Effect of composition of ruminally-infused short-chain fatty acids on net fluxes of nutrients across portal-drained viscera in underfed ewes

Pierre Nozière[1]*, Cécile Martin[1], Didier Rémond[1], Niels B. Kristensen[2], Richard Bernard[1] and Michel Doreau[1]

[1]*Unité de Recherches sur les Herbivores, INRA Theix, 63122 Saint-Genès-Champanelle, France*
[2]*Department of Animal Nutrition and Physiology, Danish Institute of Agricultural Sciences, Box 50, DK-8830 Tjele, Denmark*

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Four ewes, each fitted with a rumen cannula and with catheters in the mesenteric artery and portal and mesenteric veins, received continuous intrarumen infusions of water or of short-chain fatty acids (SCFA). SCFA infusions were isoenergetic (83 kJ/h) and provided rumen molar proportions (acetate : propionate : butyrate) of 70 : 20 : 10, 50 : 40 : 10 or 50 : 20 : 30. The rumen SCFA production rate with the basal diet was 90·0, 23·1 and 8·8 mmol/h for acetate, propionate and butyrate respectively. Portal net fluxes indicated that 74, 67 and 22–30 % of infused acetate, propionate and butyrate respectively, reached the portal vein. Portal net release of β-hydroxybutyrate increased with SCFA infusions, irrespective of the amount of butyrate infused. Portal net release of lactate decreased with high-butyrate infusion. Portal net uptake of glucose increased with the SCFA infusions. In ewes infused with water, a portal net uptake of total amino acids (AA) was observed. SCFA infusions decreased the uptake of nonessential AA (glutamate, glycine, but not glutamine) and increased the net release of tyrosine and essential AA (isoleucine, leucine). Portal net fluxes of AA were similar with both high-acetate and high-propionate infusions. Lower net uptake of glutamine and net release of most essential AA and some nonessential AA were observed with the high-butyrate infusion. Energetic summation of portal net release was not significantly different between the three SCFA infusions, although it tended to be lower with high-butyrate infusion. This may be related to the higher trophic effect of butyrate on the digestive mucosa.

Sheep: Short-chain fatty acids: Portal fluxes: Amino acids

The high oxidative activity of portal-drained viscera (PDV) contributes 15–25 % of whole-animal energy maintenance expenditure in ruminants (for review see Huntington, 1990). Changes in intake result in variations in weight of PDV and thus contribute to changes in the maintenance requirements of the animal 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. PDV metabolism appears to be closely related to the metabolizable energy (ME) intake but dietary factors involved in this relationship are not well known (for review see Goetsch, 1998). Increases in the filling of the digestive tract and in the amount of nutrients produced may both be considered to be responsible for the increase in PDV metabolism induced by the increase in intake. Moreover, the effects of the composition of rumen short-chain fatty acids (SCFA), which account for the main energy source absorbed in the portal vein and which vary considerably according to the nature of the diet, are still not well known due to the lack of direct comparisons. Intrarumen infusions of SCFA allow an increase in ME intake without change in the filling of the digestive tract. The aim of this present study was to determine whether the composition of ruminally-infused SCFA changes the amount and the composition of energy reaching the portal vein. Three different infusions were used, allowing rumen molar proportions of SCFA close to those obtained with high-forage, high-cereal or high-soluble-carbohydrate diets.

Materials and methods

Animals, feeding and infusions

Four adult (2·5 years), non-pregnant and non-lactating Lacaune ewes of 56·4 (SD 1·5) kg body weight were used. They were surgically fitted with a rumen cannula and with

Abbreviations: AA, amino acid; ME, metabolizable energy; NEFA, non-esterified fatty acid; PAH, *p*-aminohippuric acid; PDV, portal-drained viscera; SCFA, short-chain fatty acid.

* **Corresponding author:** Dr Pierre Nozière, fax +33 4 73 62 45 19, email noziere@clermont.inra.fr

long-term catheters in the portal vein, a mesenteric artery and a mesenteric vein as described by Ortigues *et al*. (1994). The animals were housed in an air-conditioned room in individual boxes (1·5 m^2). They were able to ingest the experimental diet 3 d after surgery. This diet consisted of 482 g DM/d of a first-cut of natural grassland hay, with ME and crude protein contents of 8·14 MJ and 89·5 g/kg DM respectively. This diet provided 53 and 54 % of their net energy and N requirements respectively, taken according to the French feeding systems (Institut National de la Recherche Agronomique, 1989). They also received 10 g mineral supplement/d (Ca 150, P 100 and Mg 20 g/kg) containing vitamins and trace elements and had free access to water and block salt. Hay was offered every 3 h in eight equal meals per d by an automatic feeder throughout all the experiment.

After 10 d to recover from surgery, each ewe received four intrarumen infusions (Table 1) in a 4×4 Latin square experimental design. Each infusion was maintained for 1 week with a flow rate of 3 l/d. The control infusion (C) consisted only of water. The other three infusions were prepared in a mineral buffer (36·5 g $NaHCO_3$/l, 19·0 g $KHCO_3$/l and 3·5 g NaCl/l). They were isoenergetic (0·64 kJ ME/ml) and their molar proportions of SCFA (acetate : propionate : butyrate) were 70 : 20 : 10 (infusion A), 13 : 77 : 10 (infusion P) and 0 : 20 : 80 (infusion B). The infusions were chosen in order that the amount of SCFA produced by the rumen fermentation of the hay plus the amount of SCFA infused should induce molar proportions of SCFA in the rumen of approximately 70 : 20 : 10 for infusion A (as with high-fibre diets), 50 : 40 : 10 for infusion P (as with high-cereal diets), and 50 : 20 : 30 for infusion P (as with high-soluble-carbohydrate diets).

Measurements

Digestibility, short-chain fatty acid production, rumen sampling and measurements. Digestibilities of DM, organic matter, neutral detergent fibre and acid detergent fibre were measured on five consecutive days by total faeces collection, starting 2 d after the beginning of the infusion (Laboratoire Cilal, St-Genès-Champanelle, France). For each treatment, rumen and blood sampling were carried out on the same day. To calculate the volume and the turnover rate of the rumen liquid phase, a pulse dose of CrEDTA (140 mg Cr in 50 ml water) was infused into the rumen at 08.55 hours. Rumen contents were sampled via the rumen cannula using a tube placed in the ventral sac at 08.50, 11.00, 13.00, 14.50, 17.00 and 23.00 hours the same day, 08.00 and 13.00 hours the following day. Cr in rumen contents was then measured by atomic absorption spectrophotometry (Binnerts *et al*. 1968). The pH was immediately measured on the samples taken at 08.50, 11.00, 13.00 and 14.50 hours, using a combination electrode. The osmolality was determined on two pooled samples (08.50 and 14.50 hours, 11.00 and 13.00 hours, i.e. before and 1–2 h after a meal respectively). SCFA concentration and composition were determined on the same pooled samples preserved with 0·1 volume of orthophosphoric acid (1 : 19 v/v) by GC using 4-methylvaleric acid as an internal standard (Jouany, 1982).

The SCFA production rate from hay degradation was measured for the control diet (C) on the same four ewes 1 week after the end of the Latin square. Because exchange of propionic acid C with other SCFA is quantitatively insignificant (Peters *et al*. 1990), [^{13}C]propionate was chosen as the reference to predict *in vivo* production rate of all SCFA. After sampling 30 ml rumen liquid (blank for analyses), a solution of [1-^{13}C]propionate (34·6 mg/h, i.e. 0·460 mmol [1-^{13}C]propionate/h, 99·9 % enriched; Leman, St Quentin en Yvelines, France) was continuously infused (130 ml/h) into the rumen for 24 h. After the beginning of the infusion (16 h), six samples of rumen contents (30 ml each) were taken every 1·5 h. The ^{13}C-enrichment of rumen propionate was determined on 2-chloroethyl esters of propionate by GC–isotope ratio mass spectrometry (Tetens *et al*. 1995; Kristensen *et al*. 1999).

Blood sampling and measurements. Animals were studied for 1 d per period, 5 or 6 d following the beginning of the infusion. The morning of the sampling day, *p*-aminohippuric acid (PAH) dissolved (100 g/l) in a physiological sterile saline solution (pH 7·4) was infused continuously via the mesenteric vein (7·2 ml/h) after a prime injection (2·25 ml) at 08.30 hours. Blood sampling started at 09.15 hours, i.e. 15 min after a meal, and was repeated at intervals of 30 min over two feeding cycles. At each sampling time, blood samples were taken from mesenteric artery and portal vein (7 ml each) using syringes containing EDTA-K as anticoagulant. Immediately after sampling, blood syringes were gently inverted to mix avoiding haemolysis, and

Table 1. Intrarumen infusion rate of short-chain fatty acids (SCFA) (mmol/h), metabolizable energy (ME) (kJ/h) and water (ml/h) (Means with standard errors for four ewes)

		SCFA infusion†				Contrasts	
	C	A	P	B	SE	Level	Composition
Acetate	0·00^{a}	43·03^{b}	7·25^{c}	0·00^{a}	1·60	***	***
Propionate	0·00^{a}	13·78^{b}	44·51^{c}	8·93^{d}	1·73	***	***
Butyrate	0·00^{a}	6·58^{b}	5·74^{b}	34·18^{c}	0·88	***	***
ME	0·00^{a}	73·16^{b}	87·18^{b}	88·53^{b}	4·83	***	*
Water	114·2^{a}	119·4^{a}	133·1^{a}	137·6^{a}	7·6	NS	NS

^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P < 0·10$).
***$P < 0·001$; *$P < 0·10$; NS, not significant ($P > 0·10$).
† For details of infusions see p. 522.

packed cell volume was determined in triplicate by centrifuging blood in capillary tubes. NH_3, urea and PAH were determined in blood by the phenol–hypochlorite (Weatherburn, 1967), the diacetylmonoxime (Marsh *et al*. 1965) and the *N*-α–naphtyl ethylene diamine 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 β-hydroxybutyrate (Barnouin *et al*. 1986) in blood deproteinized by 2 volumes of $HClO_4$ (0·6 N), of 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 an extraction derived from Brighenti (1997) by deproteinization with 0·26 volumes of metaphosphoric acid (400 g/l) (Prolabo, Fontenay-sous-Bois, France), SCFA were analysed in blood by GC with 2-ethylbutyric acid as internal standard. The chromatograph was a GC 8000 with a AS 800 autosampler (Fisons Instruments, Milan, Italy), a split/splitless injector in the splitless mode operated at 170° and an FID detector operated at 240°. The column was 30 m × 0·53 mm × 1 μm (HP-FFAP, Hewlett Packard, Waldbroom, Germany), programmed to increase up to 140° at a rate of 25°/min after 1 min at 85°. He gas was used as a carrier. After deproteinization with 0·1 volume of sulfosalicylic acid (400 g/l), deproteinized blood was conserved at −80° pending analysis of amino acids (AA) by ion-exchange chromatography using an autoanalyser (Beckman Instruments, Palo Alto, CA, USA) with D-glucosaminic acid as internal standard. Since two molecules of cysteine are rapidly oxidized into one molecule of cystine, cystine concentration was determined and multiplied by two to give cysteine concentration.

Calculations and statistical analyses

The volume and the turnover rate of the rumen liquid phase was calculated from the exponential decrease of Cr concentrations with time. After semi-logarithmic linearization, the slope represented the turnover rate (per h), and the volume of liquid phase in the rumen was calculated as the amount of Cr infused/$\exp^{(\text{intercept at } t=0)}$.

Production rate of propionate (mmol/h) was calculated according to the following equation:

$$(\text{AF infusate} - \text{AF background}) \times \text{infusion rate}/((\text{AF rumen} - \text{AF background}) \times 3),$$

where AF is the atomic fraction of $^{13}C = {}^{13}C/({}^{12}C + {}^{13}C)$ and infusion rate is expressed in mmol/h. Production rates of acetate and butyrate were respectively obtained by multiplying the propionate production rate by the concentration ratios acetate : propionate and butyrate : propionate.

Blood (or plasma) flow (F, l/h) through PDV was calculated as described by Katz & Bergman (1969):

$$F = I/(PAH_{pv} - PAH_a),$$

where I is PAH infusion rate (g/h) and PAH_{pv}, PAH_a are the concentrations of PAH (g/l) in the portal vein and arterial blood (or plasma) respectively. As all PAH is recovered in the plasma fraction, concentrations of PAH in plasma (PAH_p) were derived from blood values (PAH_b) and packed cell volume (PCV (%)), as described by Nozière *et al*. (1998):

$$PAH_p = (100 \times PAH_b)/(100 - PCV).$$

Net flux of metabolites (NF, mmol/h) through PDV were calculated as described by Katz & Bergman (1969):

$$NF = F\,(M_{pv} - M_a),$$

where F is blood (or plasma) flow, M_{pv} and M_a are concentrations of metabolites (mM) in the portal vein and arterial blood (or plasma) respectively. Positive net fluxes represented net release in the portal vein, whereas negative net fluxes represented net uptake by PDV.

Summations of net portal energy fluxes were based on heats of combustion of 876 (acetate), 1528 (propionate), 2310 (4-C SCFA and β-hydroxybutyrate), 2838 (5-C SCFA), 1368 (lactate) and 2001 (amino acids) kJ ME/mole.

As no significant effects of sampling time were observed on any variables, data were analysed by ANOVA with animal, period and composition of infusion as the main factors, with means per animal, per treatment as the experimental unit. No period effect was evidenced for any variables. Differences between the four treatments were examined by the Student-Newman-Keuls *t* test. The effects of energy level (treatment C *v*. A, P and B) and of energy composition (treatments A *v*. P, A *v*. B, B *v*. P) were assessed by contrast analysis. Significance was declared at $P < 0{\cdot}10$. All analyses were performed using the GLM procedure of SAS (version 6.03 (1988), Statistical Analysis Systems Inc., Cary, NC, USA).

Results

Digestibility, rumen variables and short-chain fatty acid production rate

Diet digestibility was similar among the four treatments (Table 2), on average 56·6, 58·5, 57·0 and 52·2 % for DM, organic matter, neutral detergent fibre and acid detergent fibre respectively. Both rumen pH and osmolality increased with infusions of SCFA (Table 3). The increase in osmolality was similar with A, P and B infusions, from 201 to an average of 307 mosmol/l for the three infusions of SCFA. Conversely, pH was higher with the infusions P and B (7·16 on average) than with infusion A (6·88). The volume of the rumen liquid phase increased with infusions of SCFA, irrespective of their composition, from 5·50 to an average of 6·84 litres for the three infusions of SCFA. The turnover rate increased with infusion B. The rumen concentration of NH_3 decreased from 3·02 to an average of 1·08 mmol/l for the three infusions of SCFA. The rumen concentrations of acetate, propionate and butyrate increased linearly with their infusion rate (*R* 0·91, 0·98 and 0·98 and a slope of 0·62, 0·66 and 0·63 respectively, data not shown). These relationships were similar with or without the data of the control treatment. The molar proportions of acetate : propionate : butyrate in all the SCFA were, as expected, close to 70 : 20 : 10 with infusions C and A, 50 : 40 : 10 with infusion P, and 50 : 20 : 30 with infusion B. The rumen concentration of isoacids was not significantly modified by infusion of

Table 2. Diet digestibility of dry matter (DM), organic matter (OM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) (%)*

(Means with standard errors for four ewes)

	SCFA infusion†					Contrasts	
	C	A	P	B	SE	Level	Composition
DM	56·4	57·9	55·4	56·7	2·1	NS	NS
OM	58·4	60·1	57·4	58·2	2·0	NS	NS
NDF	57·6	57·1	56·2	56·9	2·2	NS	NS
ADF	54·9	57·1	53·6	55·0	2·7	NS	NS

SCFA, short-chain fatty acid; NS, not significant ($P > 0{\cdot}01$).
* For details of composition of diet see p. 522.
† For details of infusions see p. 522.

SCFA, and were on average 0·58 mmol/l for isobutyrate and 0·32 mmol/l for isovalerate. Conversely, the rumen concentration of valerate increased with infusion of SCFA, and was the highest with infusions B and P.

The rumen SCFA production rate on the control diet (C), measured *in vivo* by a continuous infusion of [^{13}C]propionate, was 90·0 (SD 7·9), 23·1 (SD 2·1) and 8·8 (SD 1·0) mmol/h for acetate, propionate and butyrate respectively (Table 3).

Arterial concentrations

The arterial concentrations of acetate, propionate and butyrate increased linearly with their infusion rate (Table 4) (R 0·84, 0·83 and 0·98 and a slope of $6{\cdot}1 \times 10^{-3}$, $0{\cdot}9 \times 10^{-3}$ and $0{\cdot}9 \times 10^{-3}$ respectively, data not shown). Conversely, the arterial concentrations of minor SCFA did not vary between treatments. The arterial concentration of β-hydroxybutyrate was not modified by infusion A, but was decreased by infusion P and increased by infusion B. The arterial concentrations of NH_3 and glucose were similar among treatments, averaging 0·22 mmol/l blood and 3·51 mmol/l plasma respectively. Urea concentration decreased with SCFA infusions, irrespective of their composition, from 1·95 to 1·13 mmol/l blood. Arterial concentration of lactate increased with infusion P. Arterial concentration of NEFA decreased with infusions A and P. SCFA infusions induced a decrease in arterial concentration of AA. The decrease concerned mostly nonessential AA (glutamine, ornithine, glycine and asparagine) but also essential AA (methionine, lysine, arginine) and 3-methylhistidine. It was irrespective of the SCFA infused for most AA, but was more marked for glutamine and less marked for glycine and alanine with infusion B, and less marked for arginine with infusion A.

Portal blood flow and nutrient net fluxes

The portal blood and plasma flow rates were not affected by treatment, averaging 140 and 106 l/h respectively (Table 5).

Table 3. Physicochemical variables, ammonia, short-chain fatty acid (SCFA) concentration, molar proportion and production rate in the rumen

(Means with standard errors for four ewes)

	SCFA infusion‡					Contrasts	
	C	A	P	B	SE	Level	Composition
pH	6·58^{a}	6·88^{b}	7·13^{c}	7·18^{c}	0·05	***	**
Osmolality (mosmol/l)	201^{a}	305^{b}	317^{b}	299^{b}	8	***	NS
Volume (litres)	5·50^{a}	7·23^{a}	6·42^{a}	6·88^{a}	0·57	†	NS
Turnover rate (%/h)	6·02^{a}	6·41^{a}	8·38^{ab}	9·67^{b}	0·96	†	*
Ammonia (mmol/l)	3·02^{a}	1·74^{b}	0·91^{b}	0·58^{b}	0·36	**	*
SCFA (mmol/l)							
Acetate	37·04^{a}	65·28^{b}	44·78^{a}	39·88^{a}	2·35	***	***
Propionate	9·55^{a}	18·17^{b}	39·41^{c}	17·12^{b}	1·41	***	***
Isobutyrate	0·54^{a}	0·58^{a}	0·64^{a}	0·55^{a}	0·04	NS	NS
Butyrate	3·64^{a}	7·90^{b}	7·54^{b}	25·56^{c}	0·50	***	***
Isovalerate	0·30^{a}	0·32^{a}	0·32^{a}	0·34^{a}	0·05	NS	NS
Valerate	0·25^{a}	0·29^{a}	0·38^{b}	0·43^{b}	0·03	*	*
Molar proportion of SCFA (%)							
Acetate	72·20^{a}	70·53^{a}	48·15^{b}	47·05^{b}	0·01	***	***
Propionate	18·55^{a}	19·65^{a}	42·38^{b}	20·55^{a}	0·01	***	***
Butyrate	7·10^{a}	8·53^{a}	8·08^{a}	30·85^{b}	0·01	***	***
Production rate of SCFA (mmol/h)							
Acetate	90·0	–	–	–	–	–	–
Propionate	23·1	–	–	–	–	–	–
Butyrate	8·8	–	–	–	–	–	–

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0{\cdot}10$).
***$P < 0{\cdot}001$; **$P < 0{\cdot}01$; *$P < 0{\cdot}05$; † $P < 0{\cdot}10$; NS, not significant ($P > 0{\cdot}10$); –, not determined.
‡ For details of infusions see p. 522.

Table 4. Arterial concentrations of nutrients (mmol/l)
(Means with standard errors for four ewes)

		SCFA infusion‡				Contrasts	
	C	A	P	B	SE	Level	Composition
Blood							
Acetate	0·587[a]	0·814[b]	0·592[a]	0·527[a]	0·020	†	***
Propionate	0·011[a]	0·020[a]	0·051[b]	0·023[a]	0·004	**	**
Isobutyrate	0·003[a]	0·003[a]	0·004[a]	0·002[a]	0·001	NS	NS
Butyrate	0·002[a]	0·005[a]	0·005[a]	0·031[b]	0·001	***	***
Isovalerate	0·001[a]	0·001[a]	0·001[a]	0·001[a]	<0·001	NS	†
Valerate	0·001[a]	0·001[a]	0·001[a]	0·001[a]	<0·001	NS	NS
β-Hydroxybutyrate	0·244[a]	0·237[a]	0·107[b]	0·428[c]	0·020	NS	***
Ammonia	0·219[a]	0·194[a]	0·206[a]	0·237[a]	0·032	NS	NS
Urea	1·952[a]	1·162[a]	0·979[a]	1·269[a]	0·307	*	NS
Amino acids	2·734[a]	2·235[b]	2·220[b]	2·482[ab]	0·129	*	NS
Plasma							
Glucose	3·469[a]	3·525[a]	3·622[a]	3·410[a]	0·128	NS	NS
Lactate	0·367[a]	0·568[a]	0·873[b]	0·424[a]	0·106	†	*
NEFA	0·377[a]	0·272[b]	0·236[b]	0·351[a]	0·024	*	**
Blood							
Threonine	0·082[a]	0·058[a]	0·077[a]	0·071[a]	0·011	NS	NS
Valine	0·178[a]	0·159[a]	0·161[a]	0·158[a]	0·015	NS	NS
Methionine	0·013[a]	0·011[a]	0·011[a]	0·011[a]	0·001	*	NS
Isoleucine	0·085[a]	0·073[a]	0·069[a]	0·081[a]	0·005	NS	NS
Leucine	0·105[a]	0·091[a]	0·094[a]	0·094[a]	0·008	NS	NS
Phenylalanine	0·037[a]	0·035[a]	0·037[a]	0·036[a]	0·002	NS	NS
Lysine	0·184[a]	0·125[a]	0·127[a]	0·135[a]	0·018	*	NS
Histidine	0·082[a]	0·069[a]	0·071[a]	0·066[a]	0·008	NS	NS
Arginine	0·049[a]	0·046[a]	0·038[b]	0·038[b]	0·002	**	*
Essential AA	0·816[a]	0·667[b]	0·685[b]	0·688[b]	0·054	†	NS
Aspartate	0·021[a]	0·025[a]	0·026[a]	0·023[a]	0·003	NS	NS
Serine	0·073[a]	0·066[a]	0·072[a]	0·079[a]	0·010	NS	NS
Asparagine	0·043[a]	0·035[b]	0·037[ab]	0·038[ab]	0·002	*	NS
Glutamate	0·208[a]	0·230[a]	0·199[a]	0·225[a]	0·017	NS	NS
Glutamine	0·219[a]	0·162[b]	0·153[bc]	0·129[c]	0·009	***	*
Glycine	0·971[a]	0·702[b]	0·673[b]	0·955[a]	0·069	*	*
Alanine	0·157[a]	0·177[a]	0·198[a]	0·159[a]	0·012	NS	*
Tyrosine	0·041[a]	0·035[a]	0·040[a]	0·037[a]	0·002	NS	†
Ornithine	0·176[a]	0·128[a]	0·125[a]	0·138[a]	0·014	*	NS
Cysteine	0·009[a]	0·011[a]	0·010[a]	0·011[a]	0·002	NS	NS
Nonessential AA	1·918[a]	1·568[b]	1·535[b]	1·793[ab]	0·096	*	†
3-Methylhistidine	0·054[a]	0·043[b]	0·039[b]	0·042[b]	0·002	***	NS

SCFA, short-chain fatty acid; NEFA, non-esterified fatty acid; AA, amino acid.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P < 0·10$).
***$P < 0·001$; **$P < 0·01$; * $P < 0·05$; † $P < 0·10$; NS, not significant ($P > 0·10$).
‡ For details of infusions see p. 522.

The portal net release of acetate and propionate increased linearly with their infusion rate (R 0·66 and 0·87 and a slope of 0·74 and 0·67 respectively, data not shown). Linear and curvilinear regressions both indicated a portal recovery of 0·31 for butyrate (R 0·96 and 0·92 respectively, data not shown). The relationships between infusion rate and portal net release were the same with or without data from the control treatment, except for acetate, for which the closest relationship (R 0·74) was observed without data from the control treatment (slope 0·84). Net portal release of valerate increased with SCFA infusions but this was significant only with infusion B. Net fluxes of isobutyrate, isovalerate and NEFA did not vary between treatments, averaging 0·41, 0·27 and –0·59 mmol/h respectively. Net release of β-hydroxybutyrate and net uptake of glucose increased with SCFA infusions, irrespective of their composition, from 2·88 to 4·60 mmol/h for β-hydroxybutyrate and from 10·77 to 14·53 mmol/h for glucose. Net release of NH_3 and net uptake of urea decreased with SCFA infusions, from 8·32 to 4·64 and from 10·55 to 6·93 mmol/h respectively, irrespective of the SCFA infused. Net release of lactate decreased with infusion B. Portal net fluxes of AA indicated a net uptake of AA by PDV with the control treatment, mostly due to nonessential AA (glycine, glutamate, glutamine). The net uptake of AA decreased with SCFA infusions, and shifted to a net release with infusions A and P. This was related to an increase in net release of essential AA (isoleucine, leucine) and tyrosine and to a decrease in net uptake of nonessential AA such as glutamate but not of glutamine. For all AA, net fluxes were similar with infusions A and P. Conversely, a lower net release of most essential (particularly isoleucine, leucine, phenylalanine), and non-essential (particularly asparagine, tyrosine) AA, and a lower net uptake of glutamine were observed with infusion B.

Summation of net portal energy fluxes increased with SCFA infusions, irrespective of their composition, from

Table 5. Portal flows (l/h), net fluxes of nutrients (mmol/h) and energetic summation (kJ/h)
(Means with standard errors for four ewes)

		SCFA infusion‡				Contrasts	
	C	A	P	B	SE	Level	Composition
Blood flow	135·4[a]	149·0[a]	136·4[a]	139·7[a]	10·0	NS	NS
Plasma flow	104·9[a]	113·8[a]	102·0[a]	104·6[a]	8·0	NS	NS
Net fluxes							
Acetate	58·73[a]	86·38[b]	52·56[a]	49·36[a]	7·36	NS	**
Propionate	15·46[a]	24·86[b]	47·32[c]	23·02[b]	2·31	***	***
Isobutyrate	0·39[a]	0·40[a]	0·42[a]	0·43[a]	0·06	NS	NS
Butyrate	0·58[a]	2·02[a]	1·84[a]	10·77[b]	0·78	***	***
Isovalerate	0·24[a]	0·29[a]	0·28[a]	0·25[a]	0·05	NS	NS
Valerate	0·08[a]	0·10[a]	0·12[a]	0·22[b]	0·02	*	**
β-Hydroxybutyrate	2·88[a]	5·17[b]	4·06[ab]	4·56[ab]	0·58	*	NS
Glucose	–10·77[a]	–16·06[b]	–12·65[ab]	–14·89[ab]	1·33	*	NS
Lactate	10·35[ab]	10·15[ab]	11·77[a]	5·07[b]	1·63	NS	*
NEFA	–0·27[a]	0·36[a]	1·16[a]	0·05[a]	0·83	NS	NS
Ammonia	8·32[a]	5·46[ab]	3·35[b]	5·11[ab]	1·28	*	NS
Urea	–10·55[a]	–8·09[a]	–5·88[a]	–6·83[a]	1·70	†	NS
Amino acids	–7·10[a]	5·72[a]	3·23[a]	–1·54[a]	3·76	†	NS
Energetic summation	99·85[a]	158·50[b]	159·03[b]	126·11[ab]	13·74	***	NS
Threonine	0·22[a]	0·47[a]	0·47[a]	0·15[a]	0·14	NS	NS
Valine	–0·03[a]	0·55[a]	0·51[a]	0·19[a]	0·24	NS	NS
Methionine	0·06[a]	0·13[a]	0·09[a]	0·07[a]	0·02	NS	†
Isoleucine	0·04[a]	0·46[b]	0·40[b]	0·17[a]	0·07	**	*
Leucine	0·12[a]	0·74[b]	0·74[b]	0·26[a]	0·12	**	*
Phenylalanine	0·30[a]	0·44[a]	0·49[a]	0·14[a]	0·10	NS	*
Lysine	0·09[a]	1·52[a]	0·60[a]	–0·32[a]	0·61	NS	†
Histidine	–0·19[a]	0·04[a]	0·18[a]	–0·27[a]	0·17	NS	†
Arginine	–0·01[a]	0·01[a]	0·27[a]	–0·22[a]	0·18	NS	†
Essential AA	0·60[a]	4·36[b]	3·74[b]	0·19[a]	0·96	†	*
Aspartate	–0·05[a]	–0·21[a]	0·50[a]	–0·06[a]	0·34	NS	NS
Serine	0·74[a]	1·04[a]	0·86[a]	0·48[a]	0·19	NS	†
Asparagine	0·45[a]	1·03[a]	0·63[a]	0·10[a]	0·25	NS	*
Glutamate	–3·87[a]	–0·99[b]	–1·29[b]	–1·06[b]	0·51	**	NS
Glutamine	–1·76[ab]	–2·23[ab]	–3·30[b]	–1·26[a]	0·47	NS	*
Glycine	–3·90[a]	0·26[a]	0·05[a]	–1·13[a]	1·78	NS	NS
Alanine	1·19[a]	1·87[a]	1·67[a]	0·90[a]	0·38	NS	NS
Tyrosine	0·08[a]	0·33[b]	0·25[ab]	0·11[a]	0·06	*	*
Ornithine	–0·55[a]	0·16[a]	0·04[a]	0·13[a]	0·35	NS	NS
Cysteine	–0·03[a]	0·09[a]	0·07[a]	0·05[a]	0·11	NS	NS
Nonessential AA	–7·70[a]	1·36[b]	–0·51[b]	–1·73[b]	3·18	†	NS

SCFA, short-chain fatty acid; NEFA, non-esterified fatty acid.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P < 0·10$).
***$P < 0·001$; **$P < 0·01$; * $P < 0·05$; † $P < 0·10$; NS, not significant ($P > 0·10$).
‡ For details of infusions see p. 522.

99·9 kJ/h with the control treatment to an average of 147·9 kJ/h with treatments A, P and B (Table 5).

Discussion

Due to the low N content of the diet in each treatment, it is of importance to emphasize the lack of period effect on any variable measured, and particularly on AA arterial concentrations and portal net fluxes. Together with the normal comportment of animals, this suggests that no interactive effect of recovery from surgery occurred.

Digestibility, rumen variables and short-chain fatty acid production rate

To ensure a normal rumen motility and rumination, several authors have used semi-fasted animals (Krehbiel *et al*. 1992; Seal & Parker, 1994; Kristensen *et al*. 1996) instead of total intragastric nutrition (Weekes & Webster, 1975; Gross *et al*. 1990*a*,*b*) in order to study the PDV metabolism. A main concern in this situation is whether the basal production of rumen SCFA is the same in all treatments. In the present study, infusions were maintained for 7 d in order to control the digestibility of the diet. Digestibility remained constant, suggesting a constant basal production rate of SCFA in all treatments. In agreement with this suggestion, the rumen concentration of isoacids, which were not infused, remained constant. The increase in pH induced by infusions of SCFA may thus not have altered the cellulolytic microbial ecosystem. The amount of microbial N associated with particles in the rumen (Shriver *et al*. 1986) and the microbial fibrolytic activity (Mould & Ørskov, 1983/84) were shown to be stable with pH varying between 7·0 and 6·2.

It can be assumed that the small increase in pH induced by buffer infused concomitantly with SCFA may not have modified SCFA fractional disappearance from the lumen

since it is relatively stable at a pH of between 6·0 and 7·5 (Bueno, 1972). Conversely, the increase in osmolality from 200 to 300 mosmol/l may have decreased the SCFA absorption rate. However, according to the equations reported by Tabaru *et al*. (1990), this decrease in absorption rate may only be 3 %/h for both acetate, propionate and butyrate. Moreover, the turnover rate of the rumen liquid phase was not strongly modified by infusions of SCFA. In consequence, the relationships between the rumen infusion rate and rumen concentration of infused SCFA were similar with and without the data of the control treatment. The slopes of these relationships indicated, in agreement with Kristensen *et al*. (1996), similar rumen disappearance for acetate, propionate and butyrate, assuming a steady state in the rumen. This is in agreement with the equal rumen disappearance rates for these three SCFA observed at rumen pH around 7 by Dijkstra *et al*. (1993).

Assuming an equal rumen disappearance rate for these three SCFA, their rumen production rate, estimated by [^{13}C]propionate isotope-dilution technique, and expressed in mmol/g DM intake, were 4·5, 1·2 and 0·4 for acetate, propionate and butyrate respectively. These values are close to those measured by both [^{14}C]acetate, propionate and butyrate infusions, in sheep fed on a forage diet at a comparable intake level to ours (Weller *et al*. 1967). Hogan & Weston (1967) reported a close and linear relationship between organic matter digested and total SCFA rumen production rate in sheep fed *ad libitum*, with a slope of 9·2 mmol SCFA/g organic matter digested. This value is close to that measured in the present study (11·4 mmol SCFA produced/g organic matter digested). According to Huhtanen & Jaakkola (1995), the ratio intercept : slope given by the linear relationship between the SCFA infusion rate and rumen concentration may reflect the rumen SCFA production rate. With our data, this calculation may underestimate the SCFA production rate compared with the isotope dilution technique (63 *v.* 90, 15 *v.* 23 and 6 *v.* 9 mmol/h for acetate, propionate and butyrate respectively). Unfortunately, no other direct comparison between these two techniques of measurement of the SCFA production rate has been made.

Portal blood flow

As previously reported (Huntington *et al*. 1983; Gross *et al*. 1990*a*,*b*; Krehbiel *et al*. 1992; Seal & Parker, 1994; Kristensen *et al*. 1996), portal blood flow was not modified by rumen infusion of SCFA. In conventionally-fed animals, it has been shown to vary positively with ME intake (for reviews see Huntington, 1990; Rémond *et al*. 1998) but variations appear lower under *v.* above maintenance (Lomax & Baird, 1983; P. Nozière, D. Rémond, L. Bernard & M. Doreau, unpublished results). It can be expected that the amount of energy infused in fasted or semi-fasted animals is insufficient to induce changes in portal blood flow, or that the filling of the digestive tract is a predominant factor regulating portal blood flow, explaining the lack of variation in infused animals. In agreement with this suggestion, an analysis of sixty data sets from seventeen available trials in which at least two levels of the same diet were fed to sheep or cattle showed that for the same variation in ME intake (in MJ/d per kg live weight$^{0·75}$), the increase in portal blood flow (in l/d per kg live weight$^{0·75}$) is more marked with high-forage (forage : concentrate ratio $>0·7$) than with high-concentrate (forage : concentrate ratio $<0·7$) diets (R. Bernard and P. Nozière, unpublished results). This may be related to a higher filling of the digestive tract with high-forage diets. The slopes of these relationships (173 and 96 respectively) are consistent with the one (134) reported by Rémond *et al*. (1998) from data obtained on sheep, without regard to the forage : concentrate ratio. However, the various dietary chemical and physical factors controlling gut development and blood flow in PDV have to be studied more clearly because they vary between the various organs in the digestive tract, and even between the various layers of the same organ (Barnes *et al*. 1986; Nozière *et al*. 1999*a*).

Short-chain fatty acids and β-hydroxybutyrate

In this present experiment, 16, 33 and 70 % of infused acetate, propionate and butyrate respectively, did not reach the portal vein. These values are close to the lowest values found in the literature, 18 to 33 % for acetate, 30 to 70 % for propionate, 74 to 90 % for butyrate, as reviewed by Rémond *et al*. (1995). It may be suggested that metabolism of SCFA in the ruminal wall was low due to the underfed state of animals.

The portal net release of β-hydroxybutyrate did not increase with the butyrate infusion rate, averaging 4·6 mmol/h with the three SCFA infusions. A limited portal net release of ketone bodies following rumen butyrate infusion has already been reported (Krehbiel *et al*. 1992; Kristensen *et al*. 1996). Since it may result from both net release from the stomachs and net uptake by mesenteric-drained viscera (Reynolds & Huntington, 1988), the limited portal release of β-hydroxybutyrate may be attributed to both a limited enzymic capacity or availability of cofactors for ketogenesis from butyrate in the stomachs, and to an increased uptake of β-hydroxybutyrate by mesenteric-drained viscera associated with the increase in arterial concentration of β-hydroxybutyrate with the high-butyrate infusion.

Glucose and lactate

Sparing glucose is a major mechanism involved in the adaptation of ruminants to undernutrition (Chilliard *et al*. 1998). Indeed, the arterial concentration of glucose did not vary between treatments, indicating that glucose homeostasis was affected neither by the undernutrition, nor by the composition of energy infused. In contrast with our results, several authors reported that increasing rumen butyrate supply induced a decrease in glucose arterial concentration (Krehbiel *et al*. 1992; Huhtanen *et al*. 1993; Miettinen & Huhtanen, 1996). This decrease in glycaemia was not related to hyperinsulinaemia, but may be related to a depressive effect of butyrate on gluconeogenesis from propionate, as observed in isolated sheep hepatocytes by Demigné *et al*. (1991). This is consistent with Eisemann & Huntington (1994) who showed no effect of hyperinsulinaemia on propionate removal from the liver. In the present study, the lack of variation in glycaemia may be due to the much lower amount of butyrate infused, which may be too low to depress the utilization of propionate for gluconeogenesis

in the liver. Although arterial supply was unchanged, the net uptake of glucose by PDV was higher with infusions of SCFA than with the control treatment. This may be due to an increased oxidation of glucose, which can be related to an insulinaemic response.

The higher arterial concentration of lactate in high-propionate infused ewes is in agreement with Casse *et al.* (1994) who reported that propionate infusion in the mesenteric vein increased net output of lactate from the liver in lactating cows. This may be related more to an increased production of lactate from propionate in the liver, than to a decrease in hepatic net uptake of lactate, since of the glucose precursors, lactate appears to have the lowest priority in fed ruminants (Reynolds, 1995). Lactate reaching the portal vein can be from various origins (lumen or artery) and precursors (propionate (Leng *et al.* 1967) or glucose (Weekes & Webster, 1975)). With the diet used in the present work, lactate absorbed from the lumen can be assumed as negligible. As observed by others (Casse *et al.* 1994; Seal & Parker, 1994), net portal output of lactate did not increase with the infusion of propionate, suggesting that lactate production from propionate did not increase in the ruminal wall or that uptake of lactate in the wall of mesenteric-drained viscera increased, in relation to the increase in arterial concentration of lactate. The decrease in portal net output of lactate observed with the high-butyrate infusion suggests an inhibitory effect of butyrate on lactate synthesis. This is in agreement with others (for review see Rémond *et al.* 1995) who reported that propionate activation to propionyl-coA is severely depressed *in vitro* in the presence of butyrate. Thus, it seems that lactate production from propionate in PDV is not negligible but may be limited.

Non-esterified fatty acids

During fasting, the oxidation of NEFA released by adipose tissue allows the sparing of glucose and AA as oxidative fuels (Chilliard *et al.* 1998). An extensive use of NEFA by peripheral tissues is thus observed in underfed ruminants. However, little data on net fluxes of NEFA through PDV are available. Krehbiel *et al.* (1992) observed no changes in portal net release of NEFA by increasing the rumen infusion rate of butyrate in steers. In the present study, although arterial concentration of NEFA was higher in non-infused ewes, reflecting lipolysis, net flux of NEFA across PDV did not differ from 0 with the four treatments, suggesting, in agreement with Lindsay (1993), that NEFA are not a direct source of energy for PDV.

Amino acids

Infusion of SCFA decreased arterial concentration of AA and particularly of glutamine and 3-methylhistidine. This may reflect a higher net release of AA by peripheral tissues in non-infused ewes, allowing provision of AA for maintenance of vital processes in other tissues. A decrease in arterial AA with infusion of SCFA was also obtained for α-amino-N with feeding sodium propionate (Harmon & Avery, 1987) or infusion of butyrate (Krehbiel *et al.* 1992), without any change in splanchnic net flux of α-amino-N (Krehbiel *et al.* 1992). These results suggest that infusions of SCFA increased the uptake of AA by peripheral tissues. Similarly, a decrease in portal net release of AA with feeding propionate or infusing butyrate was reported by Harmon & Avery (1987) and Krehbiel *et al.* (1992), indicating that infusions of SCFA increased the uptake of AA in PDV tissues as in peripheral tissues. This may be related to an insulinaemic response.

Net portal flux of AA showed a net uptake of AA by PDV in non-infused animals. Above maintenance, the portal net appearance of AA has been shown to decrease linearly when the level of intake of the same diet decreases (Huntington & Prior, 1985). More recently, we observed that the portal net flux of AA decreases more drastically under than above maintenance, reaching from net appearance above maintenance to net uptake in underfed animals (Nozière *et al.* 1999*b*). This suggests that in the underfed state, AA have to be used to sustain energy requirements of PDV, which is consistent with the results of the present study. The net uptake of AA with the control treatment was mostly due to nonessential AA, and particularly glutamate, glycine and glutamine. Net uptake of glutamate and glutamine by PDV is classically observed in ruminants and other species (for review see Huntington, 1990). Because amino groups released from oxidation of glutamate and glutamine are transmitted to alanine, serine and glycine, the net uptake of glycine appears surprising. This result suggests that glycine may be used by PDV in ewes submitted to energy underfeeding. Similarly, net portal flux of glycine has been shown to shift from net release to net uptake in ewes fed on 1·3 and 0·5 energy maintenance requirements respectively (Nozière *et al.* 1999*b*).

SCFA infusions decreased net uptake of total AA (except glutamine) by PDV, allowing a net portal release of total AA with high-acetate and high-propionate infusions. The main AA involved were glutamate, for which uptake was decreased, and isoleucine, leucine and tyrosine, for which net portal release was increased. This may be related to a decrease in the oxidation of isoleucine and leucine by PDV. Conversely, a decrease in portal net release of α-amino-N with feeding propionate or infusing butyrate was reported by Harmon & Avery (1987) and Krehbiel *et al.* (1992), indicating an increased use of AA by PDV. However, in these experiments, the basal diet covered more than energy maintenance requirements, in contrast with the basal diet used in our trial. According to the increase in rumen pH and osmolality and the decrease in rumen NH_3 concentration induced in the present experiment by infusions of SCFA, it can be expected that microbial synthesis was increased due to the increase in energy available for microbes with infusions of SCFA. This could have provided a higher amount of digestible AA from microbial origin in the small intestine, explaining the shift from portal net uptake of AA with the control treatment to net release in ewes receiving SCFA. Moreover, compared with infusions A and P, net release of essential AA and net uptake of glutamine were reduced with infusion B, suggesting a shift from glutamine to essential AA utilization by PDV with high-butyrate-producing diets. This may be related to a higher stimulating effect of butyrate, compared with acetate and propionate, on epithelial cell proliferation (Sataka & Tamate, 1978).

Ammonia and urea

As discussed earlier, infusion of SCFA may have improved the microbial synthesis in the rumen. In association with the low N level of the diet, this may explain the observed decrease in rumen concentration of NH_3. Since NH_3 is preferentially absorbed from the rumen by simple diffusion (Bergman, 1990), the decrease in portal net release of NH_3 with SCFA infusions was related to the decrease in the rumen NH_3 concentration. This may contribute to the decrease in urea synthesis in the liver, as shown by the drop in the arterial urea. Consequently, the transfer of urea from arterial blood to lumen was decreased.

Energetic summation of portal net release

In the present experiment, 61 % of ME intake was released in the portal vein with the control treatment. This value is in agreement with Huntington (1990) and Lindsay (1993) who reviewed the partition of ME intake during absorption in conventionally fed cattle or sheep: 52–82 % are released in the portal vein as SCFA, ketone bodies, lactate and AA, 11–24 % are oxidized during absorption, the remaining (8–37 %) corresponding to heat of fermentation, absorption of long-chain fatty acids, nucleic acids and peptides. However, there is little data on energetic summation of portal net release in infused animals, since most studies based on intragastric infusions focused on only few metabolites. Gross *et al*. (1990*b*), studying growing lambs maintained by total enteral infusions, reported that substitution of propionate by an isoenergetic abomasum infusion of glucose decreased the amount of ME released in the portal vein from 84 to 64 %. This may be related to the energy cost of glucose absorption. From data from Huntington *et al*. (1983), it can be calculated that 82 % of the ME (246 kJ/h) infused as acetate into the rumen of cows fed above maintenance was released in the portal vein as SCFA and lactate. Conversely, infusion of 250 mmol/h of butyrate (i.e. 578 kJ/h) in the rumen of steers fed at 1·25 × net energy maintenance requirements did not modify the amount of energy released in the portal vein (Krehbiel *et al*. 1992), suggesting that all energy infused was used by PDV during absorption. This may be related to a stimulating effect of SCFA, particularly butyrate, on epithelial cell proliferation, as observed *in vivo* in adult ruminants (Sakata & Tamate, 1978).

Of major concern in the present study is whether the composition of SCFA may modulate the amount of energy retained by the digestive tract. Our data show that the composition of SCFA does not significantly modify the amount of energy recovered in the portal vein, averaging 67, 64 and 50 % of the total ME intake infused for treatments A, P and B respectively. Although the portal recovery of ME tended to be lower with treatment B, it can be expected that SCFA may not induce higher PDV energy expenditure with high-soluble-carbohydrate diets than with high-forage or high-cereal diets. Indeed, in most cases, high-soluble-carbohydrate diets rarely induce such a high molar proportion of butyrate in the rumen as in this experiment (30 %). Moreover, the partition of energy between SCFA and their metabolites (β-hydroxybutyrate, lactate) on one hand, and AA on the other hand, was similar with all treatments, net portal flux of AA contributing less than 7 % of the net portal flux of ME, due to the N underfeeding.

In conclusion, 60 % of ME infused into the rumen as SCFA was recovered in the portal vein, and the composition of SCFA did not significantly modify the amount of energy supplied to portal blood. However, with high-butyrate infusion, compared with high-acetate or -propionate infusions, PDV may reduce its consumption of glutamine but increase its consumption of essential AA. This may be related to the trophic effect of butyrate on the digestive mucosa.

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