isotope infused into the abomasum could have been taken up by the parasites. Additionally, Yu *et al.* (1990) observed a significant difference in whole-body leucine ILR when both [$^{13}$C]- and [$^{14}$N]leucine were infused via the jugular vein in dogs and suggested that this might be due to a faster rate of N removal than decarboxylation at the whole-body level.

The whole-body estimates of valine kinetics based on the jugular infusion of [$^3$H]valine (approximately 112 mmol/d) presented in this study are in agreement with those presented in the literature for other intravenous labelled amino acid infusions (approximately 48–200 mmol/d; Harris *et al.* 1992; Lobley *et al.* 1996; Connell *et al.* 1997). Estimates of whole-body cysteine kinetics (approximately 30 mmol/d) are lower but within the range previously reported in the literature for sheep fed fresh forage (approximately 19–58 mmol/d; McNabb *et al.* 1993; Wang *et al.* 1994). This lower value may be due either to differences in the level of feed intake (approximately 689–769 (current study) *v.* 900–1300 g DM/d (McNabb *et al.* 1993; Wang *et al.* 1994)) and/or to the route of [$^{35}$S]cysteine infusion. The [$^{35}$S]cysteine was infused into the abomasum in the current study, whereas the kinetics in the other published studies were based on intravenous infusions. The latter estimate does not include the dilution of the cysteine tracer by first-pass metabolism through the gastrointestinal tract, therefore resulting in a higher estimate of ILR. This also explains the higher estimates of whole-body valine ILR based on the abomasal infusion of [$^{13}$C]valine compared with the [$^3$H]valine intravenous infusion (approximately 300 *v.* 112 mmol/d). Additionally, whole-body valine estimates based on the infusion of [$^{13}$C]valine into the abomasum were higher than those based on the infusion of [$^{35}$S]cysteine into the abomasum (approximately 300 *v.* 30 mmol/d). This may be due to the larger pool size of valine in the whole body compared with that of cysteine in both control and parasitised lambs, resulting in a greater dilution of [$^{13}$C]valine and thus a higher ILR when valine was used as a tracer (Bermingham, 2004).

Despite reduced growth in infected lambs in the last 20 d of the experimental period, a similar FSR in the biceps femoris muscle between the control and the parasitised lambs was observed in this study. This latter data contradicts observations in the literature that indicate a decrease in muscle FSR during parasitic infection (Jones & Symons, 1982).

The muscle FSR estimates for the control lambs in this study averaged 1 %/d, and these values are low compared with observations made under similar infusion protocols (e.g. 3–5 %/d; Davis *et al.* 1981). It is possible that the 8 h infusion period in the current study was too short to allow sufficient incorporation of [$^3$H]valine into the skeletal muscle protein pool. Therefore, caution must be exercised when interpreting the effect of parasitic infection on muscle $FSR_P$ and $FSR_I$.

Despite an increased FSR in the intestinal, hepatic and immune tissues and reduced growth, infection did not change whole-body and skeletal muscle protein metabolism. It is possible that, between days 26 and 48, the metabolism has largely adjusted to the parasite burden and has returned to normal. Measurement of whole-body and tissue protein metabolism at day 48 was based on previous data from Yu *et al.* (2000), which were measured at week 5 of dosing using a trickle infection protocol (i.e. a constant exposure to infective larvae at least once a week). Changes in intestinal amino acids kinetics and protein metabolism were obtained in the Yu *et al.* (2000) study and observed in the current study. Studies indicating changes in muscle protein metabolism (synthesis and/or degradation) during parasitic infection have also used more severe infection protocols than the one used in the current study. Therefore, the infection regimes may account for the altered muscle protein turnover that was observed in those studies.

In this experiment, lambs were infected with 6000 L3 *T. colubriformis* larvae/d for 6 d to provide a total dose of 36 000 larvae, as opposed to the trickle dose infection (i.e. constant exposure to infective larvae at least once a week) that is commonly reported in the literature (e.g. Jones & Symonds, 1982; Sykes & Coop, 1976; Yu *et al.* 2000). Trickle

doses are more representative of what occurs on the farm (MacRae and Lobley, 1991) and mean that the animal is dealing with the continual onslaught of new larvae as well as the existing adult populations. Therefore, any data relating to alterations in metabolism in the trickle-infected animal may be caused by the initial challenge by the parasite larvae, the developing adult worms and finally the breeding adult worms. By utilising the single-dose method, the effect of an adult population of parasites in the small intestine on whole-body protein turnover and tissue FSR was determined without any confounding effects of new larval challenges.

It is possible that more severe metabolic alterations may have occurred earlier in the infection cycle with the single-dose method. An increase in nutrient requirements by the parasite during its growth and reproductive cycle may also incur a metabolic cost to the host, with the period leading up to peak egg production (up to day 26 post-infection) likely to alter the metabolism of the lamb.

The increase in FSR of intestinal, immune and hepatic tissues in the infected lambs suggests that additional amino acids are still required for supporting increased protein synthesis at day 48 post-infection. Thus, additional amino acids must be sourced from protein stores elsewhere in the body and/or from the diet. The decreased liveweight gain of the parasitised animals over the last 20 d of the experimental period supports this explanation. Our data on interorgan fluxes of amino acids in parasitised lambs (EN Bermingham, NC Roy, IA Sutherland, BR Sinclair, BP Treloar and WC McNabb, unpublished results) will provide some information about the source of amino acids to support the changes in the small intestine, immune tissues and liver of lambs with an established parasitic infection following a bolus dose of infective *T. colubriformis* larvae.

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## References

Baracos VE, Brun-Bellut J & Marie M (1991) Tissue protein synthesis in lactating and dry goats. *Br J Nutr* **66**, 451–465.

Beckett PR, Cadenhead A & Fuller MF (1992) Valine oxidation: the synthesis and evaluation of L-[-$^{3}$H]-valine as a tracer *in vivo*. *Br J Nutr* **68**, 139–151.

Bermingham EN () (2004) The metabolic cost of an intestinal parasite infection on amino acid kinetics in sheep fed fresh forages. PhD Thesis, , Massey University, New Zealand.

Bermingham EN, Hutchinson KJ, Revell DK, Brookes IM & McNabb WC (2001) The effect of condensed tannins in Sainfoin (*Onobrychi Viciifolia*) and Sulla (*Hedysarum coronarium* on the digestion of amino acids in sheep. *Proc NZ Soc Anim Prod* **61**, 116–119.

Bidlingmeyer BA, Cohen SA & Tarvin TL (1984) Rapid analysis of amino acids using pre-column derivatisation. *J Chromatogr* **336**, 93–104.

Breuillé D, Rose F, Arnal M, Meilin C & Obled C (1994) Sepsis modifies the contribution of different organs to whole-body protein synthesis in rats. *Clin Sci* **86**, 663–669.

Burke JL, Waghorn GC & Brookes IM (2002) An evaluation of sulla (*Hedysarum coronarium*) with pasture, white clover and Lucerne for lambs. *Proc NZ Soc Anim Prod* **62**, 152–156.

Connell A, Calder AG, Anderson SE & Lobley GE (1997) Hepatic protein synthesis in the sheep: effect of intake as monitored by use of stable-isotope-labelled glycine, leucine and phenylalanine. *Br J Nutr* **77**, 255–271.

Coop RL & Angus KW (1975) The effect of continuous doses of *Trichostrongylus colubriformis* larvae on the intestinal mucosa of sheep and on liver vitamin A concentration. *Parasitology* **70**, 1–9.

Davis SR, Barry TN & Hughson GA (1981) Protein synthesis in tissues of growing lambs. *Br J Nutr* **46**, 409–419.

Davis TA, Fiorotto ML, Burrin DG & Vann RC (1999) Protein synthesis in organs and tissues: quantitative methods in laboratory animals. In *Methods for Investigation of Amino Acid and Protein Metabolism*, pp. 49–68 [A El-Khoury, editor]. New York: CRC Press.

Gaitonde MK (1967) A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. *Biochem J* **104**, 627–633.

Harris PM, Skene PA, Buchan V, Milne E, Calder AG, Anderson SE, Connell A & Lobley GE (1992) Effect of food intake on hind-limb and whole-body protein metabolism in young growing sheep: chronic studies based on arterio-venous techniques. *Br J Nutr* **68**, 389–407.

Huntington GB, Reynolds CK & Stroud BH (1989) Techniques for measuring blood flow in splanchnic tissues of cattle. *J Dairy Sci* **72**, 1583–1595.

Jones WO & Symons LEA (1982) Protein synthesis in the whole body, liver, skeletal muscle and kidney cortex of lambs infected with *Trichostrongylus colubriformis*. *Int J Parasitol* **12**, 295–301.

Krempf M, Hoerr RA, Marks L & Young VR (1990) Phenylalanine flux in adult men: estimates with different tracers and route of administration. *Metabolism* **39**, 560–562.

Lee J, Harris PM, Sinclair BR & Treloar BP (1993) Whole body metabolism of cysteine and glutathione and their utilisation in the skin of Romney sheep: consequences of wool growth. *J Agric Sci (Camb)* **121**, 111–124.

Lee J, Knutson RJ, Davis SR, Louie K, Mackenzie DDS & Harris PM (1999) Sulphur amino acid metabolism in the whole body and mammary gland of the lactating Saanen goat. *Aust J Agric Res* **50**, 413–423.

Lobley GE (1994) Amino acid and protein metabolism in the whole body and individual tissues of ruminants. In *Principles of Protein Nutrition of Ruminants*, pp. 147–178 [JM Asplund, editor]. Boca Raton: CRC Press.

Lobley GE, Connell A, Lomax MA, Brown DS, Milne E, Calder AG & Farningham DAH (1995) Hepatic detoxification of ammonia in the ovine liver: possible consequences for amino acid catabolism. *Br J Nutr* **73**, 667–685.

Lobley GE, Connell A, Milne E, Newman A & Ewing TA (1994) Protein synthesis in splanchnic tissues of sheep offered two levels of intake. *Br J Nutr* **71**, 3–12.

Lobley GE, Connell A, Revell DK, Bequette BJ, Brown DS & Calder AG (1996) Splanchnic-bed transfers of amino acids in sheep blood and plasma, as monitored through use of a multiple U-$^{13}$C-labelled amino acid mixture. *Br J Nutr* **75**, 217–235.

MacRae JC (1993) Metabolic consequences of intestinal parasitism. *Proc Nutr Soc* **52**, 121–130.

MacRae JC & Lobley GE (1991) Physiological and metabolic implications of conventional and novel methods for the manipulation of growth and production. *Livest Prod Sci* **27**, 43–59.

MacRae JC, Walker A, Brown D & Lobley GE (1993) Accretion of total protein and individual amino acids by organs and tissues of growing lambs and the ability of nitrogen balance techniques to quantitate protein retention. *Anim Prod* **57**, 237–245.

McNabb WC, Waghorn GC, Barry TN & Shelton ID (1993) The effect of condensed tannins in *Lotus pedunculatus* on the digestion and metabolism of methionine, cystine and inorganic sulphur in sheep. *Br J Nutr* **70**, 647–661.

Ortigues I & Durand D (1995) Adaptation of energy metabolism to undernutrition in ewes. Contribution of portal-drained viscera, liver and hindquarters. *Br J Nutr* **73**, 209–226.

Sutherland IA, Brown AE, Green RS, Miller CM & Leathwick DM (1999*a*) The immune response of sheep to larval challenge with *Ostertagia circumcinta* and *O. ostertagi*. *Vet Parasitol* **84**, 125–135.

Sutherland IA, Leathwick DM, Green R, Brown AE & Miller CM (1999*b*) The effect of continuous drug exposure on the immune response to *Trichostrongylus colubriformis* in sheep. *Vet Parasitol* **80**, 261–271.

Sykes AR & Coop RL (1976) Intake and utilisation of food by growing lambs with parasitic damage to the small intestine caused by daily dosing with *Trichostrongylus colubriformis* larvae. *J Agric Sci (Camb)* **86**, 507–515.

Sykes AR, Poppi DP & Elliot DC (1988) Effect of concurrent infection with *Ostertagia circumcincta* and *Trichostrongylus colubriformis* on the performance of growing lambs consuming fresh forages. *J Agric Sci (Camb)* **110**, 531–541.

Symons LEA (1978) Epithelial cell mitosis and morphology in worm-free regions of the intestine of the rat infected by *Nippostrongylus brasiliensis*. *J Parasitol* **64**, 958–959.

Symons LEA & Jones WO (1971) Protein metabolism 1. Incorporation of $^{14}$C-L-leucine into skeletal muscle and liver proteins of mice and guinea pigs infected with *Nematospiroides dubis* and *Trichostrongylus colubriformis*. *Exp Parasitol* **29**, 230–241.

Symons LEA & Jones WO (1972) Protein metabolism 2. Protein turnover, synthesis and muscle growth in suckling, young and adult mammals infected with *Nematospiriodes dubuis* or *Trichostrongylus colubriformis*. *Exp Parasitol* **32**, 335–342.

Symons LEA & Jones WO (1975) Skeletal muscle, liver and wool protein synthesis by sheep infected by the nematode *Trichostrongylus colubriformis*. *Aust J Agric Res* **26**, 1063–1072.

Symons LEA & Jones WO (1978) Protein metabolism 5. *Trichostrongylus colubriformis*: changes of host body mass and protein synthesis in guinea pigs with light to heavy infections. *Exp Parasitol* **44**, 7–13.

Symons LEA & Jones WO (1983) Intestinal protein synthesis in guinea pigs infected with *Trichostrongylus colubriformis*. *Int J Parasitol* **13**, 309–312.

van Houtert MFJ, Barger IA, Steel JW, Windon RG & Emery DL (1995) Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubriformis*. *Vet Parasitol* **56**, 163–180.

Vlassoff A & McKenna PB (1994) Nematode parasites of economic importance in sheep in New Zealand. *NZ J Zool* **21**, 1–8.

Wang Y, Waghorn GC, Barry TN & Shelton ID (1994) The effect of condensed tannins in *Lotus corniculatus* on plasma metabolism of methionine, cystine and inorganic sulphate by sheep. *Br J Nutr* **72**, 923–935.

Waterlow JC, Garlick PJ & Millward DJ (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*. Amsterdam: North-Holland Biomedical Press.

Whitlock H (1948) Some modifications of the McMaster helminth egg-counting technique and apparatus. *J Council Sci Indusl Res Austr* **21**, 177–180.

Wykes LJ, Fiorotto M, Burrin DG, Del Rosario M, Frazer ME, Pond WG & Jahoor F (1996) Chronic low protein intake reduces tissue protein synthesis in a pig model of protein malnutrition. *J Nutr* **126**, 1481–1488.

Yang D, Diraison F, Beylot M, Brunengraber DZ, Samols MA, Anderson VE & Brunengraber H (1998) Assay of low deuterium enrichment of water by isotopic exchange with [U-$^{13}C_3$]acetone and gas chromatography-mass spectrometry. *Anal Biochem* **258**, 315–321.

Yu F, Bruce LA, Calder AG, Milne E, Coop RL, Jackson F, Horgan GW & MacRae JC (2000) Subclinical infection with the nematode *Trichostrongylus colubriformis* increases gastrointestinal tract leucine metabolism and reduces availability of leucine for other tissues. *J Anim Sci* **78**, 380–390.

Yu YM, Wagner DA, Tredget EE, Walaszewski JA, Burke JF & Young VR (1990) Quantitative role of splanchnic region in leucine metabolism: L-[1-$^{13}$C,$^{15}$N]leucine and substrate balance studies. *Am J Physiol* **259**, E36–E51.