Lipid metabolism of liquid-associated and solid-adherent bacteria in rumen contents of dairy cows offered lipid-supplemented diets

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The lipid distribution and fatty acid (FA) composition of total lipids, polar lipids and free fatty acids (FFA) were determined in liquid-associated bacteria (LAB) and solid-adherent bacteria (SAB) isolated from the rumen contents of seven dairy cows fitted with rumen fistulas. Two experiments, arranged according to a 4 × 4 and 3 × 3 Latin Square design, were performed using two basal diets consisting of one part hay and one part barley-based concentrate, and five lipid-supplemented diets consisting of the basal diet plus (g/kg dry matter): 53 or 94 rapeseed oil, 98 tallow, 87 soya-bean oil or 94 palmitostearin. For all diets used, total lipids were 1·7–2·2 times higher in SAB than in LAB ($P < 0·05$); this probably resulted from a preferential incorporation of dietary FA adsorbed onto food particles. Addition of oil or fat to the diets did not modify the polar lipid content but increased the FFA content of SAB and LAB by 150%. Lipid droplets were observed in the cytoplasm in SAB and LAB using transmission electron microscopy, which suggested that part of the additional FFA was really incorporated into the intracellular FFA rather than associated with the cell envelope by physical adsorption. Linoleic acid was specifically incorporated into the FFA of SAB, which emphasized the specific role of this bacterial compartment in the protection of this acid against rumen biohydrogenation.

Rumen bacteria: Lipid metabolism: Lipid-supplemented diets

Lipid metabolism in the rumen is characterized by intense lipolysis, fatty acid (FA) hydrogenation and *de novo* lipid cellular synthesis by micro-organisms. In the last three decades, it has been the subject of numerous studies in vitro and in vivo which have been extensively reviewed by Harfoot (1981), Noble (1981) and Moore & Christie (1984).

In the rumen contents, dietary lipids, especially triacylglycerols and the unesterified FA produced by microbial lipolysis, have been shown to be strictly adsorbed onto the surface of rumen particulate matter by hydrophobic interactions and, inversely, to be absent in the liquid-phase of digesta (Lough, 1970).

Such a specific association of lipid to feed particles may influence the lipid metabolism of rumen bacterial populations. These bacterial populations may be divided into three main compartments, i.e. bacteria associated with the liquid phase and bacteria either loosely or firmly attached to feed particles (Czerkawski, 1986; Legay-Carmier & Bauchart, 1989). These bacterial populations differ in their enzymic properties (Williams & Strachan, 1984) and their chemical composition. In particular, lipid and fatty acid concentrations are twice as high in solid-adherent bacteria (SAB) as in liquid-associated bacteria (LAB) (Merry & McAllan, 1983; Legay-Carmier & Bauchart, 1989). However, the lipid and FA compositions of rumen bacteria have been determined mainly in vitro using pure cultures (Ifkovits & Ragheb, 1968; Viviani, 1970; Emmanuel, 1978; Miyagawa *et al.* 1979; Miyagawa & Suto, 1980), whereas the available information obtained in vivo from mixed populations harvested from rumen contents refers only to LAB populations (Katz & Keeney, 1966; Williams & Dinusson, 1973; Czerkawski, 1976).

There have been attempts to use large amounts of unprotected lipids as energy sources

in concentrates for dairy cows in early lactation (Czerkawski & Clapperton, 1984; Palmquist, 1984). This practice may have important consequences for the lipid metabolism of each different rumen bacterial population. Therefore, the aim of the present work was first to describe precisely, in two 4×4 and 3×3 Latin Square design experiments, the lipid distribution and the fatty acid composition of SAB and LAB populations in rumen contents of dairy cows, and second to investigate the effects of the addition to the diet of various sources of unprotected fats or oils characterized by different degrees of unsaturation of the acyl chains.

Part of the present work was presented at the 2nd Animal meeting of the French National Institute of Agronomical Research (INRA) on Feeding and Nutrition of Herbivores (Paris, March 1986) and was published as an abstract (Legay-Carmier *et al.* 1987).

MATERIALS AND METHODS

Animals and diets

Two experiments were performed using seven high-yielding Holstein × Friesian dairy cows each fitted with a rumen cannula. At the beginning of Expt 1, animals were in their 7th or 8th week of lactation and produced 25–33 kg milk/d, whereas in Expt 2, cows were in their 15th or 16th week of lactation with a production of 22–28 kg milk/d.

Expts 1 and 2 were arranged according to a 4×4 and a 3×3 Latin Square design respectively. Treatments were three concentrate mixtures in Expt 1 (control, C1; tallow, T; rapeseed oil, R5 and R10) and two concentrate mixtures in Expt 2 (control, C2; soya-bean oil, S; palmitostearin, P).

Seven diets provided by Dievet Society (27100, le Vaudreuil, France) were used. The basal diets consisted, on a dry matter (DM) basis, of 500 g second-cut natural-grass hay and 500 g concentrate mixtures (C1 and C2 of average composition in g/kg: 450 beet pulp, 230 barley, 120 soya-bean meal, 120 rapeseed meal, 30 molasses, 10 urea, 22 calcium hydrogen phosphate, 4 magnesium sulphate, 5 sodium chloride, 9 vitamin and mineral mixes). The experimental diets consisted of the same basal diets to which were added (g/kg DM diet): 53 (R5) or 94 (R10) rapeseed oil, 98 animal tallow (T), 87 soya-bean oil (S) or 94 palmitostearin (P) (Tables 1 and 2). The vegetable oils were stabilized by an antioxidant, butylated hydroxytoluene (2·6-ditert-butyl-*p*-cresol, 0·5 g/kg DM diet). Calcium chloride (2 g/kg DM diet) was added to the lipid-supplemented diets.

Diets were given in two equal portions at 08.00 and 16.00 hours, the concentrates being offered before the hay. DM allowances were calculated according to the estimated individual metabolizable energy requirements (INRA, 1978). Mean DM intakes amounted to 15·0 (C1), 15·1 (R5), 16·4 (R10) and 16·0 (T) kg/d in Expt 1 and 12·8 (C2), 13·3 (S) and 15·1 (P) kg/d in Expt 2. Animals had continuous access to mineralized salt and water. Experiments started with an adaptation period of 3 weeks followed by a 10–15 d period of sampling rumen contents.

Isolation of bacteria from rumen contents

Rumen contents (1200 g fresh material) were sampled representatively from three depths using a sampling tube (i.d. 50 mm; length 800 mm) just before feeding and at 2·0, 4·0 and 6·0 h after feeding, and were subsequently mixed.

Preparation and treatments of solid and liquid phases of the rumen contents were carried out as described by Legay-Carmier & Bauchart (1989).

Liquid and solid phases were separated by squeezing rumen contents through a wire gauze (1 mm mesh). The rumen fluid was then centrifuged at 500 ***g*** for 30 min at 4° to isolate residual particles. The latter were subsequently mixed with the solid material retained on the gauze to constitute total rumen particles. The supernatant fraction was centrifuged at

Table 1. *Expts 1 and 2. Mean dry matter (DM) concentration and DM composition of the hays and of the basal concentrates of the control and the experimental diets supplemented with unprotected lipids*

	Expt 1					Expt 2			
Diets...	Hay 1	C1	R5	R10	T	Hay 2	C2	S	P
DM (g/kg feed)	910	878	893	900	901	850	919	938	918
Composition of DM (g/kg)									
Organic matter	898	934	940	941	940	908	899	901	929
Gross energy (MJ/kg)	18·200	17·404	17·550	20·956	22·517	18·935	17·390	20·400	20·619
Net energy value*	0·82	1·02	1·14	1·28	1·28	0·84	0·99	1·24	1·28
Crude protein (N × 6·25)	129	222	202	185	187	160	197	164	195
Lipid	65	31	103	191	202	57	27	184	194
Fatty acid	20·0	19·0	90·9	168·2	181·7	16·9	15·6	162·1	174·5
Calcium	4·5	8·6	8·7	8·7	8·7	11·0	11·6	11·5	8·1

Hays 1 and 2, natural-grass hay; C1, C2, controls; R5, R10, rapeseed oil; T, tallow; S, soya-bean oil; P, palmitostearin; for details of diets, see p. 564.
* Feed units for lactation (Vermorel, 1978).

Table 2. *Expts 1 and 2. Diet long-chain fatty acid composition (weight % of total methyl esters) and daily total fatty acid (FA) intake of dairy cows offered control (C1 for Expt 1, C2 for Expt 2) and lipid-supplemented diets (R5, R10 and T for Expt 1; S and P for Expt 2)*

(Results of FA intake are mean values and standard deviations for four cows in Expt 1 and for three cows in Expt 2)

	Expt 1				Expt 2		
Diets*...	C1	R5	R10	T	C2	S	P
12:0	4·3	1·5	0·7	1·0	1·0	0·2	0·1
14:0	2·3	0·9	0·4	2·9	1·2	6·1	0·8
16:0	18·3	9·3	7·4	21·4	19·9	12·1	48·5
16:1	1·9	1·3	0·6	3·8	2·0	0·6	0·3
18:0	3·0	2·0	2·0	15·9	2·6	3·7	4·6
18:1	10·4	42·6	49·1	33·7	12·6	18·8	24·4
18:2*n*-6	25·5	22·5	22·6	7·4	25·3	50·0	12·3
18:3*n*-3	25·8	14·3	12·0	5·3	28·4	13·0	4·8
15:0+17:0	0·9	0·5	0·9	2·0	1·4	0·3	0·6
Sum of							
NSFA	29·5	15·0	12·3	44·0	26·7	16·9	54·2
BCFA	1·8	0·6	0·3	4·0	2·6	0·5	2·6
MUFA	14·7	46·1	52·3	39·5	17·6	21·0	25·5
PUFA	52·8	38·5	35·2	13·1	53·0	61·7	17·7
Total FA intake							
(g/24 h): Mean	292[a]	833[b]	1545[c]	1622[c]	206[a]	1192[bc]	1445[c]
SD	57	101	74	187	18	204	70

NSFA, normal saturated fatty acids; BCFA, branched-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[a,b,c] Mean values with different superscript letters were significantly different ($P < 0·05$).

* Expt 1: R5, R10, diets contained 53 and 94 g rapeseed oil/kg dry matter (DM); T, diet contained 98 g tallow/kg DM; Expt 2: S, diet contained 87 g soya-bean oil/kg DM; P, diet contained 94 g palmitostearin/kg DM; for details of diets, see p. 564.

27000 ***g*** for 40 min at 4° for isolation of liquid-associated bacteria (LAB1). With the purpose of isolating the free-floating bacteria of the liquid phase that had been retained on the particles (LAB2), total particles were washed by manual shaking for 1 min in saline solution (9 g sodium chloride/l; 1 kg fresh material/l). LAB2 were separated from total washed particles by successive centrifugations as described previously. LAB2 were mixed with LAB1 to give total LAB.

Total washed particles were chilled in an ice bath and homogenized in saline (100 g fresh material/320 ml) at 4°. Detachment of SAB was partially achieved first by storage at 4° for 6 h and second by subsequent pummelling of particles for 5 min in a Colworth Stomacher 400 (Stewart & Co. Ltd, London) followed by squeezing through layers of surgical cloth. The solid residues were rinsed twice with cold saline, the filtrates were then pooled and centrifuged at 500 ***g*** for 30 min to eliminate particles and the supernatant fraction was centrifuged at 27000 ***g*** for 40 min to isolate SAB.

Analytical methods

Food components and rumen bacteria were dried for 48 h at 80° for DM determination. Their total lipids were extracted from freeze-dried samples (1 g) in chloroform–methanol (2:1, v/v) according to the method of Folch *et al.* (1957) and subsequently in hexane–ethanol–hydrochloric acid (25:10:10, by vol.) (Bauchart *et al.* 1984) and determined gravimetrically.

Table 3. *Expts 1 and 2. Lipid composition (g/kg dry matter (DM)) of solid-adherent bacteria (SAB) and liquid-associated bacteria (LAB) of rumen contents of dairy cows offered control (C1 for Expt 1, C2 for Expt 2) and lipid-supplemented diets (R5, R10 and T for Expt 1; S and P for Expt 2)*

(Results are mean values for four cows in Expt 1 and for three cows in Expt 2)

Diets*...	Expt 1								Expt 2						
	C1		R5		R10		T		C2		S		P		
	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SEM
Free fatty acids	67^{d}	15^{a}	128^{ef}	26^{ab}	164^{f}	28^{b}	139^{f}	36^{bc}	61^{d}	18^{a}	152^{f}	42^{c}	113^{e}	42^{c}	8
Triacylglycerols	tr	tr	tr	tr	5^{ab}	2^{a}	6^{b}	2^{a}	tr	tr	10^{b}	3^{c}	9^{b}	3^{a}	2
Sterols	4^{a}	3^{a}	5^{ab}	3^{a}	5^{ab}	3^{a}	6^{b}	2^{a}	3^{a}	1^{a}	4^{a}	2^{a}	5^{ab}	2^{a}	2
Diacylglycerols	7^{ab}	7^{a}	10^{b}	5^{a}	14^{bc}	7^{ab}	16^{c}	9^{b}	10^{b}	3^{a}	14^{bc}	4^{a}	12^{bc}	4^{a}	3
Polar lipids	52^{a}	41^{a}	64^{ab}	38^{a}	50^{a}	53^{a}	65^{ab}	46^{a}	46^{a}	38^{a}	51^{ab}	40^{a}	67^{b}	38^{a}	10
Other lipids	28^{ab}	32^{ab}	33^{ab}	22^{a}	44^{b}	30^{ab}	47^{b}	39^{b}	34^{ab}	28^{ab}	14^{a}	22^{a}	26^{a}	23^{a}	7
Total lipids	158^{c}	95^{ab}	240^{d}	114^{b}	282^{e}	127^{b}	279^{e}	134^{bc}	154^{c}	88^{a}	245^{d}	113^{b}	232^{d}	112^{b}	11

tr, Trace.

[a–f] Mean values with different superscript letters were significantly different ($P < 0.05$).

* Expt 1: R5, R10, diets contained 53 and 94 g rapeseed oil/kg dry matter (DM); T, diet contained 98 g tallow/kg DM; Expt 2: S, diet contained 87 g soya-bean oil/kg DM; P, diet contained 94 g palmitostearin/kg DM; for details of diets, see p. 564.

Concentrations of free FA (FFA) and esterified lipids in bacteria (polar lipids, di- and triacylglycerols) and the FA composition of total lipids, FFA and polar lipids were determined by gas–liquid chromatography (GLC) using four internal standards esterified with heptadecenoic acid purified in our laboratory (Bauchart & Aurousseau, 1981). These lipid standards (phosphatidyl choline diheptadecenoyl, heptadecenoic acid, di- and triheptadecenoyl glycerol) were added to bacterial samples before lipid extraction. Lipids were separated by thin-layer chromatography (TLC) on lipid-free Kieselgel with hexane–diethyl ether–formic acid (80:20:2, by vol.) as the developing solvent. After re-extraction from the Kieselgel, lipid classes were saponified overnight in an ethanolic potassium hydroxide solution (100 g/l) and their fatty acids were methylated by boiling and refluxing the solvent for 0·5 h with methanol–HCl (30 ml/l).

Methyl esters were separated by GLC at 195° using a glass capillary column (i.d. 0·35 mm; length 25 m) coated with FFA phase. Details of chromatographic conditions and the methods of identification of the fatty acids have been previously reported (Bauchart & Aurousseau, 1981). No attempts were made to determine unusual and minor FA containing cyclopropane, cyclopropene and cyclopentene rings or hydroxylic and keto groups.

As with the analysis of the lipid classes, the FA content of food components or of bacteria was determined by GLC using the same chromatographic conditions described earlier, using heptadecenoic acid as the internal standard (Bauchart & Aurousseau, 1981).

The sterol content of bacteria was determined from total lipids by TLC–flame ionization detection using Iatroscan TH10 coupled with an electronic integrator (Enica 10; Delsi Instruments, Suresnes, France) with methyl heptadecanoate as the internal standard (Bauchart *et al.* 1985).

Bacterial samples for transmission electron microscopy were fixed in glutaraldehyde in 0·2 M-cacodylate buffer (30 g/l) pH 7·4 for 1 h at 4°. After washing in cacodylate buffer, samples were post-fixed in a solution of osmium tetroxide in 0·2 M-cacodylate buffer (10 g/l) pH 7·4 for 1 h at 4°. The sample, rinsed in cacodylate buffer, was added to agar–agar solution in water (5 g/l) and then chilled in an ice-bath; it was subjected to successive dehydrations in a series of ethanol–water solutions followed by absolute ethanol and ending with propylene oxide. Bacterial samples were embedded in epoxy resin. Polymerization was carried out successively for 2 h at 37°, and for 48 h at 60°. Sections (70 nm) were cut using a Reichert E ultramicrotome and stained in uranyl acetate and then in lead citrate. Observations were made in a Philips EM 400 transmission electron microscope at an accelerating voltage of 80 kV.

Treatment differences were tested statistically using the Mann–Whitney U test (Mann & Whitney, 1947). The lipid and FA compositions of rumen bacteria were compared by two-way analysis of variance with diet and sample as the independent variables.

RESULTS

FA intake

Total daily FA intake in Expts 1 and 2 and the proportions of individual main FA are shown in Table 2.

Normal saturated FA were mainly provided by diets P (783 g/d including 700 g palmitic acid (16:0)/d) and T (714 g/d including 347 g palmitic acid/d and 258 g stearic acid (18:0)/d). Monounsaturated FA, which comprised essentially oleic acid and its isomers (18:1), were supplied by diets R10 (808 g/d) and T (641 g/d) and, to a lesser extent, by diets R5 (348 g/d) and P (368 g/d). Linoleic (18:2*n*-6) and linolenic (18:3*n*-3) acids constituted

the main polyunsaturated FA provided by diets S (596 and 155 g/d respectively) and R10 (349 and 185 g/d respectively) (Table 2).

Bacterial lipid composition

The lipid compositions of purified SAB and LAB of rumen contents collected in equal amounts just before and 2, 4 and 6 h after feeding are reported in Table 3.

For all diets used in both Expts, total lipids (g/kg DM) were 1·7–2·2 times higher ($P < 0·05$) in SAB than in LAB. Polar lipids and FFA dominated the lipid moiety of LAB (56–73 % of total lipids) and SAB (69–82 %) for all diets. Polar lipids consisted in both bacterial populations of phospholipids (about 90 % of total polar lipids) and galactolipids (about 10 % of total polar lipids) as estimated from values obtained by TLC using acetone–acetic acid–water (100:2:1, by vol.) as the developing solvent (D. Bauchart and F. Legay-Carmier, unpublished results).

Addition of vegetable oils or animal fat to the diets did not modify the concentrations of polar lipids and minor lipid components (diacylglycerols and sterols) of SAB and LAB, whatever the quantity and composition of the FA ingested. Similarly, the other lipids mainly associated with bacterial membranes (waxes, steryl esters and lipophilic pigments), were not altered by addition of lipids to the diets (Table 3). This fraction (referred to as 'other lipids') and its concentration in bacteria was calculated by difference between total lipids and the sum of FFA, di- and triacylglycerols, sterols and polar lipids. However, the lipid addition elevated the FFA content of LAB and SAB significantly ($P < 0·05$), the maximal effect being observed in diets R10 and S (+150 % for SAB; +130 % for LAB; Table 3). Triacylglycerols, virtually absent from bacteria in the control diets, represented 2–4 % of total lipids in both bacterial populations with the lipid-supplemented diets (Table 3).

Visual evidence of lipid accumulation in SAB and LAB was provided by transmission electron microscopy of rumen bacteria harvested from animals fed on control (Plate 1 (*a* and *b*)) and lipid-supplemented diets (Plate 1 (*c*, *d*, *e* and *f*)). Large and numerous grey areas, probably corresponding to FFA inclusions in bacterial cytosol, were clearly and frequently observed in both bacterial populations in the experimental diets, but were totally absent from these populations in the control diets (Plate 1 (*a* and *b*)).

FA composition of bacteria

Total FA content and FA composition of SAB and LAB total lipids and their main constituents (polar lipids and FFA fractions) are given in Tables 4, 5 and 6.

In total lipids, FA content of SAB was 2·5–3·1 higher than that of LAB ($P < 0·05$) and increased for both populations to the same extent in lipid-supplemented diets (Table 4). Mean proportions of 16:0 varied similarly in SAB and LAB in parallel with the 16:0 content of the diets; thus, in diet P, 16:0 was observed to be markedly accumulated in both populations ($P < 0·05$). Mean proportions of 18:0 and 18:1 in SAB were 1·7 and 1·4 times higher ($P < 0·05$) than in LAB, except in diets S and P where they were similar. Mean proportions of 18:2 in SAB were similar to those of LAB, except for diet S where 18:2 accumulated specifically in SAB ($P < 0·05$). On the other hand, mean proportions of branched-chain FA (sum of iso and anteiso forms of saturated FA plus phytanic acid) in LAB were 1·3–4·8 higher ($P < 0·05$) than in SAB and decreased systematically with FA-rich diets (Table 4).

In polar lipids, FA contents in SAB were 1·2–1·7 times higher ($P < 0·05$) than in LAB, but were unchanged by the increase in the quantities of FA ingested (Table 5). Mean FA

Table 4. *Expts 1 and 2. Fatty acid composition (weight % total methyl esters) and total fatty acid content (g/kg dry matter (DM)) of total lipids in solid-adherent bacteria (SAB) and liquid-associated bacteria (LAB) of rumen contents in dairy cows offered control (C1 for Expt 1, C2 for Expt 2) and lipid-supplemented diets (R5, R10 and T for Expt 1; S and P for Expt 2)*

(Results are mean values for four cows in Expt 1 and for three cows in Expt 2)

	Fatty acid composition of total lipids of rumen bacteria (weight % total methyl esters)														
	Expt 1								Expt 2						
Diets*...	C1		R5		R10		T		C2		S		P		
	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SEM
12:0	$2{\cdot}2^{b}$	$4{\cdot}6^{c}$	$1{\cdot}7^{ab}$	$5{\cdot}0^{c}$	$0{\cdot}5^{a}$	$1{\cdot}2^{ab}$	$0{\cdot}4^{a}$	$2{\cdot}6^{b}$	$0{\cdot}1^{a}$	$2{\cdot}5^{b}$	$0{\cdot}1^{a}$	$0{\cdot}2^{a}$	$0{\cdot}1^{a}$	$0{\cdot}3^{a}$	0·8
14:0	$2{\cdot}0^{c}$	$4{\cdot}6^{c}$	$1{\cdot}2^{b}$	$4{\cdot}2^{c}$	$0{\cdot}9^{ab}$	$3{\cdot}6^{d}$	$2{\cdot}4^{c}$	$6{\cdot}9^{f}$	$1{\cdot}1^{b}$	$2{\cdot}8^{d}$	$0{\cdot}4^{a}$	$1{\cdot}4^{bc}$	$1{\cdot}0^{b}$	$2{\cdot}1^{c}$	0·3
16:0	$19{\cdot}5^{cd}$	$20{\cdot}9^{cd}$	$12{\cdot}2^{a}$	$16{\cdot}4^{b}$	$10{\cdot}2^{a}$	$16{\cdot}3^{b}$	$24{\cdot}2^{de}$	$21{\cdot}9^{d}$	$18{\cdot}8^{c}$	$24{\cdot}6^{e}$	$12{\cdot}3^{a}$	$15{\cdot}9^{b}$	$44{\cdot}2^{f}$	$42{\cdot}9^{f}$	1·3
16:1	$1{\cdot}1^{c}$	$2{\cdot}4^{e}$	$0{\cdot}7^{b}$	$1{\cdot}6^{d}$	$0{\cdot}6^{b}$	$1{\cdot}8^{d}$	$1{\cdot}9^{d}$	$3{\cdot}7^{f}$	$0{\cdot}3^{a}$	$1{\cdot}3^{cd}$	$0{\cdot}1^{a}$	$0{\cdot}5^{b}$	$0{\cdot}3^{a}$	$0{\cdot}3^{a}$	0·2
18:0	$35{\cdot}1^{e}$	$23{\cdot}7^{b}$	$35{\cdot}2^{e}$	$26{\cdot}4^{b}$	$33{\cdot}6^{c}$	$23{\cdot}6^{b}$	$32{\cdot}0^{c}$	$18{\cdot}8^{a}$	$39{\cdot}8^{d}$	$22{\cdot}2^{ab}$	$24{\cdot}3^{b}$	$28{\cdot}5^{bc}$	$26{\cdot}0^{b}$	$26{\cdot}9^{b}$	2·0
18:1	$16{\cdot}1^{b}$	$11{\cdot}0^{a}$	$28{\cdot}2^{d}$	$22{\cdot}2^{c}$	$34{\cdot}6^{e}$	$22{\cdot}5^{c}$	$21{\cdot}0^{c}$	$15{\cdot}5^{b}$	$15{\cdot}1^{b}$	$11{\cdot}8^{a}$	$33{\cdot}2^{c}$	$33{\cdot}9^{c}$	$13{\cdot}3^{ab}$	$11{\cdot}7^{a}$	1·3
18:2*n*-6	$6{\cdot}6^{d}$	$4{\cdot}9^{c}$	$5{\cdot}5^{c}$	$3{\cdot}4^{ab}$	$3{\cdot}5^{b}$	$3{\cdot}5^{b}$	$2{\cdot}6^{a}$	$2{\cdot}8^{a}$	$5{\cdot}6^{c}$	$6{\cdot}1^{cd}$	$13{\cdot}5^{c}$	$4{\cdot}1^{ab}$	$3{\cdot}8^{b}$	$3{\cdot}1^{ab}$	0·5
18:3*n*-3	$4{\cdot}2^{c}$	$2{\cdot}1^{c}$	$2{\cdot}6^{c}$	$1{\cdot}4^{b}$	$1{\cdot}9^{bc}$	$1{\cdot}4^{b}$	$2{\cdot}5^{c}$	$0{\cdot}9^{ab}$	$3{\cdot}7^{dc}$	$1{\cdot}7^{b}$	$4{\cdot}0^{c}$	$2{\cdot}5^{c}$	$2{\cdot}0^{c}$	$0{\cdot}7^{a}$	0·3
15:0+17:0	$3{\cdot}0^{bc}$	$5{\cdot}7^{e}$	$1{\cdot}8^{a}$	$3{\cdot}6^{cd}$	$1{\cdot}4^{a}$	$3{\cdot}9^{cd}$	$2{\cdot}4^{b}$	$3{\cdot}6^{cd}$	$3{\cdot}4^{c}$	$4{\cdot}4^{d}$	$1{\cdot}8^{ab}$	$2{\cdot}4^{b}$	$2{\cdot}1^{ab}$	$2{\cdot}3^{b}$	0·5
Sum of															
NSFA	$61{\cdot}9^{dc}$	$59{\cdot}7^{d}$	$52{\cdot}8^{c}$	$53{\cdot}3^{c}$	$47{\cdot}7^{b}$	$51{\cdot}3^{bc}$	$61{\cdot}6^{dc}$	$59{\cdot}7^{d}$	$64{\cdot}9^{e}$	$56{\cdot}9^{d}$	$39{\cdot}8^{a}$	$49{\cdot}4^{b}$	$74{\cdot}3^{f}$	$74{\cdot}9^{f}$	1·6
BCFA	$5{\cdot}6^{bc}$	$12{\cdot}2^{e}$	$3{\cdot}0^{ab}$	$7{\cdot}8^{cd}$	$1{\cdot}7^{a}$	$8{\cdot}1^{cd}$	$4{\cdot}0^{b}$	$9{\cdot}8^{d}$	$6{\cdot}5^{c}$	$14{\cdot}7^{e}$	$4{\cdot}8^{b}$	$6{\cdot}2^{c}$	$4{\cdot}1^{b}$	$6{\cdot}3^{c}$	0·9
MUFA	$19{\cdot}4^{c}$	$16{\cdot}7^{b}$	$31{\cdot}0^{e}$	$26{\cdot}1^{d}$	$37{\cdot}6^{f}$	$26{\cdot}0^{d}$	$24{\cdot}3^{d}$	$21{\cdot}3^{cd}$	$16{\cdot}9^{b}$	$14{\cdot}3^{ab}$	$35{\cdot}2^{f}$	$35{\cdot}0^{f}$	$14{\cdot}6^{ab}$	$12{\cdot}5^{a}$	1·4
PUFA	$13{\cdot}2^{c}$	$11{\cdot}4^{bc}$	$13{\cdot}1^{c}$	$12{\cdot}8^{c}$	$13{\cdot}0^{c}$	$14{\cdot}6^{c}$	$10{\cdot}2^{b}$	$9{\cdot}3^{ab}$	$11{\cdot}2^{bc}$	$14{\cdot}1^{c}$	$19{\cdot}9^{d}$	$9{\cdot}6^{ab}$	$6{\cdot}4^{a}$	$6{\cdot}3^{a}$	1·5
FA (g/kg DM)	105^{d}	43^{a}	171^{e}	60^{ab}	193^{f}	68^{b}	206^{fg}	84^{c}	90^{cd}	36^{a}	214^{g}	70^{bc}	168^{e}	67^{b}	10

NSFA, normal saturated fatty acids; BCFA, branched-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA fatty acids.
[a–g] Mean values with different superscript letters were significantly different ($P < 0{\cdot}05$).
* Expt 1: R5, R10, diets contained 53 and 94 g rapeseed oil/kg dry matter (DM); T, diet contained 98 g tallow/kg DM; Expt 2: S, diet contained 87 g soya-bean oil/kg DM; P, diet contained 94 g palmitostearin/kg DM; for details of diets, see p. 564.

Table 5. *Expts 1 and 2. Fatty acid composition (weight % total methyl esters) and total fatty acid content (g/kg dry matter (DM)) of polar lipids in solid-adherent bacteria (SAB) and liquid-associated bacteria (LAB) of rumen contents in dairy cows offered control (C1 for Expt 1, C2 for Expt 2) and lipid-supplemented diets (R5, R10 and T for Expt 1; S and P for Expt 2)*

(Results are mean values for four cows in Expt 1 and for three cows in Expt 2)

	Fatty acid composition of polar lipids of rumen bacteria (weight % total methyl esters)														
	Expt 1								Expt 2						
Diets*...	C1		R5		R10		T		C2		S		P		
	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SEM
12:0	$1{\cdot}8^{b}$	$3{\cdot}9^{c}$	$0{\cdot}3^{a}$	$2{\cdot}2^{b}$	$0{\cdot}2^{a}$	$2{\cdot}7^{bc}$	$0{\cdot}5^{a}$	$2{\cdot}3^{bc}$	$0{\cdot}8^{a}$	$1{\cdot}4^{ab}$	$1{\cdot}0^{a}$	$0{\cdot}7^{a}$	$0{\cdot}5^{a}$	$1{\cdot}1^{ab}$	0·5
14:0	$6{\cdot}4^{b}$	$7{\cdot}2^{b}$	$5{\cdot}0^{ab}$	$7{\cdot}2^{b}$	$3{\cdot}5^{a}$	$6{\cdot}5^{b}$	$5{\cdot}1^{ab}$	$7{\cdot}4^{b}$	$4{\cdot}2^{a}$	$5{\cdot}2^{ab}$	$3{\cdot}6^{a}$	$4{\cdot}6^{a}$	$4{\cdot}2^{a}$	$5{\cdot}9^{b}$	1·0
16:0	$30{\cdot}0^{b}$	$26{\cdot}8^{a}$	$29{\cdot}0^{b}$	$26{\cdot}5^{a}$	$25{\cdot}5^{a}$	$26{\cdot}7^{a}$	$29{\cdot}3^{b}$	$30{\cdot}5^{b}$	$32{\cdot}2^{b}$	$28{\cdot}0^{ab}$	$29{\cdot}5^{ab}$	$30{\cdot}7^{b}$	$40{\cdot}9^{c}$	$32{\cdot}8^{b}$	1·8
16:1	$3{\cdot}9^{c}$	$3{\cdot}5^{c}$	$2{\cdot}8^{bc}$	$2{\cdot}9^{bc}$	$2{\cdot}3^{b}$	$2{\cdot}4^{b}$	$2{\cdot}8^{bc}$	$3{\cdot}8^{c}$	$1{\cdot}6^{a}$	$1{\cdot}6^{a}$	$2{\cdot}9^{bc}$	$2{\cdot}1^{b}$	$0{\cdot}8^{a}$	$1{\cdot}4^{a}$	0·5
18:0	$6{\cdot}9^{c}$	$5{\cdot}6^{b}$	$7{\cdot}3^{c}$	$5{\cdot}1^{b}$	$7{\cdot}3^{c}$	$5{\cdot}5^{b}$	$7{\cdot}8^{c}$	$6{\cdot}2^{bc}$	$6{\cdot}0^{bc}$	$5{\cdot}1^{b}$	$7{\cdot}0^{c}$	$4{\cdot}6^{b}$	$6{\cdot}9^{c}$	$3{\cdot}2^{a}$	0·8
18:1	$9{\cdot}7^{ab}$	$7{\cdot}8^{a}$	$14{\cdot}7^{d}$	$11{\cdot}4^{bc}$	$14{\cdot}3^{d}$	$11{\cdot}4^{bc}$	$12{\cdot}1^{c}$	$8{\cdot}4^{ab}$	$6{\cdot}5^{a}$	$7{\cdot}6^{a}$	$10{\cdot}6^{b}$	$10{\cdot}6^{b}$	$10{\cdot}4^{b}$	$7{\cdot}5^{a}$	1·7
18:2*n*-6	$6{\cdot}2^{ab}$	$6{\cdot}1^{ab}$	$6{\cdot}8^{ab}$	$5{\cdot}3^{a}$	$5{\cdot}2^{a}$	$4{\cdot}6^{a}$	$6{\cdot}3^{ab}$	$5{\cdot}1^{a}$	$7{\cdot}3^{b}$	$5{\cdot}9^{ab}$	$6{\cdot}9^{b}$	$7{\cdot}0^{b}$	$5{\cdot}8^{ab}$	$5{\cdot}9^{ab}$	0·8
18:3*n*-3	$4{\cdot}1^{d}$	$1{\cdot}6^{b}$	$3{\cdot}8^{d}$	$1{\cdot}1^{ab}$	$2{\cdot}7^{c}$	$0{\cdot}4^{a}$	$4{\cdot}5^{e}$	$1{\cdot}4^{b}$	$4{\cdot}3^{d}$	$0{\cdot}9^{ab}$	$2{\cdot}5^{c}$	$1{\cdot}3^{ab}$	$3{\cdot}5^{d}$	$1{\cdot}5^{b}$	0·6
15:0+17:0	$5{\cdot}0^{b}$	$7{\cdot}3^{cd}$	$5{\cdot}1^{b}$	$7{\cdot}4^{cd}$	$4{\cdot}3^{a}$	$8{\cdot}3^{d}$	$4{\cdot}1^{a}$	$6{\cdot}5^{c}$	$7{\cdot}2^{cd}$	$7{\cdot}7^{d}$	$5{\cdot}4^{b}$	$6{\cdot}2^{c}$	$4{\cdot}1^{a}$	$6{\cdot}0^{b}$	0·5
Sum of															
NSFA	$51{\cdot}3^{bc}$	$51{\cdot}4^{bc}$	$48{\cdot}2^{b}$	$49{\cdot}4^{b}$	$44{\cdot}3^{a}$	$54{\cdot}3^{c}$	$49{\cdot}8^{b}$	$53{\cdot}4^{c}$	$51{\cdot}8^{bc}$	$49{\cdot}4^{b}$	$47{\cdot}1^{ab}$	$50{\cdot}3^{b}$	$57{\cdot}1^{d}$	$51{\cdot}0^{b}$	1·7
BCFA	$16{\cdot}5^{b}$	$21{\cdot}7^{c}$	$15{\cdot}7^{b}$	$21{\cdot}2^{c}$	$15{\cdot}0^{ab}$	$20{\cdot}5^{c}$	$13{\cdot}3^{a}$	$20{\cdot}6^{c}$	$18{\cdot}6^{bc}$	$27{\cdot}4^{e}$	$18{\cdot}6^{bc}$	$22{\cdot}4^{d}$	$17{\cdot}6^{b}$	$27{\cdot}5^{c}$	1·3
MUFA	$18{\cdot}2^{bc}$	$15{\cdot}5^{ab}$	$21{\cdot}2^{c}$	$18{\cdot}6^{bc}$	$24{\cdot}9^{c}$	$17{\cdot}3^{b}$	$19{\cdot}4^{bc}$	$16{\cdot}4^{b}$	$16{\cdot}6^{b}$	$13{\cdot}4^{a}$	$19{\cdot}5^{bc}$	$17{\cdot}2^{b}$	$13{\cdot}9^{a}$	$11{\cdot}3^{a}$	2·4
PUFA	$13{\cdot}9^{cd}$	$11{\cdot}0^{bc}$	$15{\cdot}1^{cd}$	$10{\cdot}8^{bc}$	$15{\cdot}6^{d}$	$8{\cdot}2^{b}$	$17{\cdot}5^{d}$	$9{\cdot}6^{b}$	$12{\cdot}7^{c}$	$9{\cdot}9^{bc}$	$14{\cdot}6^{cd}$	$9{\cdot}8^{bc}$	$11{\cdot}1^{bc}$	$2{\cdot}5^{a}$	1·9
FA (g/kg DM)	44^{cd}	31^{ab}	51^{d}	30^{a}	40^{c}	28^{a}	44^{cd}	35^{b}	31^{ab}	27^{a}	36^{b}	28^{a}	47^{cd}	27^{a}	4

NSFA, normal saturated fatty acids; BCFA, branched-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA fatty acids.
[a–e] Mean values with different superscript letters were significantly different ($P < 0{\cdot}05$).
* Expt 1: R5, R10, diets contained 53 and 94 g rapeseed oil/kg dry matter (DM); T, diet contained 98 g tallow/kg DM; Expt 2: S, diet contained 87 g soya-bean oil/kg DM; P, diet contained 94 g palmitostearin/kg DM; for details of diets, see p. 564.

Table 6. *Expts 1 and 2. Fatty acid composition (weight % total methyl esters) and total fatty acid content (g/kg dry matter (DM)) of free fatty acids in solid-adherent bacteria (SAB) and liquid-associated bacteria (LAB) of rumen contents in dairy cows offered control (C1 for Expt 1, C2 for Expt 2) and lipid-supplemented diets (R5, R10 and T for Expt 1; S and P for Expt 2)*

(Results are mean values for four cows in Expt 1 and for three cows in Expt 2)

	Fatty acid composition of free fatty acids of rumen bacteria (weight % total methyl esters)														
	Expt 1								Expt 2						
Diets*...	C1		R5		R10		T		C2		S		P		
	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SAB	LAB	SEM
12:0	$0{\cdot}7^{ab}$	$2{\cdot}0^{b}$	$0{\cdot}4^{a}$	$1{\cdot}2^{b}$	$0{\cdot}3^{a}$	$1{\cdot}2^{b}$	$0{\cdot}4^{a}$	$1{\cdot}6^{b}$	$0{\cdot}3^{a}$	$1{\cdot}2^{b}$	$0{\cdot}1^{a}$	$0{\cdot}7^{ab}$	$0{\cdot}1^{a}$	$0{\cdot}5^{ab}$	0·4
14:0	$0{\cdot}9^{b}$	$2{\cdot}2^{bc}$	$0{\cdot}5^{a}$	$1{\cdot}1^{ab}$	$0{\cdot}4^{a}$	$1{\cdot}5^{ab}$	$2{\cdot}5^{c}$	$4{\cdot}8^{d}$	$0{\cdot}9^{b}$	$2{\cdot}1^{bc}$	$0{\cdot}4^{a}$	$1{\cdot}2^{b}$	$1{\cdot}0^{b}$	$1{\cdot}6^{b}$	0·3
16:0	$17{\cdot}8^{a}$	$16{\cdot}9^{a}$	$11{\cdot}3^{b}$	$11{\cdot}0^{b}$	$9{\cdot}7^{b}$	$11{\cdot}0^{b}$	$24{\cdot}9^{c}$	$25{\cdot}0^{c}$	$17{\cdot}4^{a}$	$24{\cdot}2^{c}$	$13{\cdot}6^{b}$	$14{\cdot}3^{ab}$	$44{\cdot}2^{d}$	$41{\cdot}8^{d}$	1·6
16:1	$0{\cdot}1^{a}$	$1{\cdot}0^{ab}$	$0{\cdot}3^{a}$	$0{\cdot}3^{a}$	$0{\cdot}3^{a}$	$0{\cdot}5^{a}$	$1{\cdot}6^{b}$	$1{\cdot}7^{b}$	$0{\cdot}2^{a}$	$0{\cdot}8^{ab}$	$0{\cdot}2^{a}$	$0{\cdot}5^{a}$	$0{\cdot}4^{a}$	$0{\cdot}7^{a}$	0·2
18:0	$48{\cdot}8^{e}$	$51{\cdot}8^{e}$	$43{\cdot}5^{bc}$	$49{\cdot}6^{bc}$	$35{\cdot}9^{b}$	$43{\cdot}3^{bc}$	$36{\cdot}8^{b}$	$37{\cdot}6^{b}$	$46{\cdot}3^{c}$	$34{\cdot}5^{b}$	$25{\cdot}2^{a}$	$32{\cdot}8^{b}$	$30{\cdot}6^{ab}$	$31{\cdot}2^{b}$	2·6
18:1	$18{\cdot}1^{c}$	$11{\cdot}1^{a}$	$30{\cdot}5^{d}$	$24{\cdot}9^{e}$	$41{\cdot}7^{e}$	$28{\cdot}8^{cd}$	$21{\cdot}9^{c}$	$15{\cdot}9^{b}$	$18{\cdot}8^{c}$	$17{\cdot}0^{bc}$	$37{\cdot}2^{e}$	$34{\cdot}4^{de}$	$15{\cdot}1^{b}$	$11{\cdot}9^{a}$	2·0
18:2*n*-6	$3{\cdot}8^{bc}$	$1{\cdot}0^{a}$	$4{\cdot}2^{c}$	$1{\cdot}3^{ab}$	$4{\cdot}7^{c}$	$1{\cdot}7^{ab}$	$2{\cdot}5^{b}$	$1{\cdot}1^{a}$	$4{\cdot}7^{c}$	$4{\cdot}6^{c}$	$11{\cdot}6^{d}$	$3{\cdot}1^{bc}$	$2{\cdot}6^{bc}$	$2{\cdot}9^{bc}$	0·7
18:3*n*-3	$2{\cdot}1^{bc}$	$0{\cdot}3^{a}$	$1{\cdot}1^{ab}$	$0{\cdot}5^{a}$	$1{\cdot}8^{b}$	$1{\cdot}1^{ab}$	$1{\cdot}0^{ab}$	$0{\cdot}4^{a}$	$2{\cdot}2^{bc}$	$1{\cdot}4^{b}$	$3{\cdot}1^{c}$	$0{\cdot}7^{ab}$	$0{\cdot}9^{a}$	$0{\cdot}4^{a}$	0·5
15:0+17:0	$1{\cdot}5^{ab}$	$3{\cdot}0^{b}$	$1{\cdot}1^{a}$	$2{\cdot}5^{ab}$	$1{\cdot}0^{a}$	$1{\cdot}5^{ab}$	$2{\cdot}4^{b}$	$2{\cdot}6^{b}$	$2{\cdot}4^{b}$	$3{\cdot}8^{b}$	$1{\cdot}4^{a}$	$2{\cdot}4^{b}$	$1{\cdot}0^{a}$	$1{\cdot}6^{ab}$	0·5
Sum of															
NSFA	$70{\cdot}6^{cd}$	$76{\cdot}9^{d}$	$57{\cdot}9^{ab}$	$67{\cdot}0^{c}$	$49{\cdot}0^{ab}$	$60{\cdot}9^{c}$	$67{\cdot}4^{c}$	$73{\cdot}2^{d}$	$68{\cdot}3^{c}$	$69{\cdot}0^{c}$	$42{\cdot}6^{a}$	$51{\cdot}5^{ab}$	$77{\cdot}6^{d}$	$77{\cdot}0^{d}$	2·2
BCFA	$2{\cdot}4^{ab}$	$4{\cdot}0^{b}$	$1{\cdot}2^{a}$	$1{\cdot}7^{a}$	$1{\cdot}2^{a}$	$1{\cdot}5^{a}$	$2{\cdot}8^{ab}$	$4{\cdot}7^{b}$	$2{\cdot}7^{ab}$	$4{\cdot}5^{b}$	$1{\cdot}6^{a}$	$3{\cdot}9^{b}$	$1{\cdot}1^{a}$	$4{\cdot}0^{b}$	0·7
MUFA	$18{\cdot}5^{ab}$	$12{\cdot}7^{a}$	$32{\cdot}1^{c}$	$28{\cdot}1^{c}$	$40{\cdot}1^{d}$	$31{\cdot}0^{c}$	$24{\cdot}6^{bc}$	$15{\cdot}9^{a}$	$20{\cdot}7^{b}$	$18{\cdot}3^{ab}$	$39{\cdot}0^{d}$	$35{\cdot}9^{cd}$	$16{\cdot}6^{a}$	$14{\cdot}2^{a}$	2·1
PUFA	$8{\cdot}6^{bc}$	$6{\cdot}6^{ab}$	$8{\cdot}9^{bc}$	$3{\cdot}2^{a}$	$9{\cdot}8^{c}$	$6{\cdot}7^{ab}$	$5{\cdot}3^{ab}$	$3{\cdot}7^{a}$	$8{\cdot}6^{bc}$	$8{\cdot}3^{b}$	$16{\cdot}9^{d}$	$8{\cdot}8^{bc}$	$4{\cdot}8^{a}$	$4{\cdot}8^{a}$	1·3
FA (g/kg DM)	67^{c}	15^{a}	128^{d}	27^{ab}	164^{e}	28^{ab}	127^{d}	30^{ab}	64^{c}	18^{a}	152^{e}	42^{b}	113^{d}	42^{b}	7

NSFA, normal saturated fatty acids; BCFA, branched-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA fatty acids.
[a–e] Mean values with different superscript letters were significantly different ($P < 0{\cdot}05$).
* Expt 1: R5, R10, diets contained 53 and 94 g rapeseed oil/kg dry matter (DM); T, diet contained 98 g tallow/kg DM; Expt 2: S, diet contained 87 g soya-bean oil/kg DM; P, diet contained 94 g palmitostearin/kg DM; for details of diets, see p. 564.

composition of SAB and LAB did not differ significantly with the diet consumed, except for 16:0 (SAB, diet P), 18:1 (SAB and LAB, experimental diets) and branched chain FA (LAB, all diets), which were significantly ($P < 0.05$) higher (Table 5).

The FFA fraction was the major source of FA in total lipids of SAB and LAB, especially in lipid-rich diets (Table 3). Taking into account the fact that FA of polar lipids were fairly stable, this explains the observation that variations with diet of FA concentrations and compositions of FFA (Table 6) exhibited in both bacterial populations displayed characteristics similar to those of total lipids (Tables 4 and 6).

Regressions between individual or total FA concentrations in FFA and polar lipid fractions of SAB and LAB populations and FA intake are shown in Fig. 1. For FFA fractions, the relation between these variables was linear, very close and significant for all FA analysed in SAB and LAB, with the exception of 18:3*n*-3 (SAB and LAB) and 18:2*n*-6 (LAB only). For polar lipid fractions, no significant relation was observed with either population, with the exception of 18:0, 18:1 and total FA in LAB.

The correlation coefficients were generally very high in FFA for 16:0, 18:2 (SAB only) and total FA (r ranging from 0·755 to 0·964) and substantially lower for 18:0 (r ranging from 0·565 to 0·679). Calculation of the slope ratio (concentrations in SAB *v.* LAB) of 16:0, 18:0, 18:1 and 18:2 present in bacteria as FFA relative to the amount of FA ingested can reflect the ability of bacteria to incorporate exogenous fatty acids. This ratio indicated that apparent deposition of these FFA in bacteria were 2·8, 4·2, 8·6, 30·5 times higher respectively ($P < 0.05$) in SAB than in LAB (Fig. 1).

DISCUSSION

Lipid analysis of purified bacterial populations from the rumen contents of dairy cows clearly showed marked differences between SAB and LAB populations, as previously reported in steers by Merry & McAllan (1983).

The higher lipid content of SAB might be interpreted as being due to (1) a selected attachment of bacteria of a specific lipid composition, (2) an intense incorporation of FFA preferentially adsorbed onto particulate matter (Harfoot, 1981) or (3) a preferential utilization of microbial lipid precursors, as volatile fatty acids (VFA), produced in the immediate surroundings of food particles.

However, in the last case it has been demonstrated that VFA utilization in vitro for lipid synthesis by rumen bacteria is partially inhibited by long-chain FA present in the medium (Emmanuel, 1978). Thus, from our results in vivo, we suggest that the large increase in FFA concentrations in SAB, and to a lesser extent in LAB populations, was more probably due to preferential utilization of long-chain FA rather than VFA incorporation. The bacterial FFA accumulation might be the result of biological uptake or physical adsorption on the cell envelope (or both) of FA resulting from microbial lipolysis. Using a suspension of mixed rumen bacteria incubated in vitro with FFA or triacylglycerols, Harfoot *et al.* (1974) estimated that preferential uptake of FFA was mainly due to physical adsorption, according to a process similar to that involved in the association of FA with food particles. However, after 4 h of incubation, these authors did not exclude a possible contribution of biological uptake since it was almost impossible to remove FFA by washing the cells with hexane or sodium hydroxide. From our results, two points would indicate that biological uptake of FFA might not be a negligible process. First, ingestion of increasing amounts of 18:2*n*-6 led to preferential and proportional incorporation of this acid in FFA of SAB (Fig. 1) and to a proportional protection against biohydrogenation as calculated by digesta flow measurement (Bauchart *et al.* 1989). Thus as food particles are known to provide sites for the adsorption and subsequent biohydrogenation of 18:2*n*-6 (Harfoot *et al.* 1973), a

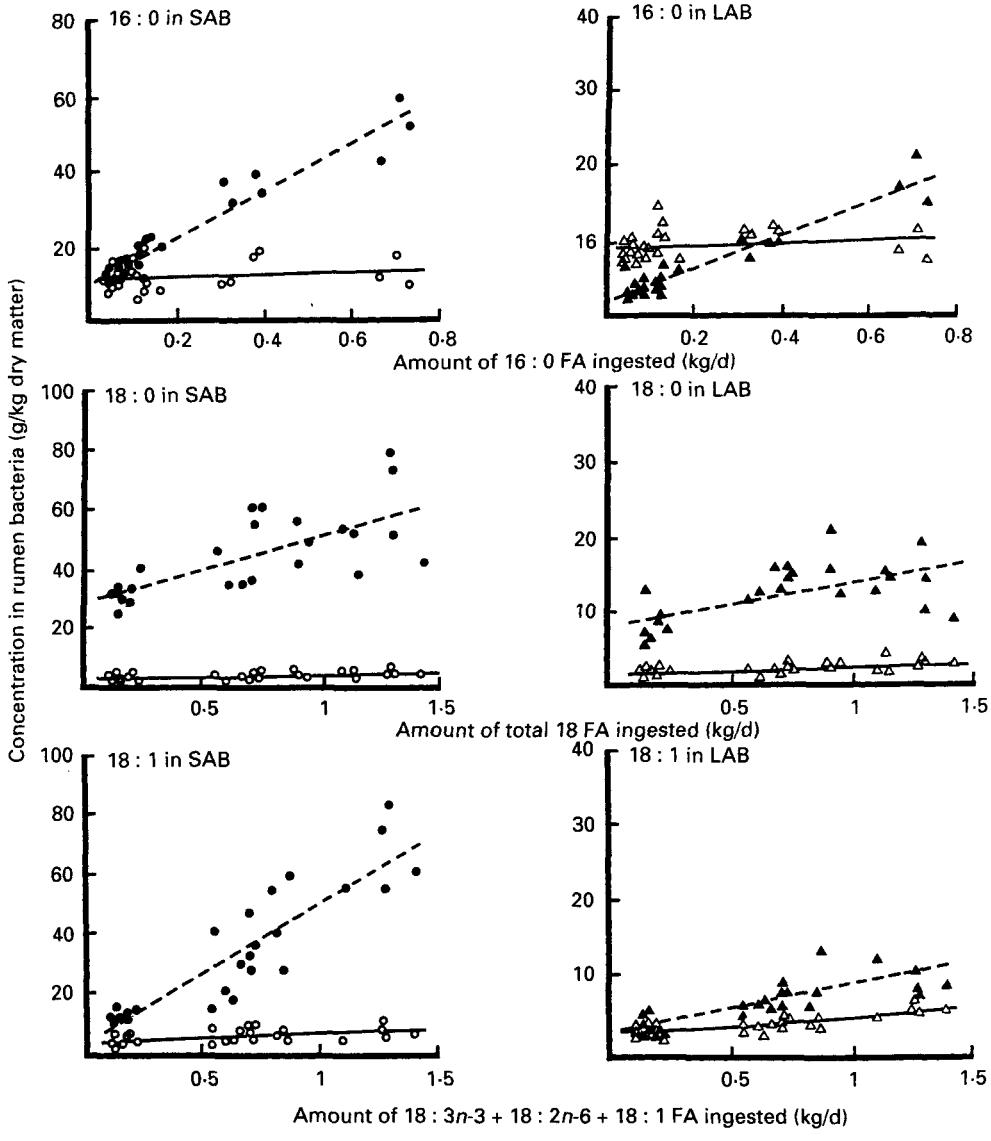

Fig. 1. Relations between the concentrations of each of the main fatty acids or groups of fatty acids in free fatty acids (FFA) (●, SAB; ▲, LAB) and in polar lipids (PL) (○, SAB; △, LAB) of rumen bacteria (y; g/kg bacterial DM) and the daily amount of individual or group of fatty acids (FA) ingested (x; kg/d). (SAB, solid-adherent bacteria; LAB, liquid-associated bacteria.) Points correspond to individual values determined in Expts 1 and 2 with the two control diets and the five experimental diets (for details, see Table 1 and p. 564).

Fatty acid		y	r	S_b
16:0 in:	SAB PL	0·919 x+11·304	0·051	3·734
	LAB PL	1·571 x+8·777	0·168	1·925
	SAB FFA	60·921 x+9·696	0·964**	3·509
	LAB FFA	21·639 x+1·840	0·939**	1·654
18:0 in:	SAB PL	0·322 x+2·520	0·129	0·518
	LAB PL	0·062 x+1·230	0·413*	0·284
	SAB FFA	21·266 x+28·680	0·679**	4·789
	LAB FFA	5·122 x+8·046	0·565**	1·561
18:1 in:	SAB PL	2·138 x+3·171	0·391	1·050
	LAB PL	1·998 x+1·707	0·749**	0·369
	SAB FFA	47·407 x+2·198	0·898**	4·838
	LAB FFA	5·501 x+2·165	0·771**	0·949

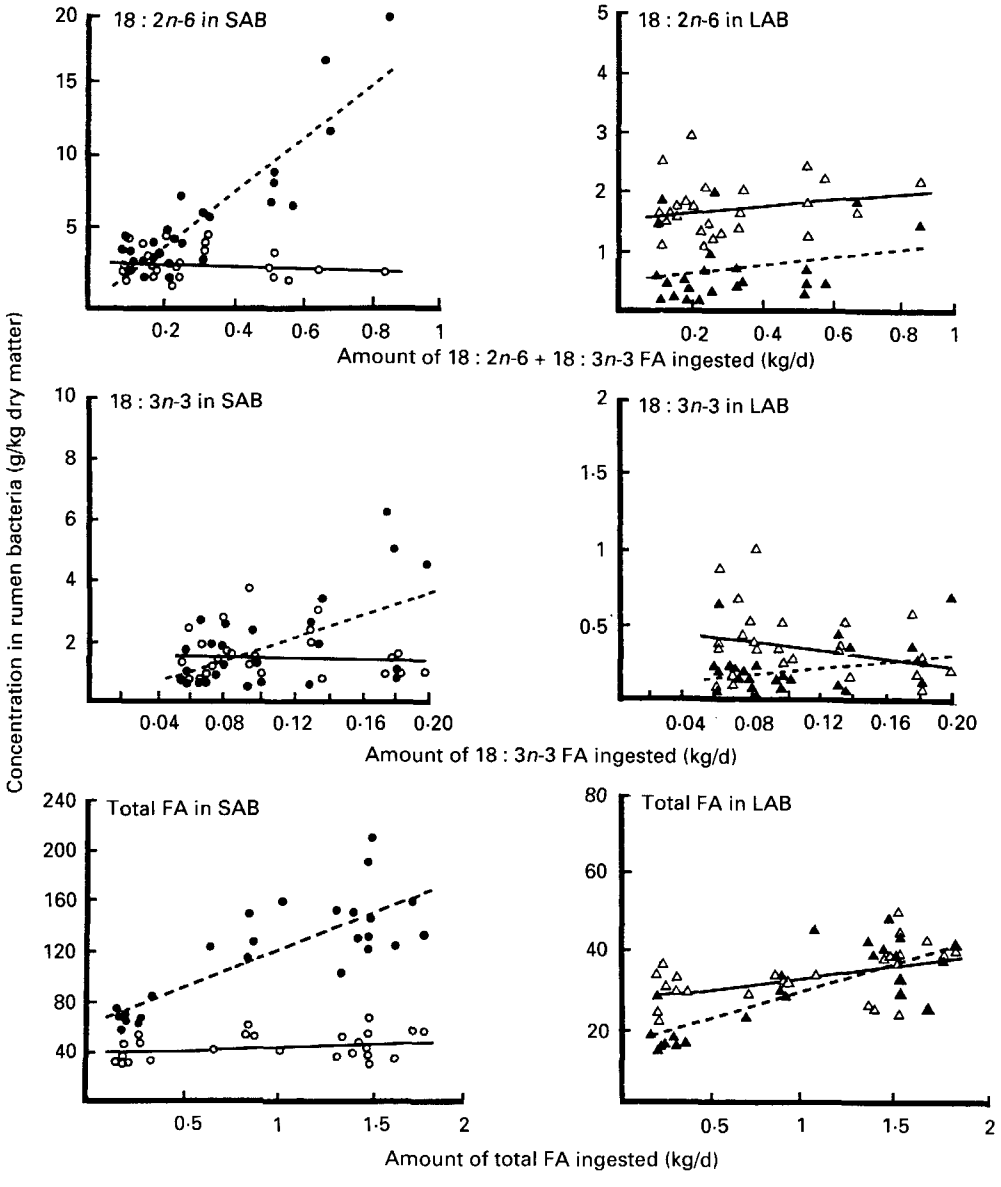

Fig. 1. (*contd.*).

Fatty acid		y	r	S_b
18:2*n*-6 in:	SAB PL	$-0{\cdot}873\,x+2{\cdot}740$	$-0{\cdot}175$	1·022
	LAB PL	$0{\cdot}380\,x+1{\cdot}635$	0·158	0·490
	SAB FFA	$18{\cdot}612\,x+0{\cdot}336$	0·876**	2·138
	LAB FFA	$0{\cdot}611\,x+0{\cdot}500$	0·209	0·595
18:3*n*-3 in:	SAB PL	$-2{\cdot}460\,x+1{\cdot}722$	$-0{\cdot}143$	3·520
	LAB PL	$-1{\cdot}610\,x+0{\cdot}520$	$-0{\cdot}324$	0·980
	SAB FFA	$9{\cdot}322\,x+0{\cdot}634$	0·283	5·231
	LAB FFA	$0{\cdot}900\,x+0{\cdot}100$	0·257	2·466
Total FA in:	SAB PL	$3{\cdot}703\,x+38{\cdot}280$	0·215	3·510
	LAB PL	$5{\cdot}120\,x+26{\cdot}954$	0·449*	2·130
	SAB FFA	$56{\cdot}283\,x+59{\cdot}623$	0·794**	8·984
	LAB FFA	$13{\cdot}610\,x+14{\cdot}710$	0·755**	2·470

S_b, standard error of regression coefficient.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

possible physical adsorption of 18:2*n*-6 on bacteria would probably result in a similar process of biohydrogenation rather than protection against biohydrogenation. Second, our transmission electron microscopy observations of rumen bacteria in cows fed on lipid-supplemented diets clearly showed lipid droplets in the cytoplasm. This confirms previous observations in Nocardia strain 721-A cell, a Gram-positive actinomycete, grown on brain–heart infusion agar (Beaman & Shankel, 1969) or in rumen bacteria grown on a lipid-rich medium (M. Minato, personal communication). This lipid accumulation indicated that part of the additional free FA was really associated with the cellular FFA of these micro-organisms.

Evidence of long-chain FA incorporation in bacterial cells was largely demonstrated in vitro with suspensions of mixed rumen populations (Hawke, 1971; Demeyer *et al.* 1978; Emmanuel, 1978; Hauser *et al.* 1979). Some of these populations, such as certain Gram-negative bacteria with the characteristics of the genus *Butyrivibrio*, presented an absolute requirement of long-chain FA for growth (Hazlewood & Dawson, 1977, 1979). High levels of incorporation of palmitic acid into lipids of SAB and LAB without desaturation confirmed previous results in vitro (Emmanuel, 1978; Hauser *et al.* 1979; Hazlewood & Dawson, 1979) which differ by the bacterial site of incorporation of this acid; in our experiments in vivo, palmitic acid was exclusively esterified into the complex phospholipids of the plasmalogen type in bacterial membranes. However, for both types of experiments palmitic acid is the predominant FA in polar lipids, which raises the question 'how do the bacteria maintain their lipid membrane fluidity?' Perhaps the phenomenon of homeo-viscous adaptation, whereby the FA composition or the repartitioning of polar lipids in membrane bilayers may be adjusted to maintain fluidity (Hauser *et al.* 1979), occurred in the rumen bacteria. Large amounts of branched-chain FA specifically observed in the polar lipids of SAB and LAB may contribute to a decrease in the rigidity of the lipid bilayers, as suggested earlier in *Butyrivibrio* S_2 by Hauser *et al.* (1979).

Emmanuel (1978) reported that six pure cultures of rumen bacteria developed adaptive mechanisms of long-chain FA synthesis in vitro that consist of the conversion of even-numbered FA (16:0; 18:0) into odd-numbered FA (15:0; 17:0) by α-oxidation reactions requiring NAD^+ but no energy. No accumulation of odd-numbered FA was noted in vivo in FFA and polar lipids for either mixed population, which seemed to indicate that the relative importance of α-oxidation reactions remains hypothetical in conditions in vivo.

It is now clear that α-linolenic acid (18:3*n*-3), the major FA of leaf lipids, and linoleic acid (18:2*n*-6) are subjected to a rapid conversion into *trans-11* octadecenoic acid and subsequently into stearic acid by rumen bacteria associated with particulate matter (Harfoot *et al.* 1973; Singh & Hawke, 1979). Whereas *trans*-octadecenoic acid is known to be an efficient growth promoter for a number of rumen bacteria (Hazlewood *et al.* 1976), its polyunsaturated precursors are less easily incorporated into bacterial cells. In our experiments in vivo, we failed to establish a significant relation between the amount of 18:3*n*-3 ingested and 18:3*n*-3 concentrations in lipids of SAB and LAB, which confirmed previous observations by Hawke (1971). By incubation of [1^{-14} C]linolenic acid with mixed bacterial fractions of rumen fluid in vitro, Hawke (1971) found that only 2·4% of the total radioactivity incorporated in the bacteria was present as 18:3*n*-3 in polar lipids.

Similar experiments in vitro using linoleic acid showed that an important part of the radioactivity was associated with the dienoic acid in the polar lipids (Demeyer *et al.* 1978). However, the major part of radioactivity was recovered in FFA associated with saturated and monounsaturated FA indicating extensive hydrogenation of linoleic acid. From our results in vivo, we did not observe any positive correlation between 18:2*n*-6 intake and 18:2*n*-6 incorporation in polar lipids even though there was a net and significant accumulation of 18:2*n*-6 in FFA of SAB. These results emphasize two specific, but opposite,

metabolic activities of these mixed bacterial populations towards linoleic acid, i.e. extensive biohydrogenation and protection against biohydrogenation by uptake and incorporation into cellular FFA.

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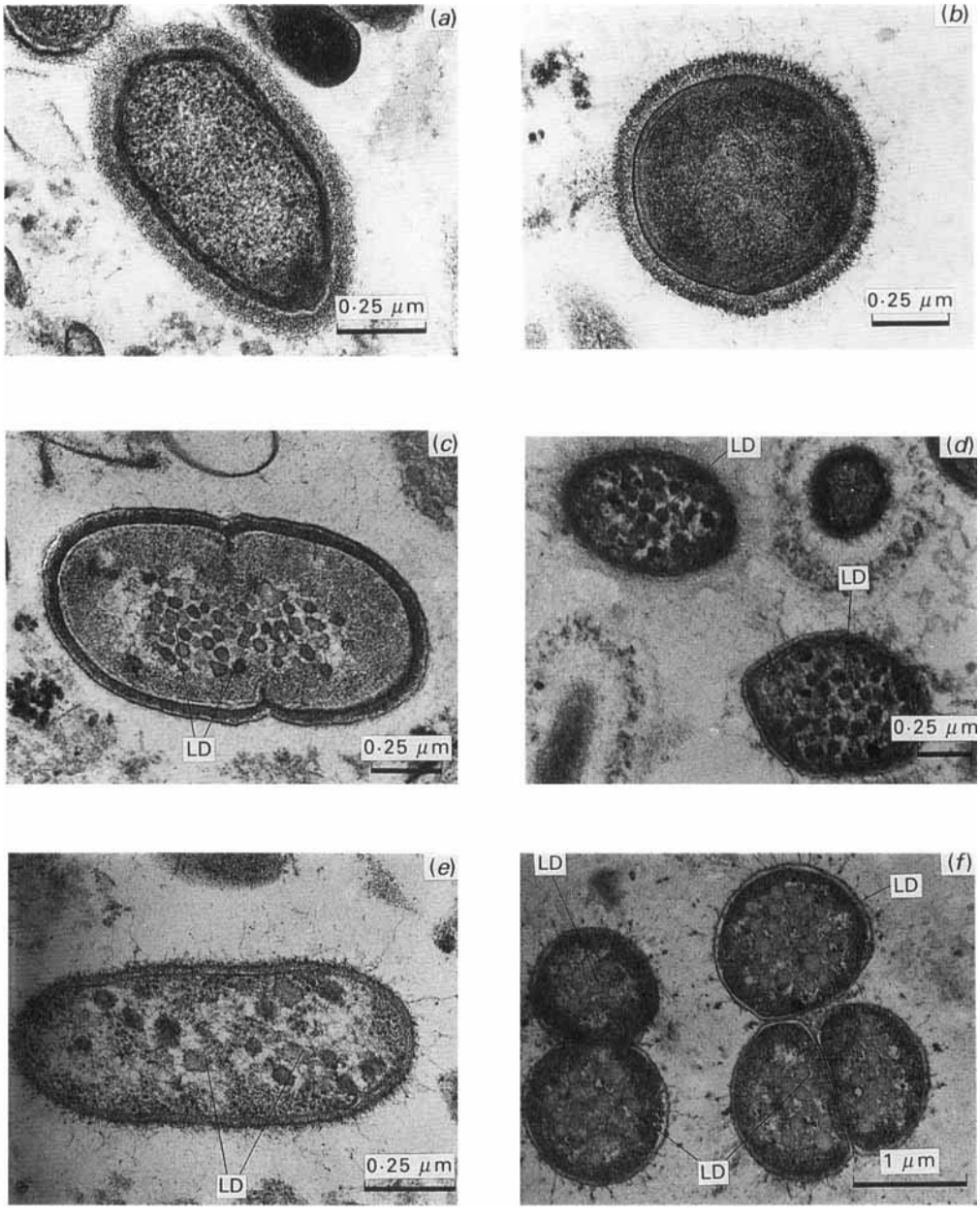

EXPLANATION OF PLATE

Plate 1. Light transmission electron mirographs of solid-adherent bacteria (*a*, *c*, *e*) harvested after washing and pummelling the total particles sampled from rumen contents in dairy cows fed on the control diet (*a*, × 63 250) or rapeseed oil- (*c*, × 51 500) or tallow- (*e*, × 66 900) supplemented diets, and liquid-associated bacteria (*b*, *d*, *f*) harvested from the liquid phase of rumen contents in dairy cows fed on the control diet (*b*, × 55 400) or palmitostearin- (*d*, × 34 200) or soya-bean oil- (*f*, × 21 800) supplemented diets. Note the presence of cytoplasmic lipid droplets (LD) in SAB and LAB harvested from rumen contents in cows fed on lipid-supplemented diets (*c*, *d*, *e*, *f*) compared with bacteria from cows fed on the control diet (*a*, *b*). For details of diets, see Table 1 and p. 564.