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Digestion and nutrient net fluxes across the rumen, and the mesenteric- and portal-drained viscera in sheep fed with fresh forage twice daily: net balance and dynamic aspects

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Digestion and portal net flux of nutrients were studied in sheep fed twice daily with fresh orchard-grass. Digestive flows were measured in six fistulated sheep using the double-marker technique. Three sheep were fitted with catheters and blood-flow probes, allowing nutrient net flux measurements across the portal-drained viscera (PDV), the mesenteric-drained viscera (MDV) and the rumen. Total tract apparent digestion of N was similar to portal net appearance of N, calculated as the sum of free amino acids (FAA), peptide amino acids (PAA), NH_3, and urea net fluxes. PAA accounted for 25 % of non-protein amino acid net release across the PDV. With the exception of glycine and glutamate, the small intestine was the main contributor to this PAA net release. The essential amino acid (EAA) apparent disappearance between the duodenum and the ileum was lower than the net appearance of EAA (FAA + PAA) across the MDV. The value of PDV:MDV flux of free EAA was, on average, 78 %. The rumen accounted for 30 % of the net uptake of EAA by the PDV tissues not drained by the mesenteric vein. Rumen net release of acetate, propionate, butyrate, 3-hydroxybutyrate, and lactate accounted for 70, 55, 46, 77 and 52 %, respectively, of their portal net releases. Conversely, the small intestine was a net consumer of arterial acetate and 3-hydroxybutyrate. Dynamic study of nutrient net fluxes across the PDV showed that throughout a feeding cycle, the liver faced a constant flux of amino acids (AA), whereas volatile fatty acid and NH_3 net fluxes varied in response to the meal. The present study specified, in forage-fed sheep, the partitioning of nutrient net fluxes across the PDV and the role of peptides in portal net release of AA.

Amino acids: Volatile fatty acids: Absorption: Portal-drained viscera: Sheep

The gastrointestinal tract, the interface between the diet and the animal, significantly modifies (quantitatively and qualitatively) the pattern of nutrients between the gut lumen and the bloodstream (Seal & Reynolds, 1993). In ruminants, although the portal-drained viscera (PDV) represent less than 10 % of body weight, and about 5 % of body protein mass, they account for one-quarter of total energy expenditure and one-third of total protein synthesis. This high metabolic activity of the gastrointestinal tract generates specific requirements in energy substrates and amino acids (AA). Thus, the portal appearance of volatile fatty acids (VFA), the main energy supply in ruminants, is lower than the amount produced in the gut, each of the individual VFA being metabolised to different extents by the gastrointestinal tract (Bergman, 1990). Similarly, the net release of essential amino acids (EAA) in portal blood is on average only two-thirds of the EAA apparent disappearance from the small intestine (Tagari & Bergman, 1978; MacRae *et al.* 1997*a*), with large variations depending upon the AA concerned. Possible improvements in ruminant feeding systems would take into account gastrointestinal tract and liver metabolism of nutrients, enabling substrate availability for peripheral tissues to be predicted. However, studies in which both digestion and absorption of nutrients are reported are scarce. Thus, establishing relationships between nutrient production, or disappearance from the gut lumen, and nutrient delivery into the bloodstream is made difficult. Furthermore, the portal

Abbreviations: AA, amino acid; BF, blood flow; EAA, essential amino acid; FAA, free amino acid; MDV, mesenteric-drained viscera; ME, metabolizable energy; OM, organic matter; PAA, peptide amino acid; PBF, portal blood flow; PDV, portal-drained viscera; VFA, volatile fatty acid.

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vein drains a set of tissues, having specific requirements, and being exposed differently to a luminal supply of substrates; the heterogeneity of these tissues increases the difficulty in interpreting PDV net fluxes.

The aim of the present study was to provide, with the same diet, a whole set of data on digestion in the different segments of the gut, and on portal net delivery of nutrients. Furthermore, it attempted to determine the contribution of the rumen (main site of energy substrate absorption) and the small intestine (main site of AA absorption) to portal net flux of nutrients. Finally, the present study aimed to determine, in sheep fed twice daily, the dynamics of portal delivery of nutrients to the liver.

Materials and methods

Animals and diet

Two groups of mature Texel wethers were used simultaneously: one for digesta flow measurement; the other for nutrient net flux measurement across the PDV. Sheep of both groups were given 6960 g fresh orchard-grass (DM content 190 g/kg, N content 18·5 g/kg DM, metabolizable energy (ME) 9·23 MJ/kg DM) distributed in two equal portions at 8.00 and 20.00 hours. This first-cycle grass, at a stage where ear emergence has occurred for 50 % of plants, was harvested the preceding day and stored at 4°C. In order to avoid refusals, feed delivery was set at 90 % of the *ad libitum* consumption. The sheep had free access to water and salt blocks. Animals were allowed to adjust to the diet during at least 2 weeks before sampling. They were housed in individual pens, in rooms under continuous lighting with controlled temperature (19–23°C). Surgical procedures and post-surgical care were conducted in accordance with national legislation on the care and use of laboratory animals.

Digestive flow. Two months before the experiment, six wethers (mean live weight 51 (SD 1) kg) were surgically fitted with a rumen cannula (silicone rubber; 50 mm internal diameter), and T-shaped cannulas (silicone rubber, 17 mm internal diameter) in the proximal duodenum (about 70 mm downstream from the pylorus) and distal ileum (about 150 mm upstream of the ileocaecal junction).

Blood fluxes of nutrients. One month before the experiment, three rumen-cannulated wethers (mean live weight 55 (SD 2) kg) were surgically fitted with a rumen cannula (35 mm internal diameter) and catheters in the right ruminal vein, the cranial mesenteric vein (20–40 mm upstream of the ileocaecocolic vein junction), the portal vein and a mesenteric artery. Transit-time ultrasonic blood flow probes (Transonic Systems, Inc., Ithaca, NY) were implanted around the portal vein, the mesenteric vein and the right ruminal artery (Rémond *et al.* 2000*b*).

Experimental procedures

Digestive flow. Solute and particle markers were ^{51}Cr-EDTA (925 kBq/d per sheep), and ^{103}Ru-phenanthroline (22 kBq/d per sheep), respectively. After a priming dose (100 ml of the infused solutions, day 1) markers were continuously infused into the rumen, via separate tubes, at a rate of 100 ml/d, until day 11. A continuous infusion of $(^{15}NH_4)_2SO_4$ (35 mg ^{15}N/d per sheep) into the rumen was started on day 4, and ended on day 11, enabling microbial protein synthesis determination. Total tract digestibility and digestive flow measurements were simultaneously performed during a 6 d period (from day 6 to day 11). For intestinal flow measurements, twelve samples were taken from the duodenum and the ileum so that each 1 h interval of the 12 h feeding cycle was represented (six sampling days, two sampling times per d). Each duodenal (160 ml) and ileal (80 ml) digesta sample was immediately sub-sampled under thorough mixing. One fraction (40 ml) was frozen as whole digesta. A second (40 ml) was squeeze-dried through a nylon gauze (250 μm pore size) and both filtrate and particulate matter were frozen. For duodenal samples, the remaining fraction (80 ml) was frozen for bacterial isolation. Fractions from the twelve sampling times were pooled to yield one sample. For bacterial isolation, after thawing, duodenal samples were centrifuged at 800 ***g*** for 10 min at 4°C to remove feed particles. The supernatant fraction was respun at 800 ***g*** for 10 min at 4°C. The second supernatant fraction was centrifuged at 27 000 ***g*** for 20 min at 4°C to precipitate the bacteria. The bacterial pellet was lyophilised.

The DM (104°C for 24 h), organic matter (OM) (550°C for 6 h), and N (by the Kjeldhal method) content were determined for feeds (fed and refusals), duodenal and ileal whole digesta and filtrate, and faeces. Samples of duodenal and ileal digesta (and their fractions), faeces, urine and infusion solutions were assayed simultaneously for ^{103}Ru and ^{51}Cr using a γ spectrophotometer (Minaxi γ 5500; Packard, Rungis, France). NH_3 concentrations were determined for duodenal and ileal whole digesta and filtrates (Weatherburn, 1967). AA determinations were performed in feed and reconstituted digesta (Faichney, 1980) by ion-exchange chromatography (6300 Beckman AA analyser; Beckman Instruments, Palo Alto, CA) after a hydrolysis step with 6 M-HCl for 24 h at 115°C. For the determination of S-containing AA, samples were submitted to an oxidative step before the hydrolysis. ^{15}N enrichment was determined on bacteria and duodenal whole digesta and filtrates. Before ^{15}N-enrichment determination, NH_3 contained within the samples was eliminated by adding an equal volume of saturated sodium tetraborate and heating at 95°C for 24 h.

Net fluxes of nutrients across the gastrointestinal tract. Blood flow (BF) was measured continuously (from 5.30 to 17.30 hours) with an ultrasonic transit time flowmeter (Transonic Systems, Ithaca, NY) interfaced with a computer for data acquisition. Blood samples (9 ml) were simultaneously withdrawn through the four catheters at 6.30, 8.30, 9.30, 10.30, 12.30, 14.30, and 16.30 hours. They were collected into cold syringes containing anticoagulant (Monovett; Starstedt, Mosheim, France). Samples were rapidly placed on ice. Packed cell volume was determined, and blood was sub-sampled. A whole blood sample (2 ml) was frozen in liquid N_2 and stored at -80°C for later VFA analysis, while another subset sample (2 ml) was used for the immediate analyses of haemoglobin, urea and NH_3.

A 2 ml fraction of blood was deproteinized by adding 0·2 ml of 6 M-$HClO_4$, and by mixing and centrifuging (10 000 ***g*** for 15 min at 4°C). The supernatant fraction (1·2 ml) was neutralized by the addition of 0·115 ml of 6 M-KOH; the mixture was stored at 4°C for 30 min, centrifuged (10 000 ***g*** for 15 min at 4°C), and the supernatant fraction was stored at −80°C for later glucose, lactate, and 3-hydroxybutyrate analyses. D-glucosaminic acid (1 ml, 0·125 mM) was added to a 1 ml blood fraction which was then deproteinized by the addition of 0·1 ml sulfosalicylic acid (40 %, w/v). Samples were mixed, and allowed to stand for 15 min at room temperature. Precipitated proteins were removed by two successive centrifugations at 10 000 ***g*** for 15 min at 4°C. The supernatant fraction was frozen (−80°C) for later free amino acid (FAA) analysis. Finally, the remaining blood fraction (4 ml) was centrifuged (1 500 ***g*** for 10 min at 4°C), and plasma was recovered. The plasma (2 ml) was then deproteinized with 0·2 ml sulfosalicylic acid (40 %, w/v) following the addition of norleucine (0·2 ml, 1·25 mM). Deproteinized plasma was frozen (−80°C) for later peptide (<3 kDa) analysis. The determination of blood urea, NH_3, haemoglobin, glucose, lactate, and hydroxybutyrate concentrations was carried out as described by Rémond *et al.* (1993*a*). Blood VFA analysis was carried out as described by Nozière *et al.* (2000*a*). Peptide amino acid (PAA) determination in plasma was achieved according to the work of Rémond *et al.* (2000*b*). Bernard *et al.* (2001) has already discussed the choice of this technique.

Rumen fluid was collected immediately after each blood sample was taken. Samples were filtered through nylon gauze. After pH measurement, rumen fluid was centrifuged (800 ***g*** for 10 min at 4°C), and the supernatant fraction was then centrifuged at 27 000 ***g*** for 20 min at 4°C. The supernatant fraction was sub-sampled for analyses of VFA, NH_3-N, FAA and PAA as described by Rémond *et al.* (1993*a*, 2000*b*).

Calculations and statistics

Duodenal, and ileal DM flows were estimated using the double-marker technique (Faichney, 1980). Bacterial N flow at the duodenum was estimated from the bacterial and the duodenal digesta ^{15}N enrichment. PAA concentration was calculated as the difference in AA concentration between samples taken before and after acid hydrolysis. Peptide-bound glutamate and aspartate were corrected for glutamate and aspartate released from glutamine and asparagine during hydrolysis. Net fluxes of metabolites in blood and plasma were calculated as described by Early *et al.* (1987). A positive net flux indicates release of a nutrient, whereas a negative net flux implies uptake. Net metabolite fluxes across the rumen wall were estimated to be twice that in the right ruminal vein. Non-MDV refers to the difference between PDV and mesenteric-drained viscera (MDV) fluxes. The quantities of metabolites transferred across the PDV tissues within the 12 h period were estimated by integrating time variations in net fluxes. The daily net flux of metabolites was estimated on the assumption that all 12 h periods were equivalent. The effect of time on studied traits was analysed statistically using the repeated option of the PROC MIXED procedure of SAS (SAS/STAT® Users Guide, Release 8.1; SAS Institute Inc., Cary, NC, 2000).

Results

In both groups, animals finished their meal within 90 min.

Digestive flow

Parameters of OM and N digestion are presented in Table 1. Total tract OM digestibility was 62·8 %. Stomachs, small intestine and large intestine accounted for 66·5, 24·0, and 9·5 % of the OM digestion, respectively. Microbial N accounted for 86 % of non-NH_3-N flow to the duodenum. Assuming an endogenous flow of N of 1·5 g N/d, the rumen degradability of dietary N was 93·6 %. Intake, duodenal flow, and small-intestine apparent disappearance of AA are given in Table 2.

Net fluxes of nutrients across the gastrointestinal tract

Rumen traits. The daily mean values of pH, and VFA and NH_3 concentrations in the rumen fluid are presented in Table 3. Variations with time in rumen pH, VFA, NH_3, FAA and PAA concentrations are given in Figs. 1 and 2. All these traits were affected by the time of day ($P<0·05$).

Blood traits. BF rates throughout the 12 h feeding cycle averaged 2804 (SD 49), 647 (SD 91), and 515 (SD 46) ml/min for PDV, MDV, and rumen, respectively. BF in the small intestine did not vary throughout the feeding cycle ($P>0·10$), whereas portal and rumen flow increased during feed intake and then gradually decreased to attain the level of the pre-feeding period ($P<0·001$; Fig. 3). The packed cell volume was not affected by the time of sampling; average values for the artery, and the portal and mesenteric veins were 26·5 (SD 1·3), 26·3 (SD 1·3),

Table 1. Organic matter (OM) digestion, and nitrogen flow at the different levels of the digestive tract in sheep fed fresh orchard-grass (*n* 6)*

(Mean values and standard deviations)

Item	Mean	SD
OM intake (g/d)	1226	0
OM apparent digestion (g/d)		
Stomachs	513	29
Small intestine	185	22
Large intestine	73	14
N intake (g N/d)	24·6	0
N duodenal flow (g N/d)		
NH_3	1·9	0·3
Non-NH_3		
Microbial	23·3	1·4
Non-microbial	3·6	0·4
N ileal flux (g N/d)		
NH_3	1·0	0·3
Non NH_3	10·5	0·8
Faecal N excretion (g N/d)	9·0	0·2
Urinary N excretion (g N/d)	13·5	0·8

* For details of diet and procedures, see p. 650.

Table 2. Intake, duodenal flow and small intestine (SI) apparent disappearance (mmol/d) of amino acids in sheep fed fresh orchard-grass (*n* 6)*

(Mean values and standard deviations)

		Duodenal flow		SI disappearance	
Item	Intake	Mean	SD	Mean	SD
Aspartic acid	128·5	116·3	6·3	79·7	6·6
Threonine	54·3	84·7	3·2	55·9	4·8
Serine	57·3	68·5	3·5	41·6	4·9
Glutamic acid	88·8	110·7	6·4	73·6	6·7
Proline	59·4	50·4	2·8	29·2	1·5
Glycine	92·0	101·7	5·2	67·1	5·6
Alanine	100·4	112·4	5·6	74·7	6·6
Valine	57·4	60·9	4·5	44·3	4·4
Cysteine	14·7	44·6	11·6	28·4	9·1
Methionine	21·1	20·3	0·3	15·2	0·5
Isoleucine	38·0	54·9	3·2	41·0	3·2
Leucine	79·0	86·2	3·8	62·7	4·2
Tyrosine	22·4	32·1	1·8	24·3	1·7
Phenylalanine	39·1	42·9	2·1	31·6	2·2
Lysine	40·7	67·0	3·6	54·6	3·3
Histidine	23·4	18·2	1·7	13·6	0·6
Arginine	36·6	36·0	2·2	27·7	1·1
Total amino acids	960·6	1108	48	765	54·5
Essential amino acids	360·6	435	20	319	20·8

* For details of procedures, see p. 650.

and 26·3 (SD 1·2) %, respectively. Values for artery haemoglobin concentration:vein haemoglobin concentration did not vary significantly with time, giving average results of 1·016 (SD 0·003), 1·012 (SD 0·006), and 1·009 (SD 0·001) for PDV, MDV, and the rumen, respectively.

Arterial concentrations. NH_3 and urea concentrations in blood were not significantly affected by the time of day (1·93 (SD 0·08) and 136·3 (SD 13·9) mg N/l, respectively). Daily means for arterial concentration of blood FAA, and plasma PAA are presented in Table 4. Total FAA concentration in arterial blood varied ($P<0·01$) throughout the feeding cycle, the highest concentration being observed during meals (Fig. 4). Conversely, the arterial concentration of plasma PAA was not affected by the time of sampling ($P>0·10$). Arterial glucose concentration decreased during meals (Fig. 4); giving a daily mean value of 2·99 (SD 0·08) mM. Arterial lactate concentration was not affected by the time of day (0·55 (SD 0·12) mM). Daily mean arterial concentrations were 1·260 (SD 0·095) mM for acetate, 32·8 (SD 8·6) μM for propionate, 8·6 (SD 1·8) μM for butyrate, 2·8 (SD 1·0) μM for isobutyrate, 0·9 (SD 0·2) μM for valerate, 2·6 (SD 0·2) μM for isovalerate, and 0·33 (SD 0·07) mM for 3-hydroxybutyrate. Arterial concentrations of each of these metabolites varied throughout the feeding cycle ($P<0·05$); their values were greater postprandially than before feed distribution. The highest values were observed 1·5 h after meal delivery for propionate, after 2·5 h for the other VFA (Fig. 5) and after 4·5 h for 3-hydroxybutyrate (Fig. 4).

Table 3. Daily values of ruminal pH, total volatile fatty acid (VFA) concentration and molar proportions of individual VFA, and ammonia-nitrogen concentration in rumen fluid of sheep fed fresh orchard-grass (*n* 3)*

(Mean values and standard deviations)

Item	Mean	SD
pH	6·67	0·02
Total VFA (mM)	85·1	4·5
Acetate (%)	64·9	1·2
Propionate (%)	21·9	1·5
Butyrate (%)	10·1	1·0
Isobutyrate (%)	1·1	0·1
Valerate (%)	1·0	0·2
Isovalerate (%)	1·0	0·2
NH_3 (mg N/l)	120·6	12·2

* For details of procedures, see p. 650.

Net fluxes of nutrients. Urea and NH_3 net fluxes across the rumen wall showed significant variations ($P<0·01$) within the feeding cycle. Urea net uptake and NH_3 net release increased during feed intake, and thereafter decreased gradually, returning to pre-feeding levels (Fig. 6). Similar variations were observed for PDV net fluxes. Net fluxes of urea and NH_3 across the MDV were not affected by the time of sampling ($P>0·10$). The daily net uptake of urea across the rumen, MDV, and PDV was 6·5 (SD 0·9), 1·9 (SD 0·2), and 10·3 (SD 0·4) g N/d, respectively. The daily net release of NH_3 was 3·5 (SD 0·1), 4·0 (SD 0·6), and 10·4 (SD 1·2) g N/d, respectively. Total and individual FAA and PAA net fluxes across the rumen, MDV and PDV did not show significant variations within the feeding cycle. Daily means of these fluxes are presented in Tables 5, 6 and 7. The dynamics of glucose, lactate, 3-hydroxybutyrate and individual VFA net fluxes across the gastrointestinal tract are presented in Figs. 7 and 8. The net fluxes of these nutrients across the MDV did not vary significantly during the feeding cycle. Across the rumen wall, net fluxes of glucose and lactate did not show significant variations with time ($P>0·10$), whereas VFA and 3-hydroxybutyrate net fluxes significantly increased

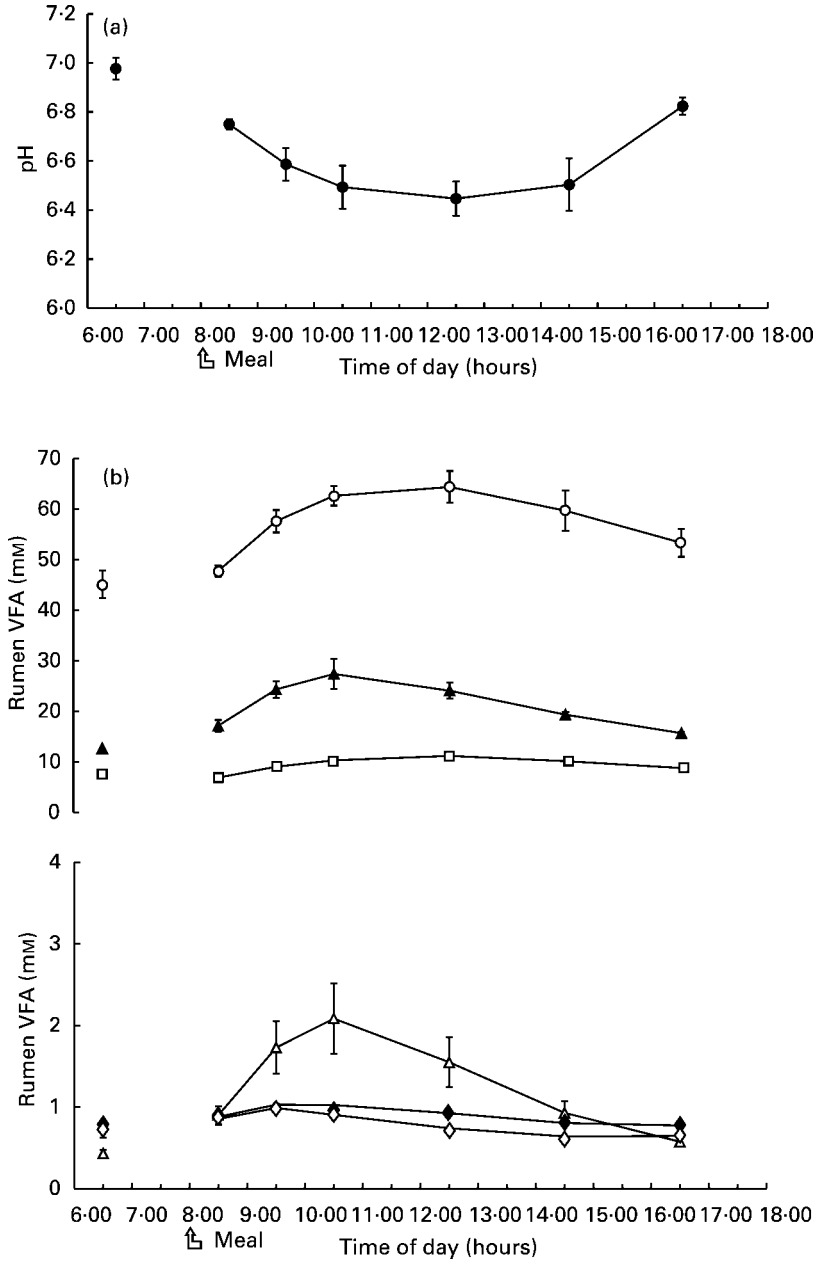

Fig. 1. (a), Patterns of rumen pH (-●-) and (b), volatile fatty acid (VFA) concentrations in sheep fed twice daily with fresh orchard-grass (*n* 3). (-○-), Acetate; (-▲-), propionate; (-□-), butyrate; (-◆-), isobutyrate; (-△-), valerate; (-◇-), isovalerate. Mean values are shown, with their standard errors represented by vertical bars.

during the meal; the highest values were observed during the hour following the meal. Similar trends were observed for the net appearance of VFA and 3-hydroxybutyrate in the portal vein. From pre-feeding until the end of the meal, a decrease in PDV glucose net uptake and lactate net release was observed ($P < 0{\cdot}05$). Daily means of glucose, lactate, 3-hydroxybutyrate and individual VFA net fluxes are presented in Table 7.

Discussion

Although digestive and blood fluxes were carried out on two different groups of animals, the homogeneity of the animals within each group (low SD for most of the studied traits) gives sense to the comparison between nutrient disappearance from the gut and its appearance in portal blood.

At the sampling site, the cranial mesenteric vein drained about 85 % of the whole small intestine (Neutze *et al.* 1994). With such a preparation, MDV flux does not take into account the duodenum and the distal ileum, and does not include pancreas drainage.

Daily net fluxes

Blood flow. The portal blood flow (PBF) recorded was consistent with the relationship observed in sheep between

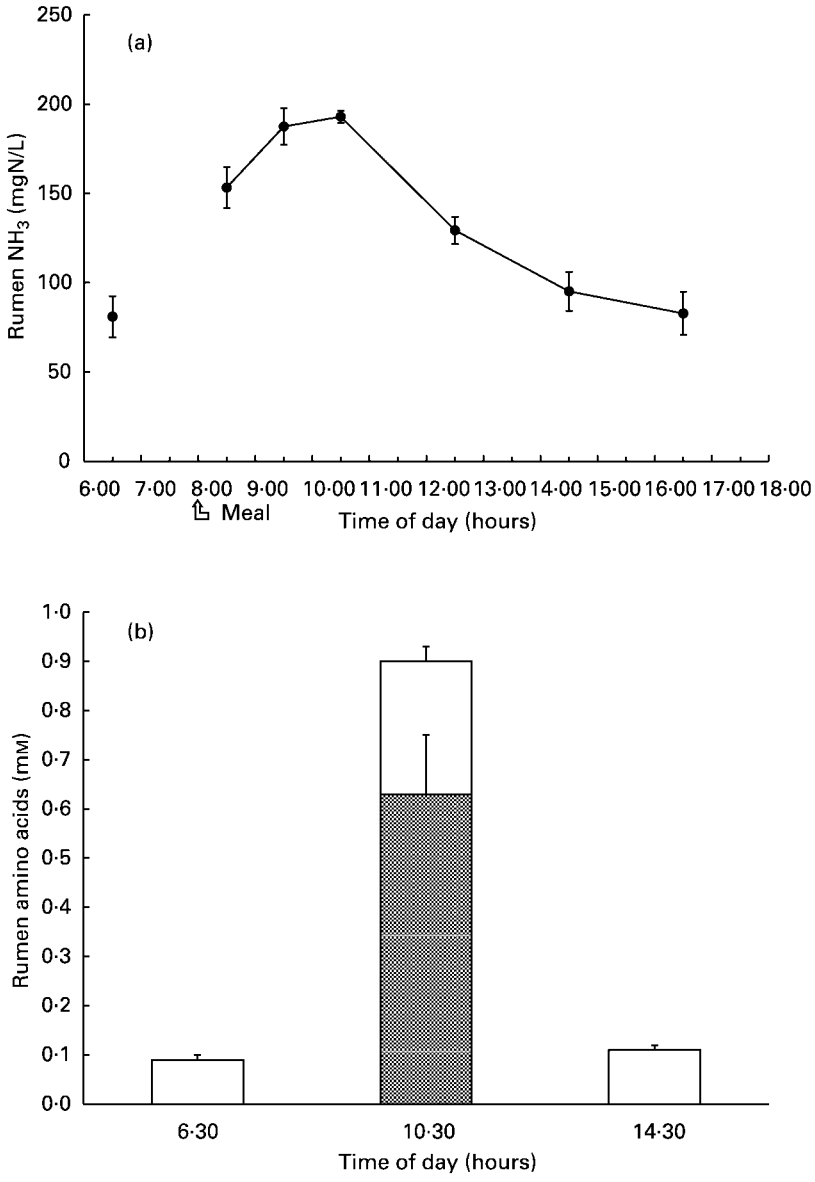

Fig. 2. (a), Patterns of rumen ammonia; (b), patterns of free amino acid (FAA; ▨) and peptide amino acid (PAA; □) in sheep fed twice daily with fresh orchard-grass (*n* 3). Mean values are shown, with their standard errors represented by vertical bars.

ME intake and PBF (Rémond *et al.* 1998). The rumen and MDV blood flows observed in the present experiment accounted for 18 and 23 % of the PBF, respectively. The rumen contribution to PBF is within the range of reported values (from 13 to 22 %; Barnes *et al.* 1983; Rémond *et al.* 2000*b*; Han *et al.* 2002). The MDV contribution is in agreement with previous studies in sheep, where BF in the cranial mesenteric vein (upstream of the junction with the ileocaecocolic vein) was recorded with transit-time ultrasonic probes (Neutze *et al.* 1994; Rémond *et al.* 2000*b*) or with the microsphere technique (Barnes *et al.* 1983). However, a greater contribution (45 % of PBF) was observed by MacRae *et al.* (1997*b*) using the dye dilution technique; such a contribution was more generally attributed to the whole intestine (Neutze *et al.* 1994; Han *et al.* 2002).

Nitrogen balance in the total tract. Total N apparently digested in the digestive tract was 15·6 g N/d while the summation of portal FAA, PAA, NH_3 and urea net fluxes (11·3, 3·5, 10·4, and −10·3 g N/d, respectively) was 14·9 g N/d. Such a close relationship between digestive and portal net balance of N was previously reported by Rémond *et al.* (2000*b*). However, these balances have to be manipulated cautiously, first because PDV measurements do not take into account salivary and biliary N secretions into the gut, and second because the net release of protein-N, high-molecular-weight peptide-N, and N from nucleic bases across the PDV was not considered. Nevertheless, the equilibrium between digestive and portal balance observed in these studies suggests that the net release of low-molecular-weight peptides could play a significant role in N transaction across the digestive

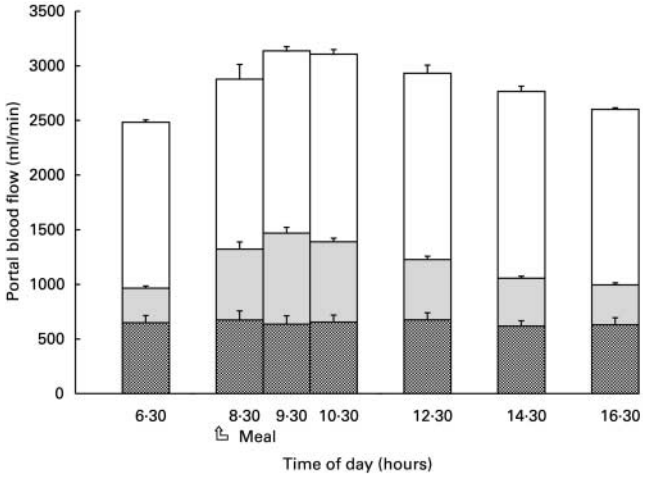

Fig. 3. Pattern of total portal blood flow, with rumen (▨) and small intestine (■) contributions, in sheep fed twice daily with fresh orchard-grass (*n* 3). Mean values are shown, with their standard errors represented by vertical bars.

tract. In the study from Rémond *et al.* (2000*b*) sheep were fed a mixture of hay and extruded peas (70:30), and PAA net release accounted for 35 % of non-protein AA net release into the portal vein. In the present study with sheep fed fresh forage it accounted for 25 %. In sheep fed lucerne pellets (Backwell *et al.* 1997; Bernard *et al.* 2002) no significant flux of PAA across the PDV tissues could be detected. Combined, these data suggest that, in adult sheep, the significance of low-molecular-weight peptide net flux across the PDV is related to the nature of the diet. Conversely, in young steers (about 130 kg body weight), PAA was observed to contribute greatly (about 65 %) to non-protein AA flux across the PDV, whatever the diet, concentrate (Koeln *et al.* 1993) or forage base (Seal & Parker, 1996). In older steers (260 kg body weight), this contribution seemed lower (32 %), and did not seem to be affected by dietary protein degradability (Han *et al.* 2001). Such data suggest that in growing cattle, animal age, rather than nutrition, determines PAA net flux across the PDV.

Table 4. Daily values of blood arterial concentration (μM) of free amino acids (FAA), and plasma arterial concentration (μM) of peptide amino acids (PAA) in sheep fed fresh orchard-grass (*n* 3)*

(Mean values and standard deviations)

	FAA		PAA	
Item	Mean	SD	Mean	SD
Aspartic acid	23·6	0·3	16·5	12·7
Threonine	142·9	17·4	33·8	17·8
Serine	89·2	2·2	16·9	8·3
Asparagine	51·0	2·2		
Glutamic acid	101·1	9·2	64·8	29·8
Glutamine	243·8	12·4		
Proline	74·3	13·5	8·5	3·4
Glycine	408·7	24·7	201·8	6·8
Alanine	166·3	9·2	34·7	1·0
Citrulline	239·3	30·5		
Valine	183·8	9·3	36·8	7·6
Cysteine	7·7	1·2		
Methionine	15·3	1·7		
Isoleucine	77·0	3·8	17·8	5·8
Leucine	102·5	12·3	27·1	4·4
Tyrosine	57·5	3·7		
Phenylalanine	40·9	6·7	14·1	5·5
Ornithine	155·4	19·7		
Lysine	160·2	16·4	20·4	2·5
Histidine	96·8	14·3	20·0	3·7
Arginine	113·3	4·7	20·0	2·8
Total amino acids	2550	185	533	112
Essential amino acids	820	82	170	48

* For details of procedures, see p. 650.

Nitrogen balance in the small intestine. Net disappearance of N from the small intestine was 17·3 g N/d while the summation of FAA, PAA, NH_3 and urea net fluxes across the MDV (15·0, 2·2, 4·0, and − 1·9 g N/d, respectively) was 19·3 g N/d (Fig. 9). Thus, at this point in the digestive tract, the calculated N net release in the portal vein was greater than N net disappearance from the gut lumen. As for total tract digestibility and portal balance, two factors may explain this discrepancy. First, N disappearance from the small intestine is significantly underestimated because of biliary and pancreatic N secretion into the duodenum (distal to the cannula, but not derived from MDV extraction of N). Indeed, in sheep (50 kg body weight), these secretions have been reported to amount to about 4 g N/d (Taschenov *et al.* 1979; Kowalik *et al.* 2001), but may reach up to 9 g N/d (Van Bruchem *et al.* 1997). Second, N appearance across the MDV tissues was also underestimated because, as at the portal level, not all the fluxes of nitrogenous substrates were recorded.

Nitrogen balance in the rumen. Rémond *et al.* (2000*a*) observed a linear increase in carnosine net release across the ruminal wall with increasing concentrations of this dipeptide in the rumen (up to 4·6 mM), demonstrating, *in vivo*, the permeability of the rumen epithelium towards small peptides. However, when the rumen epithelium faced a mixture of low-molecular-weight peptides (up to 16 mM of PAA), as can be observed during dietary protein degradation, no significant PAA net flux in the rumen vein could be detected (Rémond *et al.* 2000*b*). In the present study, low-molecular-weight peptide concentration in the rumen increased in response to the meal, but it remained far below the concentration generated by the casein hydrolysate injection in the study from Rémond *et al.* (2000*b*). For this reason, PAA net flux across the rumen was not expected in the present study, and was not considered. The summation of FAA, NH_3 and urea net fluxes across the rumen (− 1·3, 3·5, and − 6·5 g N/d, respectively) reached a net uptake of − 4·3 g N/d, which was consistent with the increase in N flux observed between the mouth and the duodenum (+4·2 g N/d), and suggested that NH_3-N absorption between the rumen and the duodenum was equivalent to endogenous N entry via saliva and secretion across the omasum and abomasum walls. Assuming that rumen utilisation of FAA is mainly linked to epithelial cell turnover, keratin and mucus synthesis, it can be estimated that about 1·3 g N/d entered the rumen as cell desquamation, which is below the previous estimation (5–8 g N/d) derived from ^{15}N tracer studies (Kennedy & Milligan, 1980). However, the present data does not take into account the reticulum and omasum, neither does it consider the probably low, but possible, uptake of

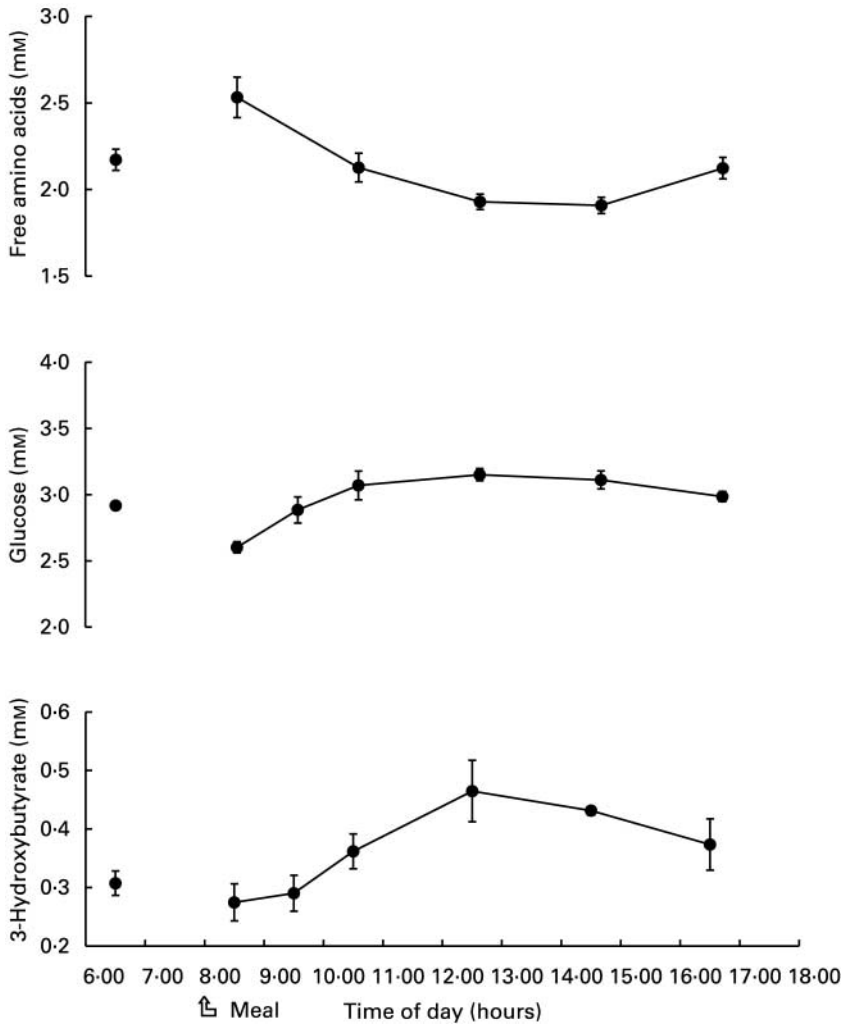

Fig. 4. Patterns of arterial concentration of free amino acids, glucose, and 3-hydroxybutyrate in sheep fed twice daily with fresh orchard-grass (*n* 3). Mean values are shown, with their standard errors represented by vertical bars.

luminal AA by the rumen epithelium. Such uptake of luminal AA may occur within the first hours following meal delivery when significant concentrations of AA are observed in the rumen (Rémond *et al.* 2000*a*), leading to a slight underestimation of N loss from the rumen wall.

Net fluxes of essential amino acids. On average, the MDV net appearance of essential FAA (359 mmol/d) was slightly greater than their apparent disappearance (319 mmol/d) from the small intestine (+13 %). When peptide EAA were added to free EAA, this difference reached +31 %. From Hamza (1976) it can be estimated that at least 40 mmol EAA/d entered the duodenum as exogenous secretion of pancreatic proteins. Similarly, from an AA profile of bile secretion (Gabel & Poppe, 1986), assuming a biliary secretion of 2 g of total N/d (Taschenov *et al.* 1979), with AA accounting for 75 % of total N (Souffrant, 1991), it can be estimated that biliary EAA secretion amounted to about 5 mmol/d. Correcting small intestine apparent disappearance of EAA for these minimal estimates of biliary and pancreatic EAA secretion led to close digestive and blood (FAA + PAA) balances. These observations are in agreement with MacRae *et al.* (1997*b*) who reported that the disappearance of EAA between the jejunum and the ileum equated with their appearance in the blood draining this gut section.

Net PDV fluxes of essential FAA averaged 75 % of net MDV fluxes. This value is in agreement with our previous measurements in sheep fed on hay and extruded peas (73 %; Rémond *et al.* 2000*b*) but is higher than the value of PDV:MDV net fluxes (61 %) reported by MacRae *et al.* (1997*b*) in sheep fed lucerne pellets with similar location of catheters. This discrepancy could be partly explained by the large difference in MDV contribution to PBF observed between the study from MacRae *et al.* (1997*b*) and the studies from our group. Nevertheless, all the studies in which PDV and MDV net flux of free EAA were recorded (Seal & Parker, 1996; MacRae *et al.* 1997*b*; Rémond *et al.* 2000*b*; Berthiaume *et al.* 2001; Han *et al.* 2002) highlighted the widespread use of arterial essential FAA by non-MDV tissues (22–45 % of the MDV net release). In the present work, the net uptake of EAA by the rumen wall accounts for 30 % of this non-MDV uptake.

The apparent digestibility of the EAA in the small intestine was on average 74 %, but a low apparent

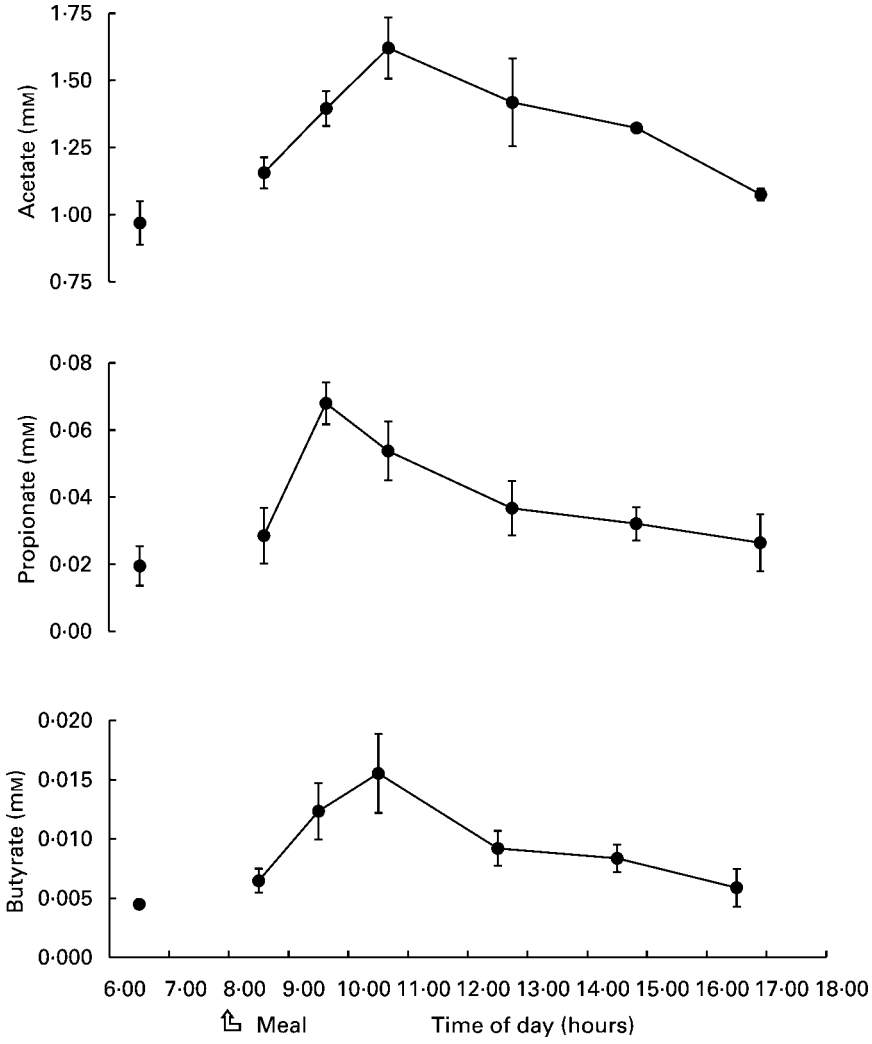

Fig. 5. Patterns of arterial concentration of acetate, propionate and butyrate in sheep fed twice daily with fresh orchard-grass (*n* 3). Mean values are shown, with their standard errors represented by vertical bars.

digestibility was observed for threonine (66 %). The large proportion of endogenous N in ileal digesta (Van Bruchem *et al.* 1997), mainly mucins, probably explains this observation. Indeed cysteine, proline and serine showed the lowest digestibility among the non-essential AA, and all these AA (threonine, cysteine, proline, serine) are present in high proportions in mucins. Conversely, the high apparent digestibility of lysine (81 %) could probably be explained by the high content in lysine of bacteria (86 % of duodenal non-NH_3-N flow) with respect to N content of the endogenous losses at the ileum.

Expressed as a percentage of the small intestine disappearance, the lowest MDV net release of EAA (FAA + PAA) was observed for phenylalanine and methionine. Conversely, the higher recoveries were observed for valine, isoleucine, lysine and histidine, whereas intermediate values were observed for threonine and leucine. Regarding the free EAA extraction rate from arterial blood by the non-MDV tissues (non-MDV net uptake divided by non-MDV arterial supply), the greatest values were recorded for isoleucine, leucine, and threonine, and the lowest for phenylalanine and methionine. As a consequence of the whole digestive tract metabolism, with respect to duodenal flow, the lower portal recoveries were observed for threonine, methionine, leucine and phenylalanine (Fig. 10). As peptide-bound methionine was not recorded, methionine recovery could be slightly underestimated (on average peptide EAA accounted for 14 % of the EAA portal net release). However, low recovery of threonine and methionine in portal blood has also been observed in pigs by Stoll *et al.* (1998), who related the great use of threonine and S-containing AA by the gastrointestinal tract for mucin and glutathione synthesis. The low recovery of phenylalanine observed in the present work is more difficult to explain. Indeed, isotope extraction measurement across the PDV (MacRae *et al.* 1997*a*; Caton *et al.* 2001; Reynolds *et al.* 2001) showed that the use of arterial phenylalanine by the PDV is generally lower than that of the other EAA, and in the present work phenylalanine is the EAA which displays the lowest arterial extraction rate across the non-MDV tissues. The low MDV recovery of the phenylalanine that apparently disappeared from the small intestine would therefore suggest an important first-pass metabolism of phenylalanine within the small intestine. This concurs

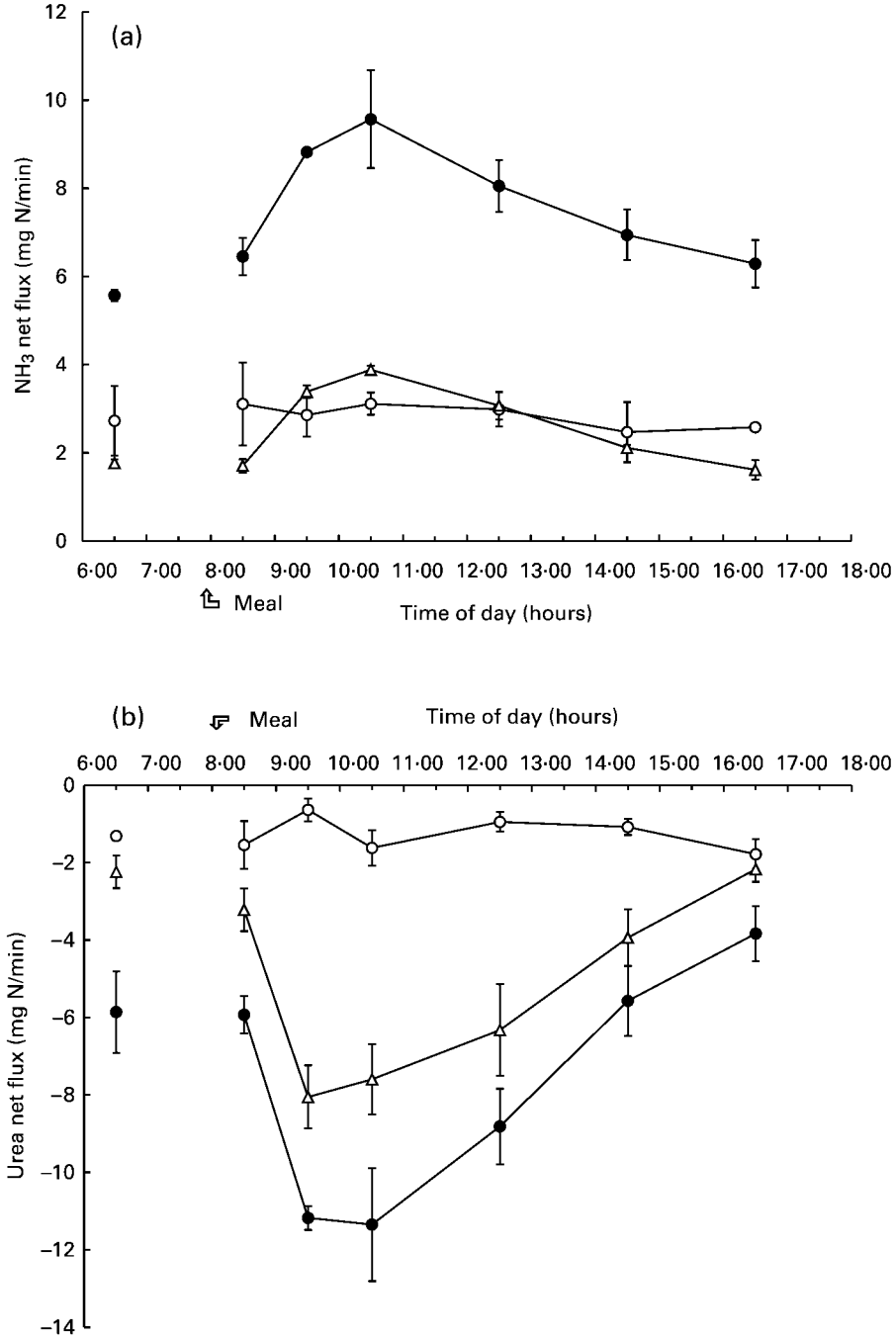

Fig. 6. Patterns of ammonia (a) and urea (b) net fluxes across the portal-drained viscera (-●-), the mesenteric-drained viscera (-○-) and the rumen (-△-) in sheep fed twice daily with fresh orchard-grass (*n* 3). Mean values are shown, with their standard errors represented by vertical bars.

with the observations in pigs showing that about 60 % of the protein-bound phenylalanine in the intestine mucosa is derived from first-pass sequestration of dietary phenylalanine (Stoll *et al.* 1999). Conversely, the high extraction rate of arterial leucine by the non-MDV tissues, together with a high MDV recovery of the leucine that apparently disappeared from the small intestine, suggests that leucine used by PDV tissues is mainly derived from the arterial pool. This observation is in agreement with isotope extraction measurements across the PDV (MacRae *et al.* 1997*a*; Yu *et al.* 2000; Caton *et al.* 2001; Reynolds *et al.* 2001).

Net fluxes of non-essential amino acids. Only 4 % of the cysteine that apparently disappeared from the small intestine was recovered in the mesenteric vein, and a negative balance was observed at the portal level. This highlights the high requirement of the gastrointestinal tract for this AA, probably in relation to glutathione synthesis in the small intestine (Reeds *et al.* 1997), and to mucin production.

Table 5. Mesenteric-drained viscera (MDV) and portal-drained viscera (PDV) net flux of blood free amino acids (FAA) and plasma peptide amino acids (PAA) in sheep fed fresh orchard-grass (*n* 3)*

(Mean values and standard deviations)

	MDV net flux (mmol/d)				PDV net flux (mmol/d)			
	FAA		PAA		FAA		PAA	
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aspartic acid	6·4	2·3	0·2	2·8	7·0	2·7	0·4	2·0
Threonine	57·8	3·5	11·1	3·3	37·9	8·3	7·3	1·7
Serine	60·0	4·2	3·3	2·7	48·8	5·8	5·0	2·7
Asparagine	46·2	2·8			31·8	3·4		
Glutamic acid	17·8	5·7	18·7	19	8·6	7·6	49·1	30·5
Glutamine	2·6	7·6			−46·4	15·8		
Proline	33·4	3·4	5·9	5·1	27·8	7·8	11·3	5·4
Glycine	76·3	10·5	29·4	16·1	90·1	11·2	56·2	14·6
Alanine	96·7	4·4	13·6	8·1	80·2	5·9	19·0	8·6
Citrulline	20·5	1·7			28·6	1·7		
Valine	57·2	3·4	12·1	6·1	40·8	9·3	11·9	4·7
Cysteine	1·3	0·4			−0·8	0·1		
Methionine	14·7	1·8			12·9	7·3		
Isoleucine	52·9	2·7	8·7	3·7	36·6	3·7	7·7	3·0
Leucine	67·5	2·8	10·2	3·2	47·6	4·1	8·3	2·8
Tyrosine	28·6	0·7			23·5	1·8		
Phenylalanine	27·4	0·8	4·4	0·6	26·3	3·2	4·5	0·6
Ornithine	2·5	0·6			7·9	6·8		
Lysine	65·0	4·9	8·3	2·5	55·9	6·6	9·1	2·0
Histidine	16·2	1·4	3·4	2·7	13·1	4·9	4·3	3·5
Arginine	33·0	1·3	6·0	3·7	24·7	6·9	9·6	1·8
Total amino acids	784	35	134	83	603	127	220	83
Essential amino acids	359	13	58	23	271	49	53	18

* For details of procedures, see p. 650.

Table 6. Non-mesenteric-drained viscera (non-MDV) net flux of blood free amino acids (FAA) in sheep fed fresh orchard-grass (*n* 3)*

(Mean values and standard deviations)

	FAA net flux (mmol/d)			
	Non-MDV		Rumen	
Item	Mean	SD	Mean	SD
Aspartic acid	0·7	0·6	−0·4	0·6
Threonine	−19·9	6·8	−3·2	1·0
Serine	−11·3	4·1	1·7	1·3
Asparagine	−14·4	3·8	−2·9	0·6
Glutamic acid	−9·2	2·5	3·1	1·1
Glutamine	−49·0	23·1	−2·3	3·0
Proline	−5·6	4·4	−2·0	1·6
Glycine	13·8	2·0	−10·0	3·3
Alanine	−16·5	10·0	−11·1	3·3
Citrulline	8·1	3·7	−0·9	0·4
Valine	−16·4	6·1	−4·0	2·1
Cysteine	−2·1	0·3	0·0	0·0
Methionine	−1·9	7·2	−1·1	0·8
Isoleucine	−16·3	3·1	−3·2	1·0
Leucine	−19·9	3·4	−5·9	1·6
Tyrosine	−5·1	1·7	−1·3	0·6
Phenylalanine	−1·1	3·0	−1·5	0·4
Ornithine	5·4	6·2	−1·7	0·4
Lysine	−9·1	8·8	−5·5	3·3
Histidine	−3·1	3·5	−2·0	0·8
Arginine	−8·3	6·2	−5·3	1·8
Total amino acids	−182·5	106·8	−59·5	22·3
Essential amino acids	−88·6	41·9	−26·4	10·9

* For details of procedures, see p. 650.

Significant net release of glycine was observed across the non-MDV tissues. However, the rumen and the tissues drained by the left gastric vein (omasum, abomasum, proximal duodenum, omental fat) appeared to be net users of glycine (Rémond *et al.* 2000*a*,*b*). Therefore, significant glycine absorption could take place in the distal duodenum and the proximal jejunum (tissues not drained by the cranial mesenteric vein).

An important release of citrulline was observed across the MDV. In the small intestine synthesis of citrulline may derive from glutamine, glutamate or proline metabolism (Wu, 1998). In the present study MDV recovery of luminal proline and arginine were high and close to the observed EAA recoveries, suggesting that most of the citrulline synthesis in the small intestine could originate from glutamine and glutamate metabolism. The non-MDV tissues released some citrulline, but the small intestine was the main contributor to PDV net release.

In their free form the net appearance of aspartate + asparagine and glutamate + glutamine in the mesenteric vein accounted for 66 and 27 % of their apparent disappearance from the small intestine. Peptide aspartate net release across the MDV was not significantly different from zero, but peptide glutamate net release was close to that observed for its free form, and when peptide glutamate was added to free glutamate the MDV recovery of glutamate + glutamine reached 52 %. Nevertheless, in agreement with observations in dairy cows (Berthiaume *et al.* 2001), MDV recovery of these AA is low. This concurs with the observation of Windmueller & Spaeth (1980),

Table 7. Daily values of glucose, lactate, 3-hydroxybutyrate and volatile fatty acid net fluxes (mmol/d) across the portal-drained viscera (PDV), the rumen (R) and the small intestine (SI) of sheep fed fresh orchard-grass (*n* 3)*

(Mean values and standard deviations)

	PDV		Rumen		Small intestine		PDV − (R + SI)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Glucose	−104	18	−24	11	−17	7	−64	27
Lactate	322	83	166	45	4	16	153	62
3-Hydroxybutyrate	259	68	229	33	−35	18	66	27
Volatile fatty acids								
Acetate	3315	66	2308	339	−275	139	1283	204
Propionate	1237	65	674	109	29	45	535	40
Butyrate	88	17	41	4	−3	1	49	16
Isobutyrate	38	4	17	4	1	1	20	16
Valerate	8	1	4	0	0	0	4	1
Isovalerate	29	6	15	6	1	0	14	1

* For details of procedures, see p. 650.

according to which aspartate, glutamate and glutamine are the major oxidative substrates for the small intestine.

At the portal level, in agreement with most of the studies performed in sheep (Heitmann & Bergman, 1978; Tagari & Bergman, 1978; Lobley *et al.* 1995; Nozière *et al.* 2000*a*,*b*; Rémond *et al.* 2000*b*; Milano & Lobley, 2001; Han *et al.* 2002), a net extraction of glutamine was observed. However, in sheep fed high levels of concentrate, a net release of glutamine across the PDV has been reported (Prior *et al.* 1981; Balcells *et al.* 1995). In accordance with this, Piccioli Cappelli *et al.* (1997) observed a shift from portal net uptake to net release of glutamine during the duodenal infusion of glucose, suggesting a sparing effect of exogenous glucose on glutamine metabolism. As generally reported (Seal & Parker, 1996; Gate *et al.* 1999; Rémond *et al.* 2000*b*; Berthiaume *et al.* 2001) a large net uptake of glutamine was observed across the non-MDV tissues. However, the rumen's contribution to this non-MDV extraction in the present study and as reported by Rémond *et al.* (2000*b*) was low. Similarly, low uptake of glutamine was observed in the left gastric vein of sheep (Rémond *et al.* 2000*a*), which drains omasum, abomasum, duodenum and omental fat. The partitioning of glutamine uptake by non-MDV tissues is yet to be elucidated, and the significance of splenic and pancreatic uptake is unknown. Similarly, the main determinants of the high removal of arterial glutamine by the gastrointestinal tract of ruminants (oxidation, purine synthesis, protein synthesis, etc) are still uncertain (Gate *et al.* 1999).

Peptide amino acids. In opposition to observations in steers (Seal & Parker, 1996), but in agreement with observations in adult sheep (Rémond *et al.* 2000*b*), the small intestine was the main contributor to peptide-bound EAA net release across the PDV. Unfortunately, the present study does not allow us to specify the origin (luminal or mucosal) of the peptide EAA released by the small intestine. The pattern of peptide EAA net flux across the PDV was not significantly different from that of free EAA, although the contribution of histidine and valine were greater for PAA than for FAA. On average, glutamate + glycine accounted for 55 % of the PDV net flux of peptides, but this contribution greatly varied between the animals. The release of peptide-bound glutamate and glycine could be linked to the splanchnic metabolism of glutathione. Whereas non-MDV release of these PAA could originate from the reabsorption of biliary glutathione in the distal duodenum and the proximal jejunum, MDV release could originate from the synthesis of glutathione in the jejunum and ileum.

Net fluxes of energy substrates. In the present study the net portal appearance of energy was 65 % of ME intake. The respective contribution of acetate, propionate, butyrate, other VFA, 3-hydroxybutyrate, lactate and AA (FAA + PAA) to ME reaching the portal vein was 37, 24, 3, 2, 8, 6, and 20 %. All these values are in agreement with Lindsay (1993) and Ortigues & Visseiche (1995) who reviewed the partition of ME intake within absorbed nutrients. The present study provides additional information on the partitioning of these energy substrate net fluxes among the PDV.

Rumen net release of acetate, propionate, four-carbon VFA, and five-carbon VFA accounted for 70, 55, 46 and 50 %, respectively, of their portal net release. This contribution reached 77 % for 3-hydroxybutyrate. The small intestine exhibited significant uptake of arterial acetate, and 3-hydroxybutyrate. These data are consistent with the data of Kristensen *et al.* (1996, 2000*b*) showing the uptake of arterial acetate and 3-hydroxybutyrate by the PDV tissues. Non-rumen and non-MDV tissues include organs where VFA absorption takes place (omasum, abomasum, and large intestine) and tissues that are potential users of arterial acetate and ketone bodies. As a consequence, net release from these tissues is characterised by a lower contribution of acetate and 3-hydroxybutyrate when compared with the rumen. All these observations explain the greater contribution of the rumen to the PDV net release of acetate and 3-hydroxybutyrate, compared with other VFA.

As usually observed in ruminants fed with forage, a net uptake of glucose was observed across the PDV. The rumen and small intestine accounted respectively for 18 and 16 % of the PDV use of arterial glucose; thus two-thirds of the uptake was attributable to glucose metabolism

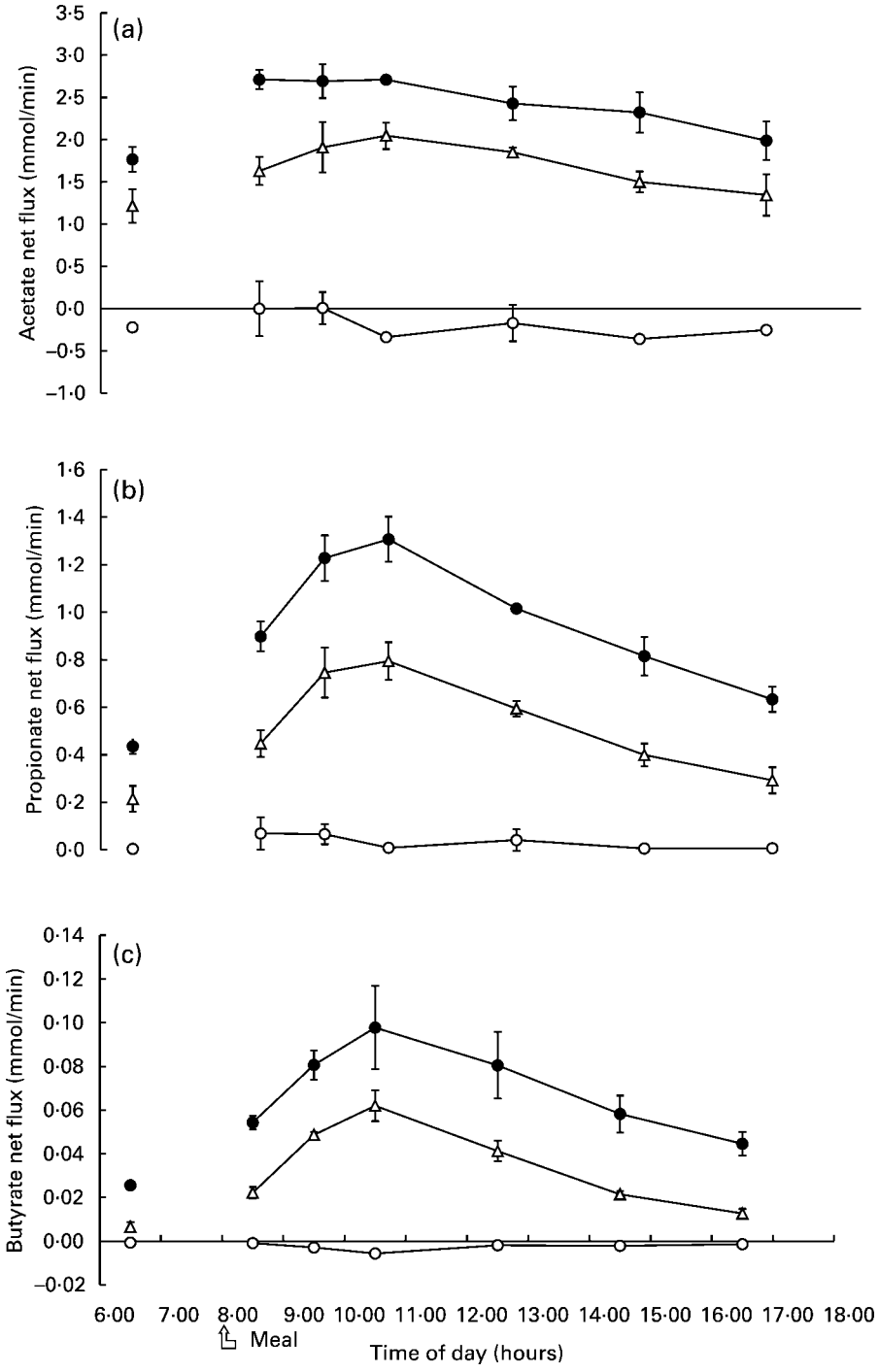

Fig. 7. Patterns of acetate (a), propionate (b), and butyrate (c) net fluxes across the portal-drained viscera (-●-), the mesenteric-drained viscera (-○-) and the rumen (-△-) in sheep fed twice daily with fresh orchard-grass (*n* 3). Mean values are shown, with their standard errors represented by vertical bars.

in the other tissues. The rate of extraction of arterial glucose by the rumen wall (0·9 %) was consistent with that previously reported by Rémond *et al.* (1993*b*).

The rumen wall accounted for 52 % of the PDV net release of lactate, whereas on a daily basis the lactate net flux across the small intestine was not significantly different from zero. Concerning the origin of lactate, glucose uptake by the rumen wall could not explain more than 30 % of the rumen lactate net release, as reported by Rémond *et al.* (1993*a*) and Han *et al.* (2002). Although its quantitative significance is subject to controversy (Kristensen *et al.* 2000*a*), propionate metabolism in the rumen wall could be the main source of lactate. However, valerate metabolism may equally contribute to lactate production. Indeed, *in vitro* studies showed that valerate metabolism might produce significant amounts of lactate (Weigand *et al.*

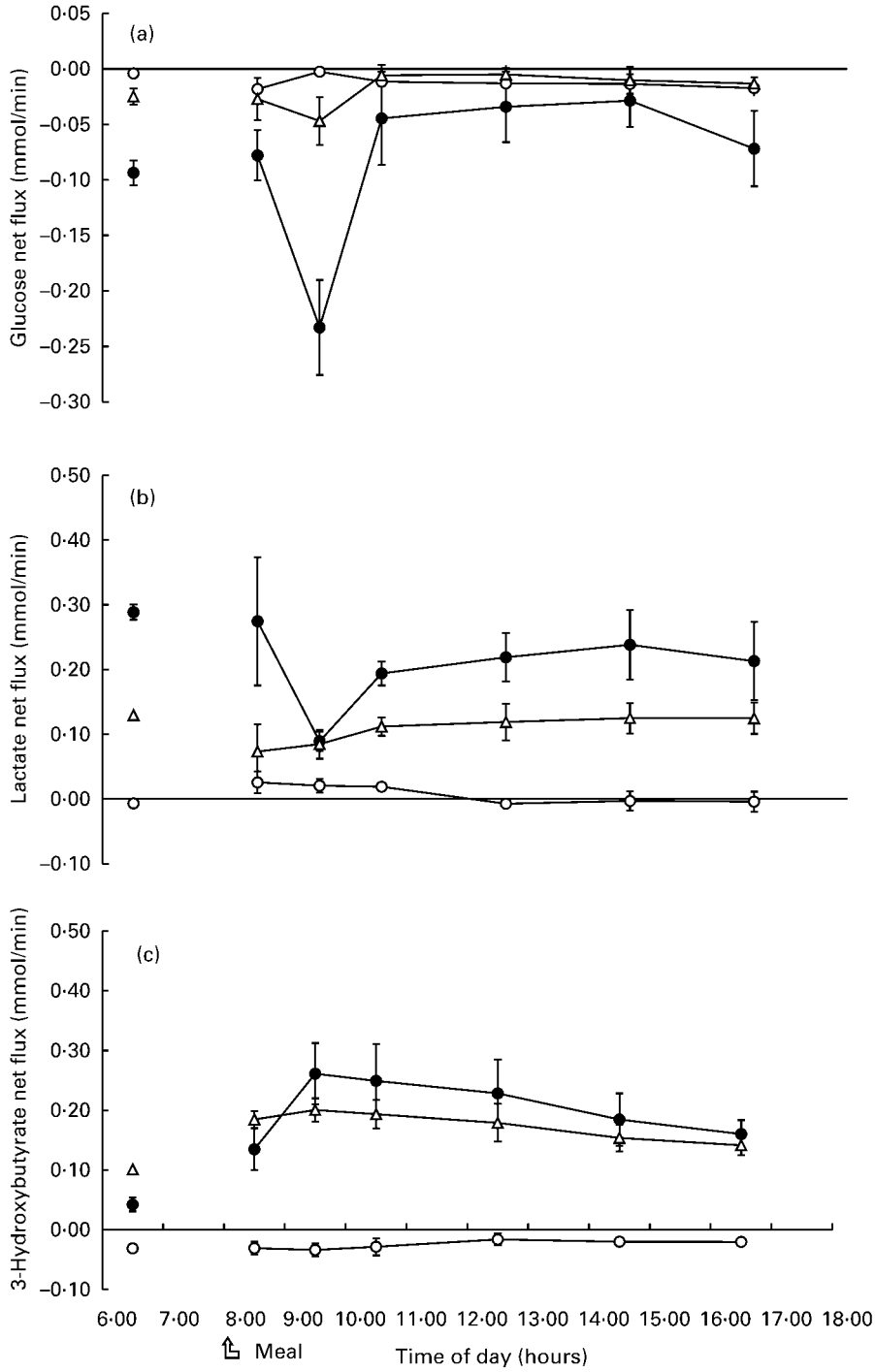

Fig. 8. Pattern of glucose (a), lactate (b), and 3-hydroxybutyrate (c) net fluxes across the portal-drained viscera (-•-), the mesenteric-drained viscera (-○-) and the rumen (-△-) in sheep fed twice daily with fresh orchard-grass (*n* 3). Mean values are shown, with their standard errors represented by vertical bars.

1975), and according to Kristensen *et al.* (2000*a*) 68 % of valerate is metabolised during absorption. The origin of lactate net release by the ruminal wall, and its relationship with VFA metabolism, is still to be elucidated.

Urea and ammonia transactions. The net release of NH_3 across the PDV accounted for 42 % of N intake. Similar amounts of urea-N were transferred across the wall of the digestive tract. Concerning the partition of PDV fluxes, the rumen and small intestine contributed respectively to 34 and 35 % of the portal net release of NH_3, and 63 and 18 % of the portal urea net uptake. These data are consistent with the previous observations of Rémond *et al.* (2000*a*). The net disappearance of NH_3 from the small intestine was low compared with the MDV net release of NH_3 (0·9 *v.* 4·0 g N/d). This difference could be linked to AA metabolism in the small intestine

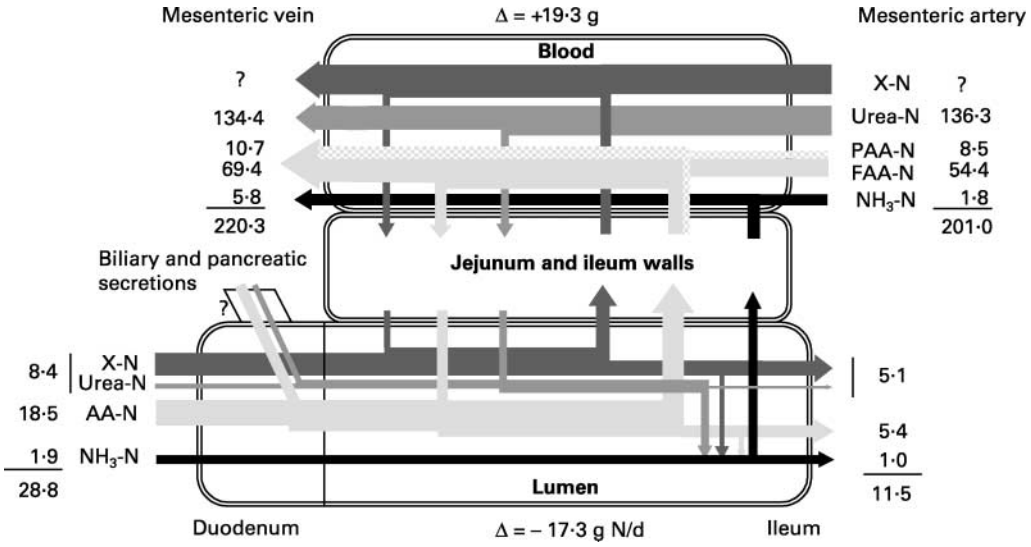

Fig. 9. Nitrogen transactions across the small intestine (g N/d). AA, amino acid; X-N, unaccounted nitrogen; FAA, free amino acids; PAA, peptide amino acids.

wall and to NH_3 production from urea by the bacterial population of the terminal ileum. Indeed, 1·9 g urea N was daily transferred across the small-intestine epithelium, and it can be estimated that about 0·5 g urea-N/d entered the small intestine with biliary and pancreatic secretions (Taschenov *et al.* 1979).

Dynamic aspects

Blood flow. As generally reported for ruminants fed twice daily (Kristensen *et al.* 1996; Han *et al.* 2001), PBF was increased by feeding, a peak value being observed around 2 h after the beginning of the meal. Concerning the partitioning of PBF, the rumen's contribution increased from 13 to 27 % when the pre-feeding and the 1·5 h post-feeding levels were compared. Conversely, within the same time lapse, mesenteric contribution decreased from 26 to 20 %. These observations are in good agreement with those of Barnes *et al.* (1983), obtained with the microsphere technique. In the present study about 80 % of the increase in PBF observed during ingestion was attributable to the rumen. As previously reported in sheep fed with hay (Rémond *et al.* 1993*b*) the rumen BF was more than doubled between pre-feeding and the end of the meal. Conversely, MDV blood flow remained stable during the feeding cycle.

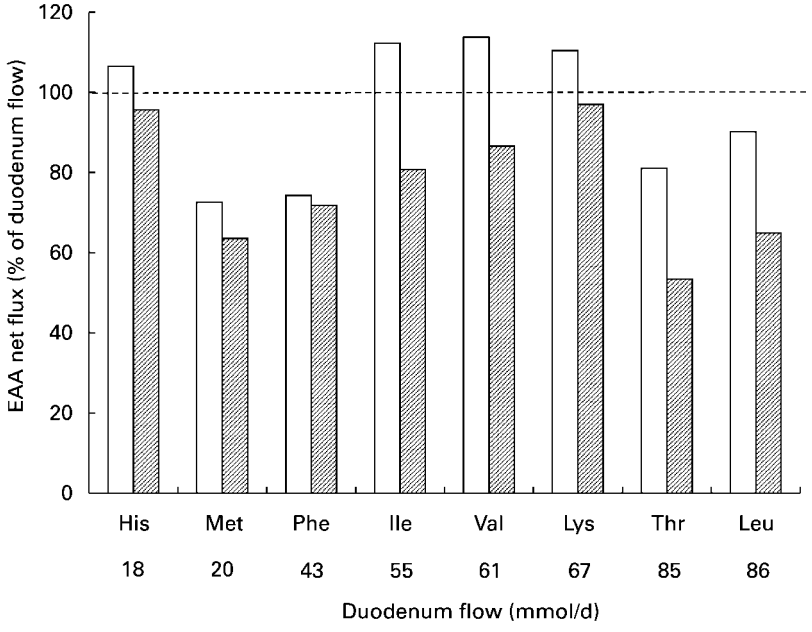

Fig. 10. Essential amino acid (free + peptide) net flux across the portal-drained viscera (▨) and the mesenteric-drained viscera (□) of sheep fed fresh orchard-grass; fluxes are expressed as a proportion of duodenal flow. His, histidine; Met, methionine; Phe, phenylalanine; Ile, isoleucine; Val, valine; Lys, lysine; Thr, threonine; Leu, leucine.

Net fluxes of urea, ammonia and amino acids across the portal-drained viscera. The portal net uptake of urea sharply increased between 30 and 90 min after feed delivery. Thus, at the end of the meal it was more than twice the pre-feeding level. About 90 % of this increase was due to the increase in rumen net uptake of urea. In sheep fed with orchard-grass hay, Rémond *et al.* (1993*b*) observed the greatest values of urea net transfer across the rumen wall around 5 h after feeding, while the rumen NH_3 concentrations were the lowest. Conversely, in the present study where sheep were fed fresh orchard-grass, the greatest values of urea net transfer were recorded only 2 h post-feeding, while rumen NH_3 concentrations were at their highest. This is in accordance with Rémond *et al.* (1993*b*) who reported that an increase of 100 mg N/l in rumen NH_3 (which corresponds to the meal-related increase observed in the present study) does not affect urea net flux across the rumen wall. Within a feeding cycle, a synchronisation between urea net transfer across the rumen wall and rumen carbohydrate fermentation has been shown (Rémond *et al.* 2002). It can therefore be suggested that the discrepancy observed in urea net flux between sheep fed fresh or dry orchard-grass was mainly related to differences in the readily fermentable carbohydrate content of the forage, because the fresh forage was harvested earlier than hay.

Within the feeding cycle, in agreement with Rémond *et al.* (1993*a*,*b*; 2002) there was a close relationship between NH_3 net release in the ruminal vein and the rumen NH_3 concentration. The increase in rumen net flux of NH_3 accounted for only 70 % of the portal increase, showing, as previously discussed, that within non-MDV tissues, the gut segment between the rumen and the distal duodenum may play a significant role in NH_3 absorption.

As previously observed by Whitt *et al.* (1996) in cattle fed twice daily, portal AA flux was not responsive to feed intake. Because, in ruminants, variations in digesta flow to the duodenum are smoothed by rumen emptying, net fluxes of AA across the small intestine did not show significant variations throughout the feeding cycle. Similarly, AA uptake by non-MDV tissues was not affected by the time of day, showing the continuous process of gut-wall protein turnover and pancreatic secretion, even in sheep fed twice daily.

Net fluxes of energy substrates across the gastrointestinal tract. Diurnal variations of portal net release of VFA across the PDV were consistent with the observations of Kristensen *et al.* (1996) in sheep fed twice daily, the highest values being recorded 2–3 h after feeding. The rumen accounted for 77 % of the portal increase in VFA net release observed in response to the meal. This suggests, in agreement with Weston & Hogan (1968), that about three-quarters of the VFA produced in the rumen were absorbed from the rumen and the remainder from the omasum and abomasum. Combining daily net fluxes and dynamics observations, it appeared that with the studied diet, the portal delivery of VFA could be distributed as 65, 15 and 20 % for the rumen, omasum + abomasum, and large intestine, respectively.

Whereas rumen net release of butyrate increased ten-fold between pre-feeding and 2·5 h after feeding, during the same interval of time rumen 3-hydroxybutyrate net release simply doubled, and a logarithmic increase in 3-hydroxybutyrate was observed with the increase in butyrate net release.

Small intestine and rumen uptake of glucose did not show significant variation with time; however the portal uptake of glucose increased during the meal, and then sharply decreased to the pre-feeding level.

As previously reported by Rémond *et al.* (1993*a*) the net release of lactate by the rumen wall was not affected by the time of day, and was therefore not affected by variations in propionate and valerate absorption. The origin of the net release of lactate by the rumen is unclear; nevertheless the present data suggest that lactate production in the rumen wall is heavily regulated.

Conclusion

The present study underlines the difficulty in comparing *in vivo* measurements of digestive and blood fluxes of AA within the intestine, because of the large amount of endogenous AA entering the duodenum with biliary and pancreatic secretions. Nevertheless, the data showed that low-molecular-weight peptides may play a significant role in AA net release by the small intestine, and that the specificity of the small intestine metabolism (high turnover rate, immune cell metabolism, mucin synthesis, glutathione turnover, etc) influences the significance of utilisation of individual AA by this organ. Furthermore, in forage-fed sheep, besides AA oxidation, the small intestine uses significant amounts of arterial acetate, 3-hydroxybutyrate and glucose to cover its energy requirements. The study of the dynamic aspects of nutrient net fluxes across the PDV showed that, with a forage diet (with which more than 85 % of the non-NH_3-N flow to the duodenum is of microbial origin), the liver faces a relatively constant flow of AA, whereas NH_3 and VFA delivery increase in response to the meal. The possible effects on liver metabolism of such diurnal variations in the pattern of the portal delivery of nutrients require further investigation.

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