

## The metabolic fate of the amido-N group of glutamine in the tissues of the gastrointestinal tract in 24 h-fasted sheep

J. J. Gate<sup>1\*</sup>, D. S. Parker<sup>1†</sup> and G. E. Lobley<sup>2‡</sup>

<sup>1</sup>*Department of Biological and Nutritional Sciences, Faculty of Agriculture and Biological Sciences, University of Newcastle, Newcastle-upon-Tyne NE1 7RU, UK*

<sup>2</sup>*Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK*

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Whole-body and gastrointestinal tract (GIT) metabolism of [5-<sup>15</sup>N]glutamine were monitored in lambs (33 kg live weight) fasted for 24 h. Animals were previously prepared with vascular catheters across the mesenteric-(MDV) and portal-drained viscera (PDV) to permit quantification of mass and isotopic transfers of metabolites by arterio-venous difference. Continuous infusions of [5-<sup>15</sup>N]glutamine into the jugular vein were conducted for 10 h and integrated blood samples withdrawn over 75 min intervals for the last 5 h of infusion. The lambs were then killed and portions from various tissues of the digestive tract and other body organs removed for determination of <sup>15</sup>N enrichment in RNA, DNA and protein (the latter obtained by difference using total acid-precipitable N). Whole-body glutamine flux was 108 μmol/min of which 23 and 47 % could be attributed to MDV and PDV metabolism ( $P < 0.001$ ) respectively. There was a small net production of glutamine across the MDV. GIT blood-flows and NH<sub>3</sub> production were partitioned 3:2 between MDV and non-MDV components. Less than 5 % of the NH<sub>3</sub> produced was derived from the amido-N of glutamine, while across the small intestine (MDV) 26 % of the glutamine flux was converted to NH<sub>3</sub>, compared with 18 % for non-MDV transfers. The <sup>15</sup>N enrichments in protein were of the order jejunum > duodenum > ileum with mucosal cells more labelled than serosal ( $P < 0.001$ ). Lesser enrichments were observed for other GIT tissues (abomasum > caecum > rumen) while liver and lymph were comparable with the abomasum; kidney, spleen and muscle were lower still ( $P < 0.05$ ). Enrichments of RNA were similar to that of protein and followed the same pattern, except for higher relative values for liver, spleen and lymphoid tissue. The lowest enrichments were observed for DNA, but again the pattern order was similar except for increased label in lymph, caecum and the spleen. For the MDV there was reasonable agreement between <sup>15</sup>N-disappearance as glutamine and appearance in NH<sub>3</sub> (24 %), protein (81 %), RNA (3.6 %) and DNA (2.1 %). For the total PDV there was a shortfall (–12 %), however, which may be due to losses in lumen components. These results show the importance of the GIT as a contributor to total glutamine plasma flux, but indicate a lesser reliance on glutamine metabolism by the digestive tract of the ruminant compared with observations from non-ruminants.

### Glutamine: <sup>15</sup>N-kinetics: Sheep: Gastrointestinal tract

It has been long-recognized that the gastrointestinal tract (GIT) has an obligate requirement for glutamine in both ruminants (Heitmann & Bergman, 1978) and non-ruminants (Windmueller & Spaeth, 1974) and the amino acid is now included as a therapeutic provision to enhance recovery from GIT-surgery (Soeters, 1995). What is not clear, however, is the metabolic basis for this need. For example, evidence from non-ruminants, particularly the vascularly-perfused preparations of rat intestine, indicated that the

majority of glutamine uptake by the GIT occurs across the small intestine (Windmueller & Spaeth, 1974). Furthermore, approximately 50–60 % of glutamine uptake was oxidized and this contributed 40–50 % of GIT-CO<sub>2</sub> production (Windmueller & Spaeth, 1980). These data led to the concept that glutamine provided an obligate source of energy, but this hypothesis has been challenged recently because alternative fuels, such as glutamate, are able to substitute (Burrin & Reeds, 1997). In ruminants, it has

**Abbreviations:** ape, atom % excess; BW, body weight; GIT, gastrointestinal tract; MDV, mesenteric-drained viscera; PDV, portal-drained viscera.

\* **Present address:** Elanco Animal Health, Basingstoke, Hants., UK.

† **Present address:** Novus Europe s.a/n.v., Rue Gulledelestraat 94, B-1200 Brussels, Belgium.

‡ **Corresponding author:** Dr G. E. Lobley, fax +44 (0) 1224 716629, email gel@rri.sari.ac.uk

recently been shown that the net utilization of glutamine by the GIT involves requirements for both the mesenteric-drained viscera (MDV; primarily small intestine) and the rumen plus caecum (Seal & Parker, 1996). Uptake by the rumen may relate, however, to more general requirements because it has been shown that 18–46% of other absorbed amino acids are also removed by the non-MDV components of the portal-drained viscera (PDV; Seal & Parker, 1996; MacRae *et al.* 1997). Energy provision for the GIT from glutamine also appears lower in ruminants compared with non-ruminants from data both *in vivo* (Heitmann & Bergman, 1978) and *in vitro* (Okine *et al.* 1995).

An alternative metabolic role for glutamine involves supply of N for key cellular products. The fates of the amido- and amino-N moieties of glutamine across the GIT do not appear to have been examined directly but, from mass transfer data based on the perfused rat intestine, Windmueller & Spaeth (1980) suggested that the N released during catabolism of glutamine matched the net appearance of NH<sub>3</sub> and certain non-essential amino acids. These values did not allow for GIT-protein synthesis, however, which is considerable in both non-ruminants (McNurlan *et al.* 1979) and ruminants (Attaix *et al.* 1992; Lobley *et al.* 1994; Neutze *et al.* 1996) with a net demand for glutamine and other amino acids, because of desquamation and protein secretions into the lumen (e.g. van Bruchem *et al.* 1997). Furthermore, glutamine amido-N also provides 50% of the N required for purine and pyrimidine synthesis and, in proliferative tissues, high rates of glutamine flux may be necessary to maintain the potential to synthesize the nucleic acids required for cell division (Szondy & Newsholme, 1989). The mucosal cells of the ovine small intestine are replaced every 2–4 d (Attaix *et al.* 1984; Attaix & Meslin, 1991), while active cell division continues in other GIT components, e.g. spleen and lymphoid material, establishing a need for continuous supply of nucleotides, which are provided either from salvage mechanisms or by synthesis *de novo* involving glutamine (Grimble, 1993). The actual magnitude of nucleotide synthesis, and thus the requirement for the amido-N of glutamine, has not been determined in ruminants. In view of these considerations, the present study attempts to identify the contribution of various GIT components to metabolism of [5-<sup>15</sup>N]glutamine in 24 h-fasted sheep. This length of fasting was selected as sufficient to markedly reduce the kinetic complications associated with glutamine transfer from the intestinal lumen, while avoiding the atrophy of the gut associated with longer periods of food deprivation. A preliminary report of some aspects of this study has previously been presented (Gate *et al.* 1997).

## Materials and methods

### *Animal procedures*

Seven cross-bred wether lambs (33–40 kg body weight (BW); 6–9 months old) were maintained on a diet of grass nuts (200 g/d) and grass hay *ad libitum*. Each lamb had silicone rubber catheters inserted in the mesenteric vein (two), hepatic-portal vein, right jugular vein and left carotid artery, as described by Balcells *et al.* (1995).

Within 2–3 weeks after surgery, feed was withdrawn from the lambs 24 h before the start of infusions. Each lamb was infused (60 g/h) for 10 h via the jugular vein with a solution of 60 mM-[5-<sup>15</sup>N]L-glutamine (99.2 atom %; Mass Trace Inc., Woburn, MA, USA) plus 130 IU/g heparin dissolved in sterile 0.15 M-NaCl.

Blood flows in the mesenteric and portal veins were measured by a modification of the technique described by Katz & Bergman (1969). *p*-Amino hippuric acid (15 g) and NaCl (3.6 g) were dissolved in 600 ml deionized water, the pH corrected to 7.4 using 10 M-NaOH and the volume adjusted to 1 litre. The infusate was then sterilized by filtration through a 0.22 µm filter followed by autoclaving. This was then infused (60 g/h) for 10 h into the distal mesenteric catheter over the same timescale as the glutamine infusion.

Before the infusion, a 10 ml blood sample was obtained from each animal to provide samples for metabolite natural abundances. Five hours after the start of the infusions, integrated blood samples (12 ml/h) were withdrawn over 75 min intervals from the mesenteric-vein-, hepatic-portal-vein- and carotid-artery-catheters using a continuous sampling technique (Lobley *et al.* 1995). Four blood samples (over 5 h) were collected in this way, with a final 20 ml sample taken directly by syringe after 10 h of infusion. Each animal was then killed immediately using an intravenous overdose of pentobarbitone, and samples of tissues rapidly obtained from the duodenum, jejunum, ileum, rumen, abomasum, caecum, liver, kidney, *m. vastis lateralis*, lymph gland and spleen. All tissues were quickly washed in cold 0.15 M-NaCl and the small intestine samples separated into mucosa and serosa components by gentle scraping with a microscope slide. All samples were then immediately frozen in liquid N<sub>2</sub>, and stored at –80° before further processing. Four sheep of similar age, breed and dietary history were slaughtered to provide tissue samples for determination of <sup>15</sup>N natural abundance.

### *Laboratory procedures*

**Blood and plasma chemical analyses.** A portion of the blood (1 g) was used for *p*-amino hippuric acid analysis based on gravimetric procedures (Lobley *et al.* 1995), but without inclusion of a heating stage as liver transfers were not quantified. Packed cell volume was determined by haematocrit. From the remainder of the blood, plasma was obtained by centrifugation at 1000 g for 15 min at 4°. This was then stored as sub-portions at –80°, except for a sample for assays of NH<sub>3</sub> and urea which were quantified immediately on a clinical analyser (Cobas Mira; Roche Ltd, Welwyn Garden City, Herts., UK) using commercial kits (Diagnostic Kit 171, Sigma, Poole, Dorset, UK; Unimate 5 Urea, Roche Ltd, Basle, Switzerland).

Concentrations of glutamine were measured in deproteinized plasma (plasma 1:1, v/v, in 1 mM-HCl, containing 0.5 mM-DL-2-amino butyric acid as an external standard). The supernatant fraction was filtered through a 10 000 MW filter (Ultrafree-MC; Millipore, Bedford, MA, USA) and amino acid concentrations determined by reversed-phase HPLC, after pre-column derivatization with phenylisothiocyanate, using a Waters Pico-Tag system (Waters, Milford, MA, USA).

**Tissue protein, DNA and RNA separations.** Tissue protein, DNA and RNA were separated using a modification of a technique described previously (Lobley *et al.* 1990). Approximately 3–5 g of tissue was freeze-dried, homogenized in 10 ml absolute ethanol containing 0.1 ml 0.1 M-HCl and centrifuged at 1500g for 10 min at 4°. The precipitate was washed twice with 10 ml ice-cold 0.23 M-perchloric acid before suspension in 6 ml water. A portion (1 g) of this was removed for drying before analysis for total acid-precipitable N (i.e. protein + RNA and DNA) and analysis for <sup>15</sup>N enrichment. To the remainder of the suspension, 5 ml 0.6 M-NaOH was added, followed by incubation at 37° for 1 h with mixing every 10 min. Then 2 ml ice-cold 2.3 M-perchloric acid was added to the mixture, which stood on ice for 10 min, followed by centrifugation. The supernatant fraction contained hydrolysed RNA and was retained for further purification. The pellet was washed in 10 ml ice-cold 0.23 M-perchloric acid before re-suspension in 0.6 M-perchloric acid and incubation at 70° for 30 min followed by standing on ice for 10 min. The supernatant fraction contained hydrolysed DNA and was retained for further purification. The precipitate, predominantly protein, was dried and ground for <sup>15</sup>N analysis.

To the RNA and DNA fractions were added 2 ml and 4 ml 2 M-K<sub>2</sub>CO<sub>3</sub> respectively, and the mixtures centrifuged at 1500g for 10 min at 4°. Then 20 ml ion exchange resin (Dowex 1, 100–200×8, OH form; Sigma Chemical Co., Poole, Dorset, UK) was mixed with the supernatant fractions and the resin fractions retained. These were then washed twice with 10 ml water, before adding 10 ml 1 M-HCl and the supernatant fractions obtained were freeze-dried and re-suspended in 400 µl water. To this was added 300 µl phenol–chloroform (1:1, v/v) and the upper phase was retained. Excess phenol was removed by three cycles of mixing with 300 µl chloroform and retaining the upper phase, which was then freeze-dried and retained for determination of <sup>15</sup>N content.

**<sup>15</sup>N analyses.** Enrichments (atom % excess; ape) of plasma urea, glutamine and other free amino acids were determined as the *t*-butyldimethylsilyl derivative, as described by Calder & Smith (1988). Plasma NH<sub>3</sub> was converted first to norvaline (Nieto *et al.* 1996), before analysis as the *t*-butyldimethylsilyl derivative. All analyses were by GC-mass spectrometry using a Hewlett Packard HP 5989A Engine (Hewlett Packard, Avondale, PA, USA).

The <sup>15</sup>N enrichments (atom %) of the total acid-precipitable N, protein, RNA and DNA fractions were determined as liberated N<sub>2</sub> gas using a 20–20 continuous flow isotope ratio mass spectrometer in conjunction with an online Roboprep CN combustion preparation system (Europa Scientific, Crewe, Ches., UK). Sample size was approximately 50–100 µg N. Corrections to ape were obtained by subtraction of natural abundance components obtained from the non-infused sheep. The final fraction (protein) caused difficulties in analysis, possibly due to the presence of acid-precipitation salts, and therefore protein enrichments were calculated from the total <sup>15</sup>N in acid-precipitable N minus the contribution from RNA and DNA. This was found to be acceptable, in the few instances where the approach could be checked, as would be expected as more than 90 % of the <sup>15</sup>N in the total acid-precipitable fraction was from protein.

### Calculations and statistics

Blood flows (kg/min) were calculated as described by Lobley *et al.* (1995) and corrected to plasma flow by multiplication with (1 – packed cell volume). Non-MDV flows and concentrations were calculated as the difference between PDV and MDV values.

Whole-body glutamine irreversible loss rate (mmol/min), based on the 5-N moiety, was determined by the standard equation:

$$((99.2/\text{arterial glutamine ape})$$

$$- 1) \times \text{glutamine infusion rate (mmol/min)}.$$

Mass (net) transfers (mmol/min; net flux) across the MDV or PDV were calculated as:

$$(C_{m \text{ or } p} - C_a) \times \text{BF (or PF)},$$

where C is the concentration (mM) of metabolite, in the artery (a) and mesenteric (m) or hepatic-portal (p) veins, BF is blood flow (kg/min) and PF is plasma flow (kg/min). Urea and NH<sub>3</sub> data were based on blood flow and plasma concentrations while transfers of glutamine and other amino acids were based on plasma flow and plasma concentrations.

Isotope transfers (mmol <sup>15</sup>N/min) were based on similar concepts, e.g. for MDV transfers:

$$(C_m E_m - C_a E_a) \times \text{BF (or PF)},$$

where E is the enrichment (ape) of the metabolite. These were then converted into gross flux (mmol metabolite/min) by:

$$\text{isotope transfer}/(\text{precursor enrichment}).$$

For this study, the ape of the arterial free metabolites were used to define the precursor. This approach will provide a minimum estimate of transfers. In practice, values based on the enrichments in venous blood would only have increased the values by 5 % or less for glutamine or NH<sub>3</sub>. No attempt was made to determine intracellular enrichments of free NH<sub>3</sub>, glutamine or other amino acids.

For secondary metabolite transfers, first, the proportion of glutamine converted to NH<sub>3</sub> was calculated as the isotopic flux of <sup>15</sup>NH<sub>3</sub> appearance across the MDV or PDV divided by the corresponding removal of [<sup>15</sup>N]glutamine. Second, the fraction of NH<sub>3</sub> derived from glutamine was estimated from:

$$\text{enrichment plasma NH}_3/\text{enrichment arterial glutamine},$$

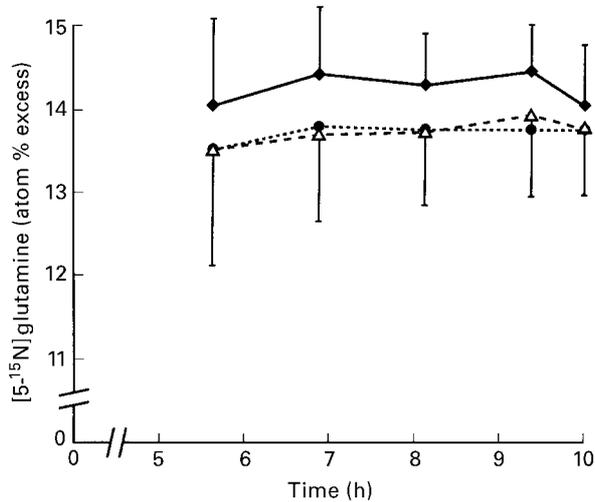
using NH<sub>3</sub> values for either mesenteric vein (for MDV) or hepatic-portal vein (for PDV) plasma.

For transfers of all metabolites (both net and gross) negative values indicate removal and positive data production by the tissues. Glutamine production was taken as net flux – gross flux.

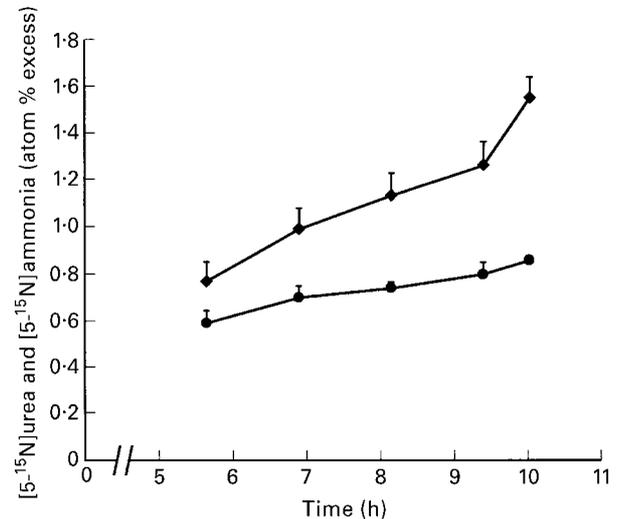
Comparisons between MDV and PDV variables were determined by one-way ANOVA with animals treated as blocks using Genstat for Windows, release 3.2 (Lawes Agricultural Trust, IACR-Rothamsted, Harpenden, Berks., UK).

### Results

Full surgical patency, which allowed both MDV and PDV transfers to be quantified, was maintained only for four



**Fig. 1.** Atom % excess enrichments of [5-<sup>15</sup>N]glutamine in plasma from artery (◆—◆), mesenteric vein (●---●) and hepatic-portal vein (△- - - △) during a 10 h infusion of 60 mM-[5-<sup>15</sup>N]L-glutamine into lambs fasted for 24–34 h. Values are means for four animals with their standard errors represented by vertical bars.



**Fig. 2.** Atom % excess enrichments of arterial [<sup>15</sup>N]urea (◆) and hepatic portal venous ammonia (■) during a 10 h intravenous infusion of 60 mM-[5-<sup>15</sup>N]glutamine into lambs fasted for 24–34 h. Values are means for four animals, with their standard errors represented by vertical bars.

lambs. For the other three animals, failure of the hepatic-portal catheter restricted the data to MDV and whole-body kinetics.

Over the last 5 h of the infusion, pseudo-plateau enrichments of [5-<sup>15</sup>N]glutamine were achieved for arterial, mesenteric and hepatic-portal plasma (Fig. 1). Whole-body flux of glutamine, based on arterial plasma enrichments, was 108 (SE 5.8)  $\mu\text{mol}/\text{min}$  ( $n$  7), or 7.2  $\mu\text{mol}/\text{kg BW}^{0.75}$  per min.  $\text{NH}_3$  enrichments in mesenteric (not shown) and hepatic-venous plasma showed a small increase over the time course of infusion (Fig. 2), probably indicative of recycling of <sup>15</sup>N through intermediary metabolites. Plasma [<sup>14</sup>N<sup>15</sup>N]urea enrichments exhibited a steady increase throughout the infusion and were always greater ( $P < 0.001$ ) than for  $\text{NH}_3$  (Fig. 2).

#### Gastrointestinal tract arterio-venous transfers

Plasma flow (which was 0.7 of blood flow) was divided between MDV and non-MDV components in an approximate ratio 3 : 2 (Table 1). The fasting condition probably lowered the contribution of the forestomachs from that observed with fed animals (Reynolds & Huntington, 1988; Seal & Parker, 1996). Net mass uptakes of glutamine, significantly different from zero ( $P < 0.001$ ), occurred across both the MDV and non-MDV components of the GIT but with the larger removal ( $P < 0.001$ ) occurring for the latter (Table 1). On average, 14% of net arterial inflow of glutamine was removed across the PDV.

There was dilution of glutamine enrichment across the MDV ( $P < 0.01$ ; Fig. 1). This represents glutamine production which arose from either uptake from the lumen and/or

**Table 1.** Plasma flow and trans-organ kinetics of [5-<sup>15</sup>N]glutamine across the mesenteric-drained viscera, portal-drained viscera and non-mesenteric-drained viscera sections of the digestive tract of lambs fasted for 24–34 h

(Mean values with their standard errors of the difference between means for four lambs)

	MDV	PDV	Non-MDV	SED	$P^*$
Plasma flow (g/min)	518	805	287	61.6	< 0.001
Glutamine kinetics ( $\mu\text{mol}/\text{min}$ )					
Net flux	-17.3	-45.5	-30.3	10.05	0.080
Gross flux	-24.5	-50.5	-26.5	6.06	0.009
Production	7.2	5.0	-2.0	5.91	NS
$\text{NH}_3$ kinetics ( $\mu\text{mol}/\text{min}$ )					
Net flux	129.7	195.3	64.0	19.75	0.002
$\text{NH}_3$ from glutamine	6.0	10.0	4.0	0.78	0.004
Interconversions (fractions)					
Glutamine flux to $\text{NH}_3$	0.269	0.209	0.177	0.0545	NS
$\text{NH}_3$ flux from glutamine	0.044	0.050	—	0.002	NS

MDV, mesenteric-drained viscera; PDV, portal-drained viscera.

\* Comparison of MDV- and PDV-values, based on one-way ANOVA, in the four animals for which full surgical preparations were maintained, treated as blocks (3 residual df).

intracellular turnover of the tissues of the small intestine. In total, this production accounted for 30 % of gross MDV flux. The gross fluxes across the MDV and PDV accounted for 23 (SE 2.2) % ( $n$  7) and 47 (SE 8.5) % ( $n$  4) of whole-body plasma glutamine irreversible loss rate respectively.

Increases in  $\text{NH}_3$  concentrations, compared with arterial plasma, were similar for mesenteric and hepatic-portal venous plasma (results not shown), and thus release of  $\text{NH}_3$  by the MDV and non-MDV was in proportion to blood flow. In terms of mass transfers, the  $\text{NH}_3$ -N released exceeded net removal of glutamine amido-N by 2–7-fold, this differential being greater for the MDV. Based on the relative enrichments (see Figs. 1 and 2), however, only 4–5 % of  $\text{NH}_3$  released was derived from glutamine. In contrast, 27 and 18 % of  $[5\text{-}^{15}\text{N}]\text{glutamine}$  removed by the MDV and non-MDV respectively, was returned to the venous drainage as  $^{15}\text{NH}_3$ . As observed in fed sheep (Lobley *et al.* 1995), there was urea removal by the GIT, but with no change in urea enrichment across either the MDV or PDV (results not shown).

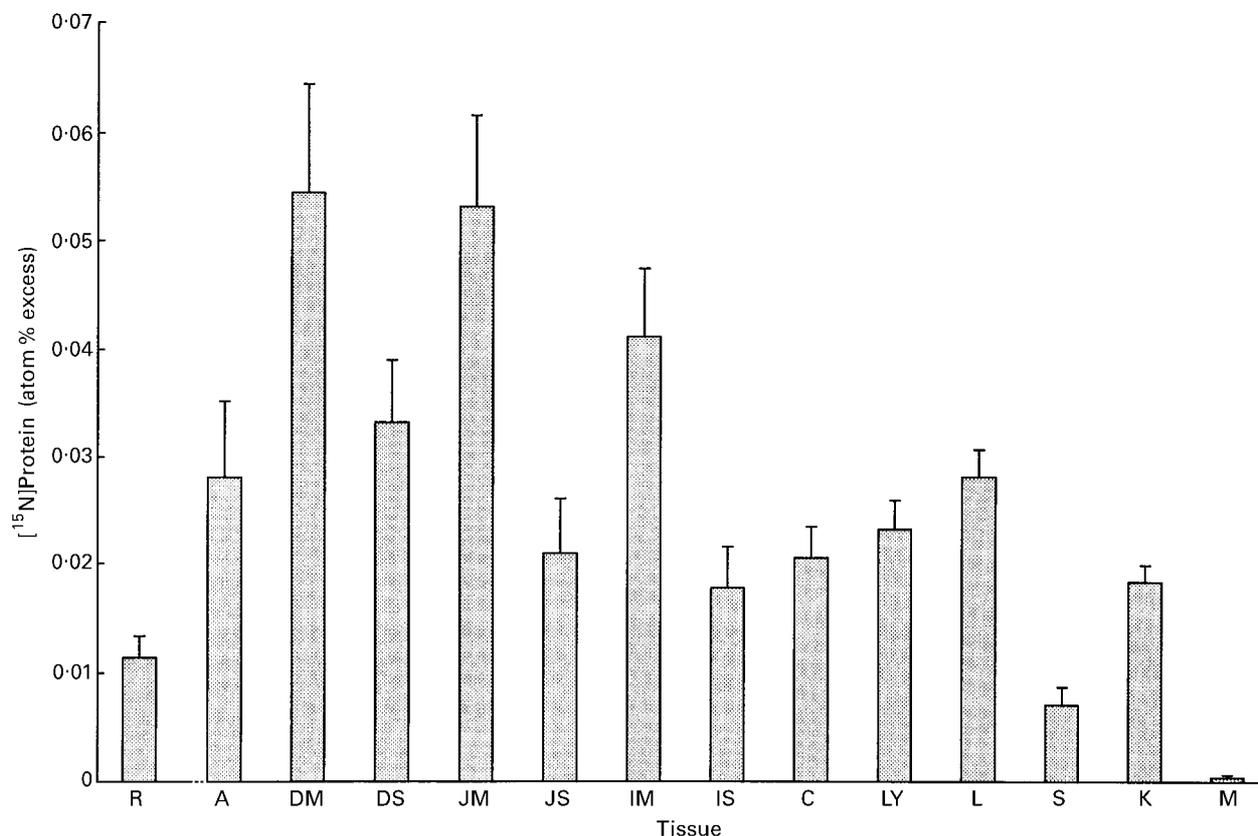
Isotopic  $^{15}\text{N}$  enrichments of other amino acids examined (aspartate, glutamate, alanine, glycine, leucine, phenylalanine) were at the limits of accurate quantification ( $0.1 \pm 0.05$  molar percent excess or less) by the GC-mass spectrometry technique and no significant differences between values for arterial and venous plasma could be detected (results

not shown). Transfers of  $^{15}\text{N}$  to other amino acids were, therefore, considered insubstantial. There was, however, net release of amino acids across the PDV; this included alanine and citrulline (14 and 11  $\mu\text{mol}/\text{min}$  respectively). The alanine probably arises from protein breakdown, because the GIT will be in a catabolic state due to food deprivation. Unfortunately, citrulline enrichment was not monitored due to technical difficulties.

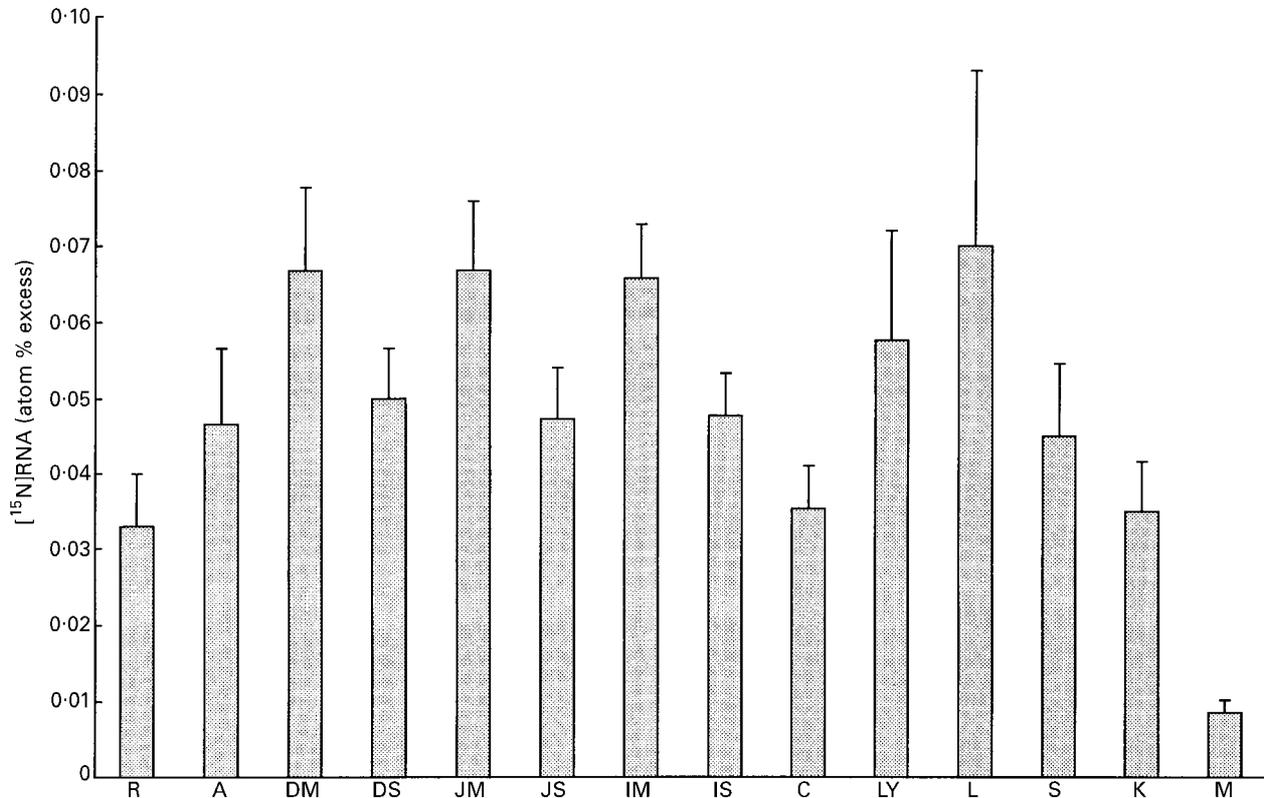
#### Tissue component enrichments

$^{15}\text{N}$ -enriched protein (either determined directly or calculated as total minus nucleotide isotope incorporation), RNA and DNA were detected in all tissues examined. Protein enrichments ( $^{15}\text{N}/\text{total protein N}$ ) were greatest in tissues of the small intestine, in the order jejunum > duodenum > ileum, with mucosa fractions more highly labelled ( $P < 0.001$ ) than the serosa (Fig. 3). For the other digestive tract tissues the order of protein enrichments was abomasum > caecum > rumen, with the latter significantly lower than the other two ( $P < 0.001$ ). Liver and the lymph node were comparable with the abomasum and higher than kidney ( $P < 0.05$ ) and spleen ( $P < 0.001$ ). Muscle had the lowest fractional incorporation ( $P < 0.001$ ) of any tissue measured.

The patterns of RNA  $^{15}\text{N}$ -enrichment were broadly similar to those for protein (Fig. 4), although there was a



**Fig. 3.** Protein-bound  $^{15}\text{N}$  enrichment (atom % excess) in various tissues from lambs fasted for 24–34 h after a 10 h intravenous infusion of 60 mM- $[5\text{-}^{15}\text{N}]\text{glutamine}$ . Values are means for seven animals with their standard errors represented by vertical bars. D, duodenum; J, jejunum; I, ileum; D, J and I used in combination with M, mucosa or S, serosa. A, abomasum; C, caecum; K, kidney; L, liver; LY, lymphoid tissue; M, muscle (*vastus lateralis*); R, rumen; S, spleen.



**Fig. 4.** RNA-bound <sup>15</sup>N enrichment (atom % excess) in various tissues from lambs fasted for 24–34 h after a 10 h intravenous infusion of 60 mm-[5-<sup>15</sup>N]glutamine. Values are means for seven animals with their standard errors represented by vertical bars. D, duodenum; J, jejunum; I, ileum; A, abomasum; C, caecum; K, kidney; L, liver; LY, lymphoid tissue; M, muscle (*vastus lateralis*); R, rumen; S, spleen.

tendency for the variance to be greater. The differential between mucosal and serosal enrichments was lower than for protein but still significant ( $P < 0.01$ ). Relative to protein there were increased enrichments for liver, spleen and lymphoid tissue (cf. Figs. 3 and 4).

Enrichments for DNA were approximately half those for RNA (Fig. 5). Again the general order of enrichments established for protein was observed for DNA, but with relatively higher incorporation for lymph node, caecum and, particularly, the spleen (cf. Figs. 3 and 5).

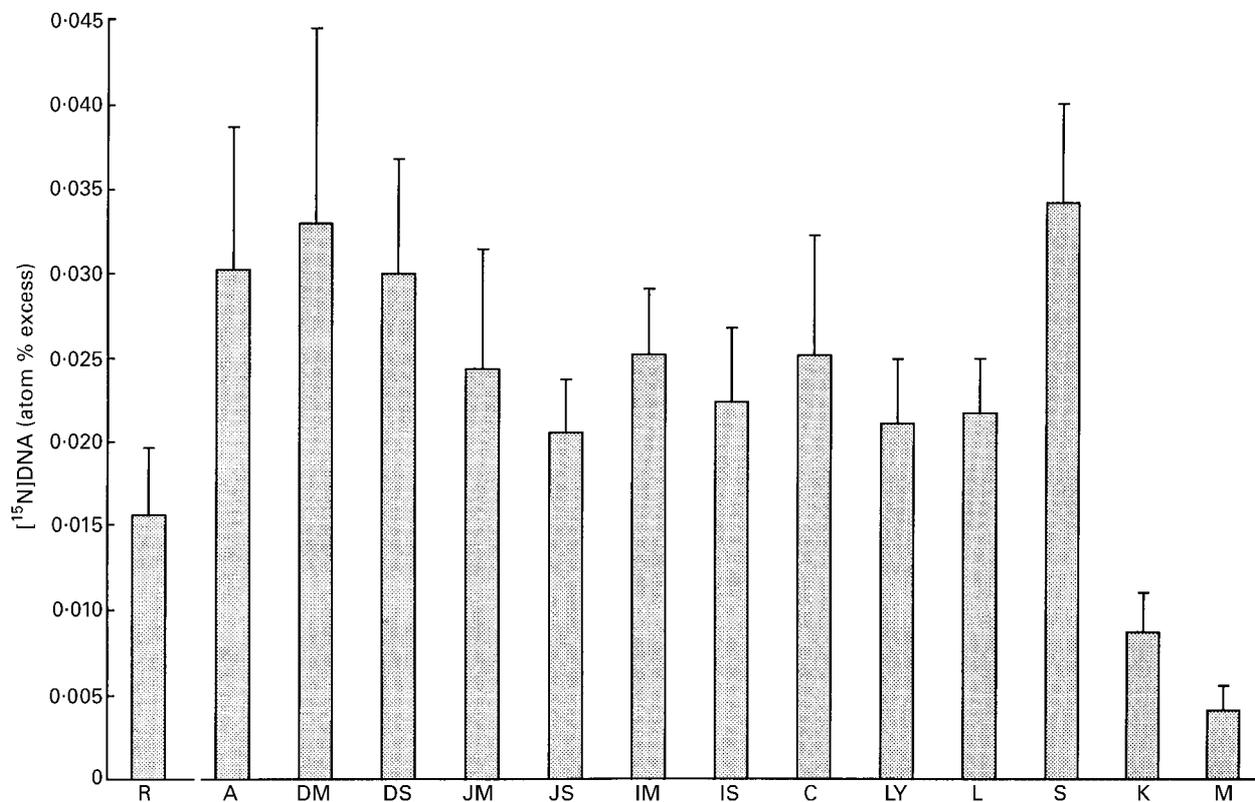
Tissue weight and composition were not determined in this study but, based on values available from the literature, an estimate of the distribution of <sup>15</sup>N incorporated into the individual tissues could be calculated (Table 2). For the small intestine, i.e. MDV-drained tissues, the jejunal and ileal sections each incorporated approximately twice the amount of <sup>15</sup>N as the duodenum. The protein:RNA ratio incorporation averaged 26:1. By this estimated summation approach, of the total [<sup>15</sup>N]glutamine extracted by the MDV, as assessed by the arterio-venous measurements, 81% was incorporated into protein, 3.6% into RNA and 2.1% into DNA. When <sup>15</sup>NH<sub>3</sub> appearance was included in the calculation, slightly more than the total [<sup>15</sup>N]glutamine uptake could be accounted for (+11%). In contrast, similar calculations for the non-MDV components yielded values for protein, RNA, DNA and NH<sub>3</sub> of 47, 1.8, 0.9 and 15% respectively,

i.e. only 65% of the total [<sup>15</sup>N]glutamine extracted. Total <sup>15</sup>N recovery, including <sup>15</sup>NH<sub>3</sub>, across the PDV was calculated as 88%.

## Discussion

### Whole-body kinetics

Data on glutamine isotope kinetics in ruminants are limited to the studies of Heitmann & Bergman (1978), using [U-<sup>14</sup>C]glutamine as tracer, and where whole-body irreversible loss rate values were unaffected by a variety of treatments, including acidosis and intake. These earlier values were greater, on both an absolute (192 v. 108 μmol/min) and metabolic-BW (9.5 v. 7.2 μmol/min) basis, compared with data from the current study using [5-<sup>15</sup>N]glutamine. This is somewhat surprising because glutamine is known to undergo transamination (Meister, 1979; Darmaun *et al.* 1986), and both de- and re-amidation reactions should leave the C skeleton intact (Meister, 1979). In consequence, the fluxes of both glutamine 2-N and 5-N should at least equal, and probably exceed, those of the C moieties. Without recourse to experiments where both C- and N-labelled glutamine are co-infused, it is impossible to say whether the differences are of a technical or biological nature, although recent studies (GD Milano and GE Lobley, unpublished results) have revealed higher rates of [5-<sup>15</sup>N]glutamine irreversible



**Fig. 5.** DNA-bound <sup>15</sup>N enrichment (atom % excess) in various tissues from lambs fasted for 24–34 h after a 10 h intravenous infusion of 60 mM-<sup>5-<sup>15</sup>N</sup>glutamine. Values are means for seven animals with their standard errors represented by vertical bars. D, duodenum; J, jejunum; I, ileum; D, J and I used in combination with M, mucosa or S, serosa. A, abomasum; C, caecum; K, kidney; L, liver; LY, lymphoid tissue; M, muscle (*vastus lateralis*); R, rumen; S, spleen.

**Table 2.** Estimated net incorporation (nmol/min) of [<sup>15</sup>N]glutamine into DNA, RNA and protein across the digestive tract of lambs fasted for 24–34 h and infused for 10 h with [<sup>5-<sup>15</sup>N</sup>]glutamine\* (Mean values and standard deviations for seven lambs)

	Net incorporation of <sup>15</sup> N (pmol/min)							
	DNA		RNA		Protein		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MDV	508	17	879	33	20052	429	21438	466
Non-MDV	249	22	501	46	12368	1177	13118	1216
Rumen	55	12	251	61	3239	629		
Abomasum	59	15	61	16	2188	825		
Duodenum								
Mucosa	24	8	57	14	1767	450		
Serosa	90	43	99	16	2556	681		
Jejunum								
Mucosa	65	31	106	20	3901	860		
Serosa	106	29	210	34	4857	1858		
Ileum								
Mucosa	66	23	114	27	2828	597		
Serosa	157	75	293	53	4144	1413		
Caecum	136	50	189	50	6941	1131		

\* Calculations are based on the observed enrichments from the current study and tissue masses plus protein, RNA and DNA contents detailed in Masters (1962), Hopkins & Tulloh (1985), Colebrook *et al.* (1988), Kolb *et al.* (1992) and Burrin *et al.* (1992).

loss rate (175–202  $\mu\text{mol}/\text{min}$ ) in fed animals compared with the fasted sheep examined in the current study. If there are nutritional effects on metabolism of the amido-N of glutamine then this would differ from the fate of the C skeleton, the irreversible loss rate of which was unaltered between fed and fasted states (Heitmann & Bergman, 1978).

#### *Digestive tract kinetics*

The data obtained from this experiment clearly show that the PDV is a net consumer of glutamine in fasted sheep. The net extraction was greater than observed in the earlier studies of Heitmann & Bergman (1978), whether based on either an absolute (2.7 *v.* 1.3–1.8 mmol/h) or metabolic-BW (196 *v.* 65–90  $\mu\text{mol}/\text{h}$  per  $\text{kg}^{0.75}$ ) basis. This may be due to the different period of food withdrawal (34 *v.* 72 h) between the studies. Furthermore, the animals in the earlier report were older and heavier and tissue energy and protein metabolism are both known to decline with age (Lobley, 1993).

The similarity in net glutamine requirements for the MDV and non-MDV tissues observed in the current study contrasts with data from post-absorptive cattle (Reynolds & Huntington, 1988), where the net glutamine transfer was smaller across 'stomach' (non-MDV) tissues. In fed sheep, however, the minimum net requirements of the non-MDV tissues for glutamine are 63–75  $\mu\text{mol}/\text{min}$  (from Seal & Parker, 1996), an even greater absolute amount than observed for our fasted lambs (30  $\mu\text{mol}/\text{min}$ ). Indeed, for sheep fed on grass pellets, all the glutamine absorbed across the small intestine is removed, and presumably utilized, by the remainder of the PDV tissues (Seal & Parker, 1996), compared with extraction values of only 18–46% for the other absorbed amino acids (Seal & Parker, 1996; MacRae *et al.* 1997).

A small amount of glutamine production occurred across the MDV, but not the remainder of the PDV, and this must be ascribed to either uptake from lumen, release from protein breakdown or synthesis *de novo* by cells of the small intestine. The digestive tract contains both glutaminase (*EC* 3.5.1.2) and glutamine synthetase (*EC* 6.3.1.2) with, in rodents, the former dominant, especially in the mucosal cells of the small intestine, while the latter is restricted primarily to the lower stomach (James *et al.* 1998). If a similar enzyme distribution occurs in the ruminant then synthesis *de novo* would not be a serious contributor to glutamine production, at least for the MDV tissues.

#### *Transfer to ammonia*

Deamidation of glutamine liberates  $\text{NH}_3$  which can either be used for other purposes within tissues, e.g. ureagenesis, glutamate and glutamine synthesis, or enter the plasma pool. Previous experiments, involving perfused jejunal segments of fasted rats, revealed that the total (amino- + amido-) N of glutamine extracted was balanced by increased mass outputs of  $\text{NH}_3$  (40%), citrulline, alanine (25%), proline (25%), with the remainder as glutamate and ornithine (Windmueller & Spaeth, 1980). Calculation of such mass balances was not possible in the current study, because there were no incremental additions of glutamine, while  $\text{NH}_3$  production, across both the MDV and non-MDV, exceeded net glutamine-N removal by several fold. From the relative

enrichments in arterial plasma, however, it was apparent that only 5% of  $\text{NH}_3$  arose from glutamine, although 25% of the glutamine flux across the PDV was converted to  $\text{NH}_3$ . These metabolic conversions were not exclusive to the small intestine (Windmueller & Spaeth, 1974) because both  $\text{NH}_3$  and glutamine  $^{15}\text{N}$ -enrichments were similar between MDV- and PDV-venous drainage. The comparisons based on both mass and isotope transfers are consistent with the concept that glutamine is a relatively minor contributor to ammoniogenesis across the digestive tract of the fasted ruminant.

#### *Other fates of extracted [5- $^{15}\text{N}$ ]glutamine*

Due to the method of analysis, it is difficult to resolve how much of the protein-bound  $^{15}\text{N}$  was in glutamine but this was probably the majority because other released free amino acids had low enrichments. The pattern of label incorporation (which approximates to relative fractional rates of protein synthesis) follows that reported for ovine digestive tract tissues (Southorn *et al.* 1992), with the order jejunum > duodenum > ileum the same as determined by a large dose procedure (Lobley *et al.* 1994). Differences did exist, however, with the protein synthetic activity of caecum and rumen in the reverse order between the current fasted animals and those fed in the earlier study (Lobley *et al.* 1994). Although enrichments of mucosal cells exceeded those of serosal origin for all components of the small intestine, mucosal : serosal ratio was also greater than reported previously (Lobley *et al.* 1994). This may be due to either a genuine biological difference related to nutritional state, or to more technical reasons, e.g. mucosal and serosal cells differ either in the relative amounts of glutamine and valine (the tracer amino acids used between the two studies) in their proteins, or in the enrichments of the intracellular glutamine and valine pools which provide the precursors for protein synthesis.

The observation that incorporation of  $^{15}\text{N}$  into RNA showed a similar pattern to protein, but with slightly higher enrichments, may be misleading because the amido group of glutamine provides 50% of the N to both purines and pyrimidines. In contrast, the proportion of glutamine in mixed proteins probably ranges between 5 and 10% and, thus, their fractional synthesis (turnover) rates would be approximately 5–10-fold greater than that of RNA. This lower rate of incorporation into RNA (and also DNA) fits with current hypotheses on salvage of nucleic acids. Thus, although a minimum of 60% of synthesized protein involves re-utilization of amino acids from cellular, as opposed to dietary, sources (Reeds & Lobley, 1980), there is debate about whether an exogenous supply of nucleotides is even required for the adult condition (see Grimble, 1993; López-Navarro *et al.* 1996). Even with growing animals, there is no clear consensus on the relative importance of endogenous salvage, synthesis *de novo* and dietary intake of nucleic acids (cf. Murray, 1971; Moyer *et al.* 1985; Kim *et al.* 1992; Grimble, 1993; Boza *et al.* 1996). That incorporation of  $^{15}\text{N}$  into RNA and DNA was observed does support the concept of tissue synthesis *de novo*, but only to a limited extent.

The lower values for DNA enrichments, compared with the corresponding RNA fraction, would be expected because intracellular turnover of the RNA species occurs (Roeder,

1973; Siimes & Dallman, 1974) in addition to proliferative requirements. The DNA : RNA differences in  $^{15}\text{N}$  incorporation between serosa and mucosal sections were somewhat surprising because the proliferative nature of the mucosa is well known (Attaix *et al.* 1984; Attaix & Meslin, 1991). The majority of the incorporation relating to cell proliferation occurs in the crypt and it is not known if the rather crude physical separation of serosa and mucosa yielded satisfactory and consistent fractions. In weaned lambs (Attaix & Meslin, 1991), cell renewal times of mucosal villi cells were similar (72–75 h; range 60–91 h) between the proximal duodenum, jejunum and ileum whereas the current data yielded similar DNA synthesis for the mucosa of the jejunum and ileum but slightly lower ( $P < 0.01$ ) for the duodenum. Other proliferative tissues, notably lymphoid, spleen (e.g. Aschkenasy, 1973; Dugan *et al.* 1994) and the caecum (e.g. Howard *et al.* 1995) also had elevated levels of  $^{15}\text{N}$  incorporation into DNA.

#### Gastrointestinal-tract $^{15}\text{N}$ balance

Calculations of total  $^{15}\text{N}$  incorporation were based on literature values of tissue mass and component distribution and, as such, can only be taken as approximate. Nonetheless, the quantitative accounting for  $^{15}\text{N}$  disappearance and appearance across the MDV was encouraging. Conversion of glutamine to  $\text{NH}_3$  was much lower than for non-ruminants (cf. Windmueller & Spaeth, 1974, 1980, and present study). Glutaminolysis would produce glutamate and subsequent energy-yielding metabolites but again supporting evidence suggests a more limited role for this function in ruminants. For example, the fraction of glutamine extracted by the digestive tract which is catabolized for energy needs appears to be lower for ruminants than non-ruminants, based on direct evidence both *in vivo* (cf. Heitman & Bergman, 1978; Windmueller & Spaeth, 1980) and for enterocytes *in vitro* (cf. Wu *et al.* 1991; Okine *et al.* 1995). A similar reduced dependency on glutamine as an energy source for bovine compared with rodent lymphocytes has also been reported (Wu & Greene, 1992).

Balance calculations need to be treated with care, however, especially as mass and isotopic transfers can yield different patterns, particularly when loss of glutamine (or products) into the lumen of the digestive tract may occur. This latter concern may account for the 'failure' of the non-MDV components of the digestive tract to exhibit balanced  $^{15}\text{N}$  appearance and disappearance. As already discussed, substantial net uptake of glutamine does occur for the fore- and hindgut and this may be used by the micro-organisms as a N source, analogous to the situation with urea (Egan *et al.* 1986). The relatively short period of food deprivation adopted in the current study may have left sufficient microbial mass present in the rumen and caecum to influence glutamine metabolism. Even under more complete fasting, losses into the lumen of N-containing metabolites still occur, by either desquamation or secretion, but estimates of the magnitude are variable (e.g. 25–60% of GIT-protein synthesis; from Lobley *et al.* 1994; Neutze *et al.* 1996; van Bruchem, 1997). Based on the lower value, this would account for most of the apparent glutamine shortfall but needs to be tested directly.

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