Effect of underfeeding on metabolism of portal-drained viscera in ewes

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We investigated whether short-term underfeeding could induce adaptative mechanisms in portal-drained viscera (PDV) that would allow nutrients to be spared for vital functions in adult ewes. Six ewes (three of them fitted with catheters in the mesenteric artery and portal and mesenteric veins) were fed, in a double 3×3 Latin square design (2 weeks per experimental period), a regrowth of natural grassland hay at 143 (high; H), 88 (medium; M) and 51 (low; L) % of their energy maintenance requirements. The digestibility of the diet was measured in all six ewes and the net portal fluxes of nutrients in the three catheterized ewes. The organic matter content and N digestibility of the diet were not affected by underfeeding. Urinary and faecal N losses and N balance were linearly related to feed intake. Arterial concentration of acetate was linearly related to feed intake. Arterial concentrations of the other volatile fatty acids, 3-hydroxybutyrate, lactate, glucose, NH_3, urea and total amino acids were not affected by underfeeding. Arterial concentration of non-esterified fatty acids (NEFA) increased with underfeeding. The portal net release of all volatile fatty acids, 3-hydroxybutyrate and NH_3 were linearly related to intake. The portal net flux of both essential and non-essential amino acids, and thus total amino acids, remained unchanged between levels H and M, and decreased between levels M and L. A significant net uptake for glycine and total non-essential amino acids occurred at level L. The portal net uptake of glucose, urea, glutamate and glutamine, and the portal net release of lactate and NEFA were not affected by underfeeding. Summation of portal energy fluxes indicated that 51 % of the metabolizable energy intake was recovered in the portal blood with the three levels of intake. In conclusion, no quantitative adaptation to spare energy, in terms of percentage of intake, occurred in PDV of short-term underfed ruminants, but the pattern of absorption of energetic nutrients was modified.

Ruminant: Underfeeding: Portal fluxes

The high oxidative activity of portal-drained viscera (PDV) contributes 15–25 % of the whole-animal energy maintenance expenditure in ruminants (for reviews, see Huntington, 1990; Ortigues, 1991). Variations in the weight of PDV with changes in intake may thus contribute to the adaptations in the animal's maintenance requirements according to its feeding level (for review, see Ortigues & Doreau, 1995), and to the amount of energy metabolizable by peripheral producing tissues such as muscles or mammary glands. The effects of feeding level on PDV metabolism have been studied mostly in animals fed at levels above maintenance (for review, see Goetsch, 1998). However, because most domesticated ruminants are subjected to periods of food restriction due to economic, husbandry, physiological or climatic reasons, it is of particular interest to analyse whether the drop in energy expenditure of PDV that occurs under maintenance could be an adaptative mechanism that would allow the animal to spare nutrients for vital functions. Data on PDV metabolism in underfed ruminants indicate that PDV reduce their O_2 consumption during the first days of underfeeding (Freetly *et al.* 1995; Ortigues & Durand, 1995) or fasting (Lomax & Baird, 1983). However, it is still unclear whether qualitative changes in nutrient absorption and uptake occur, as few data on net portal fluxes of nutrients under maintenance have been reported (Lomax & Baird, 1983; Eisemann & Nienaber, 1990). The aim of the present study was to determine the portal net fluxes of nutrients in ewes fed at three different feeding levels ranging from 50 to 140 % of their energy maintenance requirements.

Abbreviations: AA, amino acids; BW, body weight; L, M, H, low, medium and high levels of intake respectively; ME, metabolizable energy; NEFA, non-esterified fatty acids; PDV, portal-drained viscera; VFA, volatile fatty acids.

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Material and methods

Animals and feeding

Six adult (mean 6·2 years) non-pregnant and non-lactating Limousin × Romanov ewes (body weight (BW) 55·6 (SD 7·2) kg) were used. Three of them were surgically fitted with catheters in the portal vein, a mesenteric artery and a mesenteric vein as described by Ortigues *et al.* (1994). The animals were allowed 10 d to recover from surgery and were then housed in an air-conditioned room in individual boxes (1·5 m^2). Animals were fed, according to a Latin square design with one catheterized ewe per block, 357 (low; L), 624 (medium; M) or 1049 (high; H) g DM of a second cut of natural grassland hay, with organic matter, metabolizable energy (ME) and crude protein (N × 6·25) contents of 913 g, 10·19 MJ and 129 g/kg DM respectively. These diets supplied 51, 88, and 143 %, and 64, 107, and 173 % of the maintenance requirements for net energy and digestible protein respectively according to the French feeding systems (INRA, 1989). They also received 10 g/d of a mineral supplement (Ca:P:Mg 15:10:2 %, by weight) containing vitamins and micronutrients, and had free access to water and block salt. Hay was offered every 3 h in eight equal meals daily with an automatic feeder.

Measurements

Digestibility and nitrogen balance. After a 9 d period of adaptation to the level of intake, hay samples, total faeces and total urine were collected daily for five consecutive days. Urine was collected daily into 1·8 M-H_2SO_4. After faeces were homogenized and weighed, a portion from each daily faecal collection was dried to determine the total faecal DM production. A representative sample of fresh hay, faeces and urine was obtained for each animal and period, then for hay and faeces it was ground. The organic matter and neutral- and acid-detergent contents (Goering & Van Soest, 1970) were determined using dried (48 h at 80°C) hay and faeces samples. The N content was determined by the Kjeldahl method, using fresh hay, faeces and urine.

Portal net fluxes of metabolites. Blood sampling and collection was performed following an 11–13 d period of adaptation to level of intake. On the sampling day, a physiological sterile saline solution (9 g NaCl/l; pH 7·4) containing *p*-aminohippuric acid (10 %, w/v) was continuously infused into the mesenteric vein catheter (7·2 ml/h), following a prime injection (2·25 ml) at 08.30 hours to allow determination of portal blood flow by downstream dilution. Blood samples were taken at 30 min intervals during two successive feeding cycles and started at 09.15 hours, i.e. 15 min after a meal. At each sampling time, mesenteric artery and portal vein blood samples (7 ml each) were simultaneously taken using syringes containing KEDTA as anticoagulant. Immediately after sampling, packed cell volume was determined in triplicate by centrifuging blood in capillary tubes. Blood NH_3 and urea, and blood and plasma *p*-aminohippuric acid were determined by the phenol–hypochlorite (Weatherburn, 1967), the diacetylmonoxime (Marsh *et al.* 1965) and the *N*-α-naphthyl ethylenediamine dichlorhydrate (Bratton & Marshall, 1939) automated methods respectively using a continuous autoanalyser (Alliance; Méry-sur-Oise, France) as described by Rémond *et al.* (1993) and Isserty *et al.* (1998). Enzymic determinations of 3-hydroxybutyrate (Barnouin *et al.* 1986) in blood deproteinized by using 2 vol. $HClO_4$ (6 %, w/v), glucose (Merckotest kit; Merck, Nogent-sur-Marne, France), lactate (BioMérieux SA kit; Marcy-l'Etoile, France) and non-esterified fatty acids (NEFA; Wako kit; Biolyon, Lyon, France) in plasma were performed using a multianalyser (Elan; Merck-Clevenot, Nogent-sur-Marne, France). After extraction according to Brighenti (1997), by deproteinization with 0·26 vol. metaphosphoric acid (40 %, w/v; Prolabo; Fontenay-sous-Bois, France), volatile fatty acids (VFA) were analysed in blood by G with 2-ethylbutyric acid as an internal standard, as described by Nozière *et al.* (2000). Blood samples were deproteinized with 0·1 vol. sulfosalicylic acid (40 %, w/v) and amino acid (AA) analyses were performed by ion-exchange chromatography on the physiological column of a Beckman autoanalyser (model 6300; Beckman Instruments, Palo Alto, CA, USA), using D-glucosaminic acid as an internal standard.

Blood (or plasma) flow (l/h) and net flux of metabolite (mmol/h) across PDV were calculated as described by Katz & Bergman (1969). Positive net fluxes represented net release in the portal vein, whereas negative net fluxes represented net uptake by PDV. Summation of net portal energy fluxes was based on heats of combustion of 876 (acetate), 1528 (propionate), 2310 (C_4 VFA and 3-hydroxybutyrate), 2838 (C_5 VFA), 1368 (lactate) and 2001 (AA) kJ ME/mol.

Statistical analyses

Data were analysed by ANOVA with animal, period and level of intake as factors, with means per animal and per level of intake as the experimental unit. Since no period effect was observed for any variables, period was removed from the analysis. Linear and quadratic effects of intake level were tested in this model by orthogonal contrasts. Differences between the levels of intake were assessed by the Student-Newman-Keuls *t* test. Significance was accepted at $P < 0{\cdot}05$. All analyses were performed using the GLM procedure (SAS/STAT® Users Guide, Release 6.03; SAS Institute Inc., Cary, NC, USA).

Results

Digestibility and nitrogen balance

The digestibility of the diet was similar for the three levels of intake (Table 1), and averaged (%): DM 70·7, organic matter 72·6, energy 69·5, neutral-detergent fibre 74·4, acid-detergent fibre 72·8, N 64·5. Decreasing feed intake resulted in a linear decrease in faecal and urinary N losses and N retention.

Arterial concentrations and portal net fluxes of metabolites

Decreasing feed intake decreased the arterial concentrations of acetate, isoleucine, tyrosine and ornithine. The

Table 1. Effects of level of intake on DM, organic matter (OM), energy, neutral (NDF)- and acid (ADF)-detergent fibre, and N digestibility (%) and nitrogen balance (g/d) in ewes*

(Values are means for six ewes per treatment and means for the three catheterized ewes)

Level of intake†…	Low		Medium		High			
	Overall	Catheterized	Overall	Catheterized	Overall	Catheterized	SE	*P*=
Digestibility								
DM	70·9[a]	69·2	71·1[a]	71·8	70·0[a]	71·7	1·1	0·7738
OM	73·7[a]	72·5	72·7[a]	73·3	71·5[a]	73·2	1·0	0·3140
NDF	75·5[a]	75·3	74·8[a]	75·8	72·9[a]	74·8	1·2	0·2519
ADF	74·1[a]	73·0	72·6[a]	72·7	71·7[a]	74·4	1·5	0·5622
Energy	70·6[a]	71·2	69·6[a]	68·7	68·3[a]	67·2	1·0	0·3138
N	64·6[a]	63·2	65·0[a]	66·1	64·0[a]	65·6	1·4	0·8790
N balance								
Faeces‡	2·54[a]	2·72	4·81[b]	4·31	7·48[c]	7·59	0·35	0·0001
Urine‡	5·60[a]	6·85	7·50[b]	6·64	9·09[c]	9·52	0·51	0·0023
Retained‡	−0·97[a]	−2·19	1·39[b]	1·74	4·21[c]	4·97	0·54	0·0002

[a,b,c]Means within the same row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see p. 822.
† 357 (low), 624 (medium) and 1049 (high) g DM of a second cut of natural grassland hay.
‡ Linear intake effect was significant ($P < 0·001$).

same trend was observed for some other essential AA (valine and methionine), while the arterial concentration of histidine tended to increase, as did the concentrations of NEFA (Table 2). Except for histidine, these variations were linearly related to intake. The arterial concentrations of propionate, isobutyrate, butyrate, isovalerate, 3-hydroxybutyrate, NH_3, urea, glucose, lactate, and the total, essential and non-essential AA were not modified by the level of intake.

Portal blood flow did not vary significantly with decreasing intake (Table 3). The portal net release of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, 3-hydroxybutyrate and NH_3 decreased linearly to intake with underfeeding. The portal net flux of some essential (methionine, leucine) and non-essential (asparagine, glycine, alanine, tyrosine, proline) AA, and consequently total AA, decreased with underfeeding. The same trend was observed for threonine, isoleucine, phenylalanine, lysine and serine. Portal net flux reached a significant net uptake for glycine and total non-essential AA at level L ($P < 0·05$). Although the decrease in portal net release of AA occurred mainly between levels M and L, a linear effect of intake was observed for portal net release of total, essential and non-essential AA. The portal net uptake of glucose, urea, glutamate and glutamine, and the portal net release of lactate, NEFA and the other AA were not affected by the level of intake. Summation of net portal energy fluxes showed a linear response of portal net appearance of ME to intake (Fig. 1), indicating that 51 % of the ME intake was recovered in the portal blood at all three levels of intake.

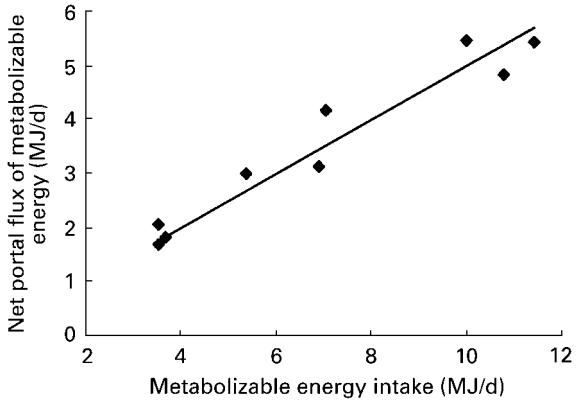

Fig. 1. Relationship between intake (x) and portal net appearance (y) of metabolizable energy in ewes: y = (0·468 (SE 0·046))x + (0·279 (SE 0·347)), $r = 0·965$; $n9$; $P < 0·001$. For details of diets and procedures, see p. 822.

Discussion

Portal blood flow and energy metabolism of portal-drained viscera

Portal blood flow at levels H (6·0 l/h per kg $BW^{0·75}$) was consistent with the relationship between ME intake and portal blood flow by Rémond *et al.* (1998) from data on sheep fed above maintenance levels (6·3 l/h per kg $BW^{0·75}$) reported in the literature. The lack of a significant decrease in portal blood flow with underfeeding was in good agreement with the findings of Webster *et al.* (1975), who reported that portal blood flow is only slightly modified in sheep fed below maintenance after adaptation periods of 1 or 2 weeks. However, this finding may be partly due to the short period of food restriction, since a large decrease in total blood volume in the whole animal has been observed in ewes underfed at 20 % maintenance for 23 weeks (Atti *et al.* 2000).

Few authors have attempted to establish a relationship between the level of intake of a diet and the net portal absorption rate of VFA. In beef heifers receiving the same high-concentrate diet at three different levels of intake, averaging 0·73, 1·36 and 1·96× ME maintenance requirements, Huntington & Prior (1983) reported linear relationships between ME intake and net portal appearance of VFA. However, the effects of underfeeding remained unclear. In the present study, the linear relationships

Table 2. Effects of level of intake on arterial concentrations of nutrients (mmol/l) in ewes†

(Mean values for three ewes per treatment group)

Level of intake‡...	Low	Medium	High	SE	*P*=
Blood					
Acetate*	0·488[a]	0·802[b]	0·979[c]	0·050	0·0057
Propionate	0·013[a]	0·010[a]	0·019[a]	0·005	0·4802
Isobutyrate	0·003[a]	0·003[a]	0·002[a]	0·0003	0·9103
Butyrate	0·008[a]	0·006[a]	0·007[a]	0·003	0·9574
Isovalerate	0·002[a]	0·002[a]	0·002[a]	0·0002	0·9559
Valerate	0·0004[ab]	0·0005[a]	0·0003[b]	0·00003	0·0430
3-Hydroxybutyrate	0·300[a]	0·287[a]	0·351[a]	0·019	0·1586
NH_3	0·144[a]	0·152[a]	0·159[a]	0·018	0·8429
Urea	4·577[a]	3·993[a]	4·354[a]	0·451	0·6783
AA	2·205[a]	2·184[a]	2·376[a]	0·154	0·6564
Plasma					
Glucose	3·225[a]	3·506[a]	3·527[a]	0·096	0·3747
Lactate	0·462[a]	0·487[a]	0·626[a]	0·078	0·3301
NEFA*	0·339[a]	0·228[a]	0·167[b]	0·019	0·0167
Blood					
Threonine	0·085[a]	0·105[a]	0·106[a]	0·011	0·3627
Valine*	0·165[a]	0·183[ab]	0·223[b]	0·014	0·0934
Methionine*	0·019[a]	0·020[a]	0·026[b]	0·002	0·0696
Isoleucine*	0·070[a]	0·089[ab]	0·105[b]	0·007	0·0496
Leucine	0·104[a]	0·114[a]	0·128[a]	0·007	0·1950
Phenylalanine	0·038[a]	0·039[a]	0·044[a]	0·004	0·4915
Lysine	0·150[a]	0·141[a]	0·169[a]	0·008	0·1428
Histidine	0·127[a]	0·089[b]	0·100[b]	0·008	0·0559
Arginine*	0·104[a]	0·119[a]	0·160[b]	0·012	0·0721
Aspartate	0·012[a]	0·012[a]	0·013[a]	0·002	0·9246
Serine	0·072[a]	0·067[a]	0·062[a]	0·011	0·7999
Asparagine	0·039[a]	0·040[a]	0·045[a]	0·003	0·3031
Glutamate	0·102[a]	0·116[a]	0·098[a]	0·006	0·2114
Glutamine	0·255[a]	0·230[a]	0·238[a]	0·015	0·5113
Glycine	0·524[a]	0·459[a]	0·408[a]	0·069	0·5391
Alanine	0·115[a]	0·110[a]	0·127[a]	0·010	0·4972
Cysteine	0·014[a]	0·015[a]	0·018[a]	0·001	0·1435
Tyrosine**	0·034[a]	0·046[a]	0·069[b]	0·004	0·0128
Ornithine**	0·083[a]	0·100[a]	0·132[b]	0·009	0·0433
Proline	0·093[a]	0·091[a]	0·105[a]	0·008	0·5191
Branched chain AA**	0·338[a]	0·386[ab]	0·456[b]	0·025	0·0704
Essential AA	0·861[a]	0·899[a]	1·061[a]	0·055	0·1180
Non-essential AA	1·344[a]	1·286[a]	1·315[a]	0·110	0·9340

[a,b,c]Means within the same row with unlike superscript letter were significantly different ($P < 0·05$).
AA, amino acids; NEFA, non-esterified fatty acids.
Linear intake effect was significant: $*P < 0·05$, $**P < 0·01$.
† For details of diets and procedures, see p. 822.
‡ 357 (low), 624 (medium) and 1094 (high) g DM of a second cut of natural grassland hay.

Table 3. Effects of level of intake on portal flows (l/h), net fluxes of nutrients (mmol/h) and total energy (kJ/h) in ewes†

(Mean values for three ewes per treatment group)

Level of intake‡	Low	Medium	High	SE	*P*=
Blood flow	105·5[a]	105·4[a]	126·1[a]	6·2	0·1236
Plasma flow	71·1[a]	85·7[a]	92·0[a]	5·9	0·1388
Net fluxes					
Acetate**	34·80[a]	62·94[a]	99·29[b]	11·17	0·0371
Propionate***	12·65[a]	20·29[b]	33·41[c]	1·35	0·0010
Isobutyrate**	0·53[a]	0·65[a]	1·11[b]	0·08	0·0130
Butyrate**	1·75[a]	2·82[a]	4·61[b]	0·48	0·0328
Isovalerate*	0·53[a]	0·83[ab]	1·24[b]	0·14	0·0531
Valerate***	0·13[a]	0·22[b]	0·42[c]	0·03	0·0049
3-Hydroxybutyrate**	4·90[a]	6·60[b]	9·18[c]	0·45	0·0064
Glucose	−8·87[a]	−7·59[a]	−7·63[a]	1·79	0·8528
Lactate	5·87[a]	4·70[a]	5·59[a]	0·85	0·6322
NEFA	0·27[a]	1·10[a]	0·64[a]	0·74	0·7446
NH_3**	14·48[a]	19·50[b]	26·25[c]	1·20	0·0059
Urea	−14·22[a]	−11·59[a]	−17·78[a]	2·63	0·3474
AA*	−4·00[a]	12·08[b]	17·60[b]	4·02	0·0417
Total energy***	77·10[a]	142·93[b]	220·03[c]	12·01	0·0029
Threonine	0·03[a]	0·92[b]	1·11[b]	0·27	0·0884
Valine	0·20[a]	1·13[a]	1·07[a]	0·33	0·2064
Methionine**	0·01[a]	0·32[b]	0·47[b]	0·08	0·0417
Isoleucine***	0·20[a]	0·96[b]	1·19[b]	0·20	0·0510
Leucine**	0·30[a]	1·33[b]	1·81[b]	0·27	0·0373
Phenylalanine**	0·39[a]	0·77[a]	1·44[b]	0·21	0·0585
Lysine**	−0·26[a]	0·84[b]	1·59[b]	0·36	0·0516
Histidine	−1·07[a]	0·30[a]	1·48[a]	0·71	0·1484
Arginine	−0·10[a]	0·38[a]	0·40[a]	0·14	0·1146
Aspartate	−0·10[a]	0·16[a]	0·16[a]	0·11	0·2519
Serine***	−0·42[a]	1·28[b]	1·62[b]	0·52	0·0989
Asparagine***	0·20[a]	0·68[b]	0·84[b]	0·11	0·0358
Glutamate	−0·53[a]	−0·42[a]	−0·54[a]	0·15	0·8126
Glutamine	−1·98[a]	−1·79[a]	−2·17[a]	0·24	0·5869
Glycine***	−0·92[a]	1·66[b]	1·72[b]	0·45	0·0226
Alanine**	0·43[a]	2·11[b]	3·07[b]	0·50	0·0473
Cysteine	−0·03[a]	0·01[a]	0·01[a]	0·03	0·5003
Tyrosine**	0·08[a]	0·54[b]	0·98[c]	0·11	0·0124
Ornithine	−0·31[a]	0·16[a]	0·39[a]	0·22	0·1933
Proline***	−0·12[a]	0·74[b]	0·98[b]	0·20	0·0393
Branched chain AA***	0·71[a]	3·42[b]	4·07[b]	0·79	0·0783
Essential AA***	−0·30[a]	6·95[b]	10·55[b]	2·14	0·0532
Non-essential AA***	−3·70[a]	5·13[b]	7·05[b]	1·91	0·0330

[a,b,c]Means within the same row with unlike superscript letters were significantly different ($P < 0·05$).
AA, amino acids; NEFA, non-esterified fatty acids.
Linear intake effect: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.
† For details of diets and procedures, see p. 822.
‡ 357 (low), 624 (medium) and 1094 (high) g DM of a second cut of natural grassland hay.

between OM digested, which is proportional to the rate of VFA production (Hogan & Weston, 1967), and portal net appearance of VFA and 3-D-hydroxybutyrate suggest that the proportion of absorbed VFA metabolized by PDV does not change with underfeeding. With high-forage diets, the slopes of the linear relationships between ME intake (MJ/d per kg $BW^{0·75}$) and net portal appearance of VFA (mmol/h per kg $BW^{0·75}$) ranged from 256, 69 and 6 in sheep (Bergman & Wolff, 1971) to 461, 170 and 48 in cattle (Huntington *et al.* 1988; Reynolds *et al.* 1993) for acetate, propionate and butyrate respectively. The slopes obtained in the present experiment (232, 74 and 10 for acetate, propionate and butyrate respectively) are in good agreement with those reported for sheep. The respective proportions of each VFA appearing in the portal blood, which are consistent with those reported recently in a review by Huntington (1999), were similar among treatments, averaging 70·6, 24·0, 4·2 and 1·2 % of total VFA for acetate ($P = 0·795$), propionate ($P = 0·818$), *iso*- and *n*-butyrate ($P = 0·792$), and *iso*- and *n*-valerate ($P = 0·873$) respectively.

Several studies based on rumen infusion of butyrate reported that the 3-hydroxybutyrate:butyrate portal net outputs decreased when the rumen infusion rate of butyrate increased (Krehbiel *et al.* 1992; Kristensen *et al.* 1996; Nozière *et al.* 2000). These results indicated that ketogenesis from butyrate in the rumen wall was limited. In the present experiment, 3-hydroxybutyrate:butyrate portal net outputs did not vary with intake ($P = 0·357$), showing that ketogenesis from butyrate was not limited in the rumen wall under such dietary conditions. This finding may be

related to the low amount of butyrate produced in the rumen on this hay diet.

Saving glucose is a major mechanism involved in the adaptation of ruminants to undernutrition (Chilliard *et al.* 1998). Arterial concentrations observed in the present study confirm that glucose homeostasis was not altered and glucose demand by PDV was not modified by the level of intake. Since the absorption of glucose from the lumen can be assumed to be negligible in this experiment, net portal flux of glucose can be considered as uptake from the arterial supply, averaging 3 % at all three levels of intake. This value is in good agreement with other data obtained in cattle (Reynolds & Huntington, 1988) or sheep (Patil *et al.* 1995) fed forage diets. In experiments where both net portal flux of glucose and whole-body glucose turnover rate have been measured, net portal uptake has been reported to account for 9–21 % of the total glucose demand in sheep and cattle fed forage diets (Huntington *et al.* 1981; Janes *et al.* 1985; Seal *et al.* 1992; Seal & Parker, 1994). In agreement with these values, net portal uptake of glucose measured in the present study may account for 10–16 % of the whole-body glucose turnover rate, as calculated from the equation of Herbein *et al.* (1978). Unfortunately, no effect of intake could be observed, since glucose turnover rate was not measured directly. The constant net uptake of glucose may be related to the net portal appearance of lactate, which was not affected by the level of intake. Indeed, glucose is a major precursor of lactate in PDV (Weekes & Webster, 1975). Taken proportionally to intake, this factor may result in a high level of lactate entering the liver as precursor for gluconeogenesis in underfed animals.

The increase in the arterial concentration of NEFA with underfeeding measured in the present study reflects lipolysis which is classically observed in underfed ruminants (Chilliard *et al.* 1998), allowing NEFA to be a source of oxidative fuel for peripheral tissues. However, the potential utilization of NEFA by PDV remains questionable, because only few data on net portal fluxes of NEFA are available, and because PDV include gut fat. In the present study net portal flux of NEFA did not differ significantly from zero even in underfed animals, as observed previously (Nozière *et al.* 2000). It is possible that the use of NEFA from the arterial supply and the release of NEFA by gut fat mobilization were of the same magnitude.

Net portal flux of AA at level H exhibited a classical pattern, characterized by a net uptake of glutamate and glutamine, and a net release of the other AA. Indeed, in the well-fed state, glutamate and glutamine are the main AA used by PDV for oxidative metabolism (Huntington, 1990). Our results show that underfeeding did not modify net portal uptake of glutamine and glutamate, but induced a decrease in net portal appearance of both essential and non-essential AA. This finding is in agreement with that of Huntington & Prior (1985), who reported no effect of feed intake on glutamate and glutamine net portal uptake, and a positive relationship between level of intake and net portal appearance of most other AA. In the present experiment, in underfed animals, total non-essential AA exhibited a significant net uptake, and net flux of total essential AA did not differ from zero. These results show that, in the short-term underfed state, the supply of AA to PDV cannot keep up with AA expenditure. In agreement with this result, the uptake of AA by the small intestine wall has been shown to exceed the amount of AA disappearing from the lumen in lambs fed at low intake (Neutze *et al.* 1997). In the present work it may be due to the decrease in both dietary and endogenous N. Indeed, the high decrease in net absorption of glycine, a major component of biliary salts (Gabel & Poppe, 1986), may be related to a decrease in biliary secretions which are absorbed in the small intestine. Intake had a linear effect on the net portal fluxes of most AA, because effects were dominated by low and high levels in the analysis. Indeed, variations occurred mainly between levels M and L. Assuming proportionality between N intake and absorbable AA, the high decrease in net portal appearance of AA between levels M and L may reflect the fact that AA consumption by PDV is only slightly different at these two levels of intake. It is likely that changes in the mass of splanchnic tissues occur rapidly, then stabilize, while body reserves decrease more slowly for a longer time (Koong *et al.* 1982; Johnson *et al.* 1990; Ortigues & Doreau, 1995). However, the length of time before stabilization of PDV metabolism remains undetermined. The stabilization of PDV O_2 consumption in sheep occurred 1 week (Ortigues & Durand, 1995) or 4 weeks (Freetly *et al.* 1995) after feed restriction in sheep. Nevertheless, it is possible that the sparing of AA by PDV in underfed animals is of short-term usefulness when compared with other tissues such as skeletal muscle, which is the main labile protein reserve in the body but in which there is a lower turnover rate (for review, see Lobley, 1993).

The net portal appearance of energy was linearly related to feed intake, reaching 51 % of ME intake with all three levels of intake. This value is in agreement with the findings of Lindsay (1993), who reported that 52–59 % of ME intake is released in the portal vein as VFA, ketone bodies, lactate and AA in sheep. The loss in ME (49 % intake) corresponds mainly to heat of fermentation, and heat production by PDV tissues. Under comparable experimental conditions, Ortigues & Durand (1995) reported that heat production by PDV tissues as a percentage of ME intake increased in response to underfeeding, accounting for 30 and 44 % ME intake respectively in ewes fed at maintenance and 0·5 times maintenance for 2 weeks. The respective contribution of acetate, propionate, butyrate, other VFA, 3-hydroxybutyrate, lactate and AA to ME reaching the portal vein at level H averaged 40, 23, 5, 3, 10, 3 and 16 % respectively, which is consistent with other data on sheep (Lindsay, 1993) or lactating dairy cows consuming forage-based diets (De Visser *et al.* 1997). Underfeeding did not modify the contribution of VFA to ME absorbed ($P = 0{\cdot}690$). Conversely, the contribution of AA to ME absorbed decreased from 16 % (on average at levels H and M) to 1 % at level L ($P = 0{\cdot}052$), and the contributions of 3-hydroxybutyrate and lactate increased from 10 to 15 % ($P = 0{\cdot}023$) and from 4 to 10 % ($P = 0{\cdot}032$) of ME absorbed respectively. It can be concluded that no quantitative adaptation to spare

energy, in terms of percentage of intake, occurs in PDV of short-term underfed ruminants, but that the pattern of absorption of energetic nutrients is modified.

N balance and net transfer of ammonia and urea across portal-drained viscera

The decrease in urinary N loss with underfeeding is in good agreement with the findings of Harmeyer & Martens (1980), who reported a positive relationship between N intake and renal urea clearance. Under normal feeding conditions, uraemia appears to be the main factor implicated in urinary urea excretion (Harmeyer & Martens, 1980). In the present study the arterial concentration of urea was not modified. A reduced blood flow and N uptake in the kidney, as reported in underfed sheep by Cirio & Boivin (1990), may thus also contribute to the decrease in N loss in urine with underfeeding. The net transfer of urea across PDV increased, as a percentage of N intake, from 27 % on average for levels H and M, to 65 % for level L ($P = 0{\cdot}010$). Assuming that hepatic urea synthesis increases with N intake (for review, see Reynolds, 1995), our results are in good agreement with those of Harmeyer & Martens (1980), who reported a negative relationship between N intake and the percentage of hepatic urea synthesis recycled from blood to gut lumen. Previous measurements of the respective contribution of the different stomachs and post-stomachs to the net fluxes from blood to gut lumen indicated that stomachs accounted for 33–95 (average 70) % of net urea transfer across PDV (Huntington, 1989; Seal *et al.* 1992; Huntington *et al.* 1996; Rémond *et al.* 2000). The transfer from blood to the lumen of omasum and abomasum appears to be low (Siddons *et al.* 1985; Obara *et al.* 1991, Rémond *et al.* 2000), so that a large proportion of net urea transfer across PDV occurs in the rumen. This contribution, together with that of the saliva, comprises approximately 40 % of the urea entering the rumen in sheep fed forage (Norton *et al.* 1982), may thus improve the availability of fermentable N for rumen microbes. However, NH_3-N absorption, which tended to increase from 41 % to 67 % of the N intake between levels H and L ($P = 0{\cdot}088$), suggests that rumen NH_3 was not a limiting factor for microbial synthesis, and thus that the efficiency of recycling of urea-N was limited.

Conclusion and perspectives

The present study provides a better understanding of the contribution of PDV to the adaptation of mature ruminants to short-term periods of food restriction. It should be emphasized that the proportion of ME intake which is absorbed through the portal vein remains unchanged. Although it is likely that splanchnic metabolism stabilizes after 1–3 weeks following the beginning of feed restriction, extrapolation of these results to long-term underfed animals needs to be examined in order to improve animal husbandry under extensive conditions and to make intervention programmes in less-developed countries more effective.

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