The utilization of diets containing acetate, propionate or butyrate salts by growing lambs

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1. In a comparative slaughter experiment growing lambs were given concentrate diets in which 7, 15 or 22% of the metabolizable energy (ME) provided by barley was replaced by sodium and calcium salts of acetic acid, or 22% of ME was replaced by Na and Ca salts of propionic or butyric acids.

2. The efficiency of utilization for fattening (k_f) of the diets containing 0, 7, 15 or 22% of ME as acetate was 57·2, 59·6, 54·1 and 48·8 (SE ±1·8) respectively, the last value being significantly lower ($P < 0.001$) than the first. The k_f for successive increments of acetate was 90, 37 and 19% (SE ±13), the decrease being significant ($P < 0.001$).

3. The k_f values of the diets containing 22% of ME as propionate or butyrate respectively were 48·7 and 50·6 (SE ±1·8), both values being significantly lower than the control ($P < 0.01$). The partial k_f of propionate was 19±13, and of butyrate 28±13%.

4. It is concluded that the experiment provided evidence that the efficiency with which acetate is utilized for energy retention is not constant, but varies with its contribution to ME. The experiment also provided some evidence that large amounts of propionate and butyrate may be inefficiently utilized by growing lambs, although poor utilization of high levels of volatile fatty acid (VFA) salts *per se* cannot be entirely excluded.

In a previous paper (Hovell, Greenhalgh & Wainman, 1976) we reported that when growing lambs were given diets in which 14 or 19% of the metabolizable energy (ME) was provided by salts of acetic acid, they deposited less fat than lambs given an equivalent amount of ME as barley. In a second experiment growing lambs utilized diets containing 4 or 16% of ME as acetate with equal efficiency for energy retention when evaluation was by indirect calorimetry. It was suggested that the results of these two experiments, together with apparently conflicting results reported in the literature, would be reconciled if the partial efficiency with which acetate is utilized for lipogenesis were not constant, but decreased as the proportion of ME provided by acetate was increased. A possible reason for such a relationship might be that the efficient utilization of acetate is dependent on the supply of glucose or glucose precursor.

In the first experiment reported here, the comparative slaughter technique was used to measure the utilization of diets in which ME provided by barley was progressively replaced by ME (0, 7, 15 or 22%) provided by salts of acetic acid; two further treatments were included in which 22% of ME was provided by propionate or butyrate. In the second experiment the rumen volatile fatty acids (VFA) of sheep given diets containing salts of acetic, propionic and *n*-butyric acids were measured.

EXPERIMENTAL

Animals

Expt 1. Fifty-six entire male Suffolk × Scottish Blackface lambs were adapted for approximately 1 month to a diet of rolled barley and extracted soya-bean meal. They were then ranked according to live weight and divided into seven blocks of eight animals. They weighed between 14 and 31 kg and were 10–14 weeks old when placed on experiment.

Expt 2. Two Suffolk × Greyface wether sheep, approximately 3 years old and fitted with permanent rumen cannulas, were used.

Treatments and design

Expt 1. The eight lambs in each block were allocated at random to an initial slaughter group or to one of six dietary treatments as shown in Table 1; two lambs in each block were allocated to the control diet. The lambs remained on their allocated treatment until slaughter.

Expt 2. No formal design was possible since only two animals were available. Four of the diets used in Expt 1 were examined (the control diet and those which contained 22% of ME as salts of VFA). Each sheep received each diet for a period of 4 weeks. Sheep *A* was given the diets in the order: control (control-1)–acetate at 22% of ME (acetate-22)–propionate–butyrate–control (control-2). Sheep *B* was also given the control diet in the first and last periods, but the VFA salt diets in the reverse order.

Diets

The composition of the diets used is given in Table 1. The VFA were added as anhydrous salts and were assumed to have an ME value equal to their heat of combustion. Two values for the ME content of the diets are given in Table 1. The assumed ME was calculated from published values for the constituents, and the corrected ME from the measured digestibility of dietary energy multiplied by a factor (0·74) which was derived from the direct measurement of the ME of a similar diet (Hovell *et al.* 1976), and which was corrected for heat of fermentation.

The relative proportions of protein and assumed ME were kept constant by adjusting the proportionality of rolled barley with a mixture of extracted soya-bean meal and white-fish meal (80:20, w/w). Chopped barley straw was added as a constant proportion (30 g/kg) of these basal ingredients.

The diets were as follows: control; 7, 15 and 22% ME as acetate (referred to as acetate-7, -15 and -22, respectively); 22% ME as propionate (propionate) and 22% ME as butyrate (butyrate). They were offered as loose mixes prepared weekly for each lamb in a bakery-type mixer (Hovell *et al.* 1976). Water was added to counteract the powdery nature of the salts, and a uniform and apparently palatable diet was produced. Selection was a problem with only one lamb on the propionate diet in Expt 1.

Management

Expt 1. All animals were housed indoors in individual pens with slatted floors. They were fed twice daily at 08.30 and 16.30 hours and were weighed once weekly immediately before the morning meal. Water was always available. The lambs were given 774 kJ assumed ME/kg live weight $W^{0.75}$ per d, W being based on the value at the start of the experiment and an assumed growth rate of 200 g/d. (The mean growth rate actually achieved was 211 g/d.) Food refusals, which were small and infrequent, were mixed into the next day's food. The propionate lamb referred to previously refused considerable amounts of food and was discarded.

Expt 2. Rationing and management were as for Expt 1, except that the sheep were bedded on sawdust.

Experimental technique

Digestibility of diets. In Expt 1 digestibility was measured by collecting faeces from each animal for two 10 d periods separated by a period of 10 weeks. General methods have already been described (Hovell *et al.* 1976).

Slaughter and processing (*Expt* 1). The lambs were slaughtered, one of the 'replicate' animals at a time, at intervals of 1 week at a local abattoir. The general methods were those of Hovell *et al.* (1976) but with two differences. The lambs were shorn before slaughter, the

Table 1. *Composition of experimental diets given to lambs*

	Diet					
	Control	Acetate-7	Acetate-15	Acetate-22	Pro-pionate	Butyrate
Constituent (g DM/kg dietary DM)*						
Rolled barley	820	733	649	566	612	632
Extracted soya-bean meal	120	129	137	145	157	162
White-fish meal	30	32	34	36	39	41
Barley straw (chopped)	30	28	25	23	25	26
Sodium salt of VFA	—	47	93	138	100	83
Calcium salt of VFA	—	31	62	92	67	56
Composition (/kg DM)						
Crude protein (nitrogen × 6·25) (g)	171	169	166	164	178	184
Organic matter (g)	960	912	866	820	868	891
Na (g)†	3	16	29	42	32	21
Ca (g)†	19	14	22	30	21	17
Phosphorus (g)†	7	7	7	6	6	6
Gross energy (MJ)	18·40	17·82	17·25	16·69	18·14	18·74
Energy from VFA salts (MJ)	—	0·84	1·65	2·45	2·73	2·82
Assumed ME (MJ)‡	12·51	12·33	12·18	12·00	12·99	13·42
Corrected ME (MJ)§	11·07	11·25	11·22	11·24	12·36	12·79
Energy from VFA salts, kJ/MJ corrected ME	0	74	147	218	221	220

DM, dry matter; ME, metabolizable energy; VFA, volatile fatty acids.

* In addition the lambs were given (/MJ assumed ME): 777 mg $CaHPO_4.2H_2O$, 480 mg NaCl, 16 mg MgO, 12 mg $ZnSO_4.7H_2O$, 6 mg $MnSO_4.4H_2O$, 34 μg $CoSO_4.7H_2O$, 16 μg KIO_3, 1·6 mg DL-α-tocopheryl acetate, 120 μg retinyl palmitate, 2 μg cholecalciferol. Lambs on the control diet were also given 1435 mg $CaCO_3$.

Initially all lambs were given an additional 29 mg MgO, but this was stopped after the first few weeks on experiment (see p. 175).

† Calculated from published values (Evans, 1960) and chemical composition of salts.

‡ Calculated as (MJ/kg DM): barley 12·92, soya-bean meal 11·54, fish meal 9·96, straw 7·36; VFA salts (kJ/mol acid equivalent) acetate 874, propionate 1535, butyrate 2192.

§ Calculated from digestible energy (Hovell, Greenhalgh & Wainman, 1976).

5–10 mm wool left on the skin being included in the 'non-carcass remainder'. The second difference was that the 'non-carcass remainder' was weighed as the components given in Table 4 before bulking for processing and chemical analysis. Two fat samples were taken before processing: (1) subcutaneous, from the full depth of the backfat just above-the last lumbar vertebra; (2) perinephric, a core sample taken just posterior to the kidney. These samples were stored at −20° until analysed.

Rumen sampling (*Expt* 2). General methods have been described (Hovell *et al.* 1976). The sheep were sampled at 08.00, 09.30, 11.30, 13.00, 16.00, 17.30 and 21.30 hours on days 26 and 28 of the experimental periods.

Chemical analysis. The methods used have been described (Hovell *et al.* 1976). Each dietary component was analysed separately, and the composition of the complete diet calculated from this basic information. Fat samples were analysed as described by Garton, Hovell & Duncan (1972).

RESULTS

Health

Expt 1. The health of the lambs generally remained good. On two occasions the animals developed a mild respiratory infection which responded to antibiotic (Strypen; Glaxo

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Table 2. *Expt* 1. *Digestibility coefficients of the diets containing different volatile fatty acids (VFA) as sources of metabolizable energy for lambs*

(Mean of two determinations with each lamb (at beginning and end of experiment))

	Diet†						Approximate SE of difference‡	Effect of acetate	
	Control	Acetate-7	Acetate-15	Acetate-22	Propionate	Butyrate		Linear	Quadratic
Nitrogen									
In complete diet	0·778	0·792	0·794	0·809**	0·816***	0·827***	0·0106	**	—
Organic matter									
In complete diet	0·839	0·851	0·858*	0·868***	0·866***	0·876***	0·0072	***	—
In basal components§	0·839	0·846	0·848	0·851	0·853*	0·865**	0·0068	*	—
Energy									
In complete diet	0·824	0·841	0·850*	0·854**	0·857**	0·866***	0·0106	**	—
In basal components§	0·824	0·828	0·829	0·834	0·844*	0·847**	0·0081	*	—

* $P<0·05$, ** $P<0·01$, *** $P<0·001$. Significance of comparison of VFA-salt treatments with controls or of effect of acetate.

† For details of diets, see Table 1 and p. 172.

‡ SE of difference of VFA-salt treatment with control. (For comparison between VFA-salt treatments, multiply by 1·18.)

§ Calculated with the assumption that the energy and organic matter of the salts were completely digestible (Hovell *et al.* 1976).

Laboratories Ltd, Greenford, Middlesex). One lamb died of an acute bacterial toxaemia during the first week (control diet), and one with a ruptured bladder after 12 weeks (acetate-7 diet). Post mortem examination revealed that the ureter of this second animal was found to be inflamed and blocked with numerous small calculi. Just before this incident two animals had been suspected of having calculi. Previous experience at this Institute had implicated magnesium as the cause of calculi in barley-fed lambs (E. R. Ørskov, personal communication), and the Mg content of the diet was therefore reduced (Table 1). No further trouble was experienced.

Utilization of diets

Expt 1. *Comparative slaughter*

As the agreement between the two control groups was always very good, their results have been combined.

Digestibility. There were no significant differences between the values obtained from the collections made at the beginning and end of the experiment, and the values given in Table 2 are the means of the two determinations.

The apparent digestibility of nitrogen, organic matter and energy increased as the level of salt inclusion was increased. In the instance of organic matter and energy, much of the increase could be accounted for if the salts were assumed to be completely digestible. However, the basal component (barley + soya-bean meal + fish meal + straw) of the propionate and butyrate diets was significantly more digestible than the control diet, and increasing additions of acetate were also associated with an increase in the digestibility of basal component. The effect of these differences in digestibility was to induce significant differences between treatments in corrected ME intake as is shown by Table 3.

Weight of body and components at slaughter. There were no significant differences in the final live weights of the lambs with the exception of those on the propionate diet which were lighter (Table 3). However, this was largely due to differences in the contents of the gastro-intestinal tract, for the apparent difference was removed when the comparison was as the 'shorn and ingesta-free' body (carcass + 'non-carcass remainder').

There was a significant quadratic effect of acetate on the weight of the 'hot' carcass, carcasses from lambs given the acetate-7 diet being the heaviest. Lambs given the butyrate diet also produced heavier carcasses. There was a decrease in the weight of the liver and of abdominal fat as the level of acetate was increased. Lambs given the propionate diet had a heavier liver, and a lighter reticulo-rumen than lambs given the control diet. Lambs given the diets with the VFA salts generally had heavier kidneys and feet than those given the control diet.

Rates of gain in live weight and its components. These are shown by Table 4.

The lambs given the acetate-7 diet made significantly greater carcass gains than those on the control. The effect of acetate was progressively reduced and lambs on the acetate-22 diet made similar gains to the control lambs, the over-all quadratic effect of acetate being highly significant. Lambs on the propionate and butyrate diets had greater rates of carcass gain than those given the control or acetate-22 diets (the control *v.* propionate comparison only approached statistical significance). Growth of the 'non-carcass remainder' tended to reflect that of the carcass, though there were no statistically significant differences.

Although there were no statistically significant differences between treatments in the rate of fat deposition, the lambs given the acetate-7 diet tended to deposit rather more fat, and those given the acetate-22 diet rather less fat than those given the control diet. This quadratic effect of acetate on fat deposition was statistically significant.

Lambs given the propionate and butyrate diets deposited significantly more protein daily than lambs given the control diet, and the difference in protein deposition between the

Table 3. *Expt* 1. *Effect of diets containing* 7 (*acetate-7*), 15 (*acetate-15*), 22 (*acetate-22*)% *of their metabolizable energy* (ME) *as acetate or* 22% *of* ME *as propionate or butyrate on the weight of the body and body components of male lambs initially approximately* 12 *weeks old*

	'Initial slaughter' group†	Diet‡						Approximate SE of difference§	Effect of acetate	
		Control	Acetate-7	Acetate-15	Acetate-22	Propionate	Butyrate		Linear	Quadratic
ME (MJ/d)										
Assumed	—	11·13	11·22	11·21	11·14	11·20	11·22	0·062	—	—
Corrected	—	9·93	10·23***	10·34***	10·44***	10·72***	10·69***	0·075	***	—
From salts	—	—	0·76	1·52	2·28	2·37	2·35	—	—	—
Wt (kg)										
Live weight: Initial	22·3	22·8	22·1	22·6	22·7	22·3	22·6	0·50	—	—
Final	—	52·4	52·0	50·9	51·0	49·9*	51·4	0·75	—	—
Hot carcass	9·8	26·2	27·5*	27·0	25·9	26·7	27·2*	0·51	—	**
'Non-carcass remainder'‖	8·0	17·2	17·5	17·5	17·0	17·5	17·4	0·49	—	—
Wt of body components (g)										
Liver	450	821	779	789	682**	919*	801	41	**	—
Lungs+trachea	455	728	724	723	710	711	814*	32	—	—
Heart	130	218	231	242	243	255*	235	15	—	—
Rumen	541	968	880	920	945	851*	937	47	—	—
Omasum	49	89	97	89	100	87	82	8	—	—
Abomasum	120	190	193	258***	262***	223*	197	16	***	—
Small intestine	697	640	661	678	658	657	660	42	—	—
Large intestine	279	344	292	327	336	326	301	27	—	—
Abdominal fat	84	1480	1636	1399	1215*	1466	1362	121	*	—
Right kidney	50	62	84**	85**	84**	78*	78*	7	**	—
Head	1541	3353	3587	3270	3432	3472	3395	163	—	—
Feet	721	1018	1029	1050	1087*	1099*	1102*	32	*	—
Skin	1231	3959	4106	4519	3957	4043	4235	312	—	—
Blood	1146	2048	1971	1991	2006	2169	2026	70	—	—

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$. Significance of comparison of VFA-salt treatments with control or of effect of acetate.
† Composition of 'initial slaughter' group not included in analysis of variance.
‡ For details of diets, see Table 1 and p. 172.
§ SE of difference of VFA-salt treatments with control. (For comparison between VFA-salt treatments, multiply by 1·18.)
‖ Excluding fleece.

Table 4. *Expt* 1. *Effect of diets containing* 7 (*acetate*-7), 15 (*acetate*-15) *or* 22 (*acetate*-22)% *of metabolizable energy* (ME) *as acetate, or* 22% *of* ME *as propionate or butyrate on the rate of growth, and the composition of the growth made by male lambs initially approximately* 12 *weeks old*

	Diet†						Approximate SE of difference‡	Effect of acetate	
ME intake compared with control (MJ/d)	Control	Acetate-7	Acetate-15	Acetate-22	Propionate	Butyrate		Linear	Quadratic
Assumed ME	(11·13)	+0·09	+0·09	+0·01	+0·07	+0·09	0·063	—	—
Corrected ME	(9·93)	+0·30***	+0·31***	+0·51***	+0·79***	+0·76***	0·075	***	—
Daily gain (g)									
Live wt	216	219	208	207	212	211	5·8	—	—
Hot carcass	117	130*	125	116	124	126*	4·5	—	**
'Non-carcass remainder'	66	69	69	65	69	68	3·3	—	—
Empty body‡									
Fat	55·6	61·7	55·0	50·6	52·7	54·6	3·14	—	*
Crude protein (nitrogen × 6·25)	30·4	33·2	33·2	31·4	33·7*	33·9*	1·55	—	—
Water	93·6	100·8	101·2	94·4	102·0	102·3	4·73	—	—
Ash	6·6	7·4*	8·0**	8·2***	8·1**	8·1**	0·43	***	—
Wool fibre	5·3	4·5	5·6	5·6	4·3	4·3	0·68	—	—
Wool grease	0·8	1·1**	1·1**	0·8	1·0*	1·0*	0·10	—	**
Energy retained (MJ/d)§	2·98	3·27*	3·02	2·81	2·91	2·99	0·126	—	*
Ash: crude protein (mg/g)	216	222	245*	264***	241*	241*	11	***	—

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$. Significance of comparison of VFA treatments with control or of effect of acetate. (For comparison between VFA salt treatments, multiply SE by 1·18.)

† For details of diets, see Table 1 and p. 172.

‡ Excluding fleece.

§ Including wool fibre, but excluding wool grease, calculated from values of Paladines, Reid, Bensadoun & Van Niekerk (1964).

Table 5. *Utilization of dietary energy by male lambs initially approximately* 12 *weeks old, and given the control diet, or diets containing* 7 (*acetate–*7), 15 (*acetate–*15) *or* 22 (*acetate–*22) % *of metabolizable energy* (ME) *as acetate, or* 22 % *of* ME *as propionate or butyrate*

	Diet†						Approximate SE of difference‡	Effect of acetate	
	Control	Acetate-7	Acetate-15	Acetate-22	Propionate	Butyrate		Linear	Quadratic
Median live wt ($kg^{0.75}$)§	15·06	15·24	15·24	14·93	15·14	15·25	0·22	—	—
Energy (kJ/$kg^{0.75}$ per d)									
Correct ME intake	659	671	679*	699***	708***	702***	10·8	***	—
ME used for maintenance‖	311	311	311	311	311	311	—	—	—
ME used for gain (*A*)	348	360	368*	388***	397***	391***	9·9	***	—
Energy retained (*B*)	199	214	199	189	193	197	9·0	NS	—
Efficiency (k_f) (100 *B*/*A*)	57·2	59·6	54·1	48·8***	48·7***	50·6**	2·5	**	NS
Energy (kJ/$kg^{0.75}$ per d)									
ME used for gain from basal	348	333	314	304	310	305	8·5	—	—
ME used for gain from VFA salt (*C*)	—	27	54	85	88	86	1·9	—	—
Energy retained from basal	199	190	179	173	177	174	7·5	—	—
Energy retained from VFA salt (*D*)	—	24	20	16	16	23	8·1	—	—
Partial k_f of VFA salt (100 *D*/*C*)	—	90	37	19	19	28	18·3	***	—
Efficiency calculated on assumed ME									
Assumed ME intake (kJ/$kg^{0.75}$ per d)	740	736	737	746	740	737	9·7	—	—
k_f of complete diet %¶	50·8	55·2*	51·2	47·6	49·3	50·9	22	***	*
Partial k_f of VFA salt %	—	110	53	36	44	51	16·2	***	NS

NS, $P < 0{\cdot}1$; * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$. Significance of comparison of VFA treatments with control, or of effect of acetate.

† For details of diets, see Table 1 and p. 172.

‡ SE of difference between means of seven values. (For VFA salt versus control comparison, multiply by 0·85.)

§ Corrected to constant ingesta weight (Hovell *et al.* 1976).

‖ Hovell *et al.* (1976).

¶ Maintenance at 348 kJ assumed ME/$kg^{0.75}$ per d (Hovell *et al.* 1976).

Table 6. *Expt 2. Average volatile fatty acid (VFA) levels (mmol/mol VFA) in the rumen of 3-year-old wether sheep given the control diet, or diets containing 22% of their metabolizable energy as acetate or propionate or butyrate*

	Diet*				
	Control-1	Control-2	Acetate-22	Propionate	Butyrate
Rumen pH	5·4	5·4	6·6	6·2	6·3
VFA (mmol/l)	140	131	130	150	131
Molar composition (mmol/mol VFA)					
Acetic	443	568	780	445	471
Propionic	347	234	112	396	142
n-Butyric	114	105	73	86	331
Isobutyric	24	36	18	23	26
n-Valeric	47	18	2	17	5
Isovaleric	13	31	15	28	20
Caproic	12	8	0	5	5
Acetic:propionic	1·3	2·5	7·5	1·3	3·3
Acetic:*n*-butyric	4·2	5·8	12·7	5·2	1·7
n-Butyric:propionic	0·3	0·4	0·6	0·3	2·5
C2:C3†	1·9	3·2	8·1	1·6	7·1

* For details of diets, see Table 2 and p. 172.
† (Acetate + 2 butyrate + valerate + 3 caproate):(propionate + valerate) expressed as molar proportions.

control diet, and the acetate-7 and acetate-15 diets (in favour of the acetate diets) approached significance ($P < 0{\cdot}1$). The rate of ash deposition was considerably greater ($P < 0{\cdot}05$–$P < 0{\cdot}001$) on all the VFA salt diets.

Utilization of dietary energy. Fat and protein gains expressed as energy retention are also shown by Table 4. Lambs given the acetate-7 diet retained more energy than those given the control diet, the over-all effect of acetate being quadratic and significant. No other differences were statistically significant.

The efficiency (k_f) with which dietary energy was utilized for energy retention is given in Table 5. All diets containing 22% of ME as salts of VFA were utilized less efficiently than the control diet when the basis of the calculation was corrected ME. With the acetate diets there was a suggestion ($P < 0{\cdot}1$) of a quadratic relationship between the utilization of dietary energy and ME intake, the acetate-7 diet being utilized slightly more efficiently than the control diet.

The partial k_f values of the VFA salts are also given in Table 5. There was a clear and highly significant decrease in the partial k_f of acetate as its contribution to ME was increased, acetate being utilized with an apparent efficiency 4·7 times better in the acetate-7 diet than in the acetate-22 diet. Propionate and butyrate were utilized with efficiencies which did not differ significantly from that of acetate at the same level of inclusion (22% of corrected ME). When assumed ME was used as the basis for calculation, the effect of acetate remained the same, but the partial k_f of propionate and butyrate was similar to the k_f of the control diet.

Expt 2. Rumen pH and VFA

The agreement between the values obtained for samples from the two sheep was good, and therefore the values have been combined. However, there were clear differences between the two sampling periods for the control diet (control-1 and control-2), and therefore these two assessments of the control diet are presented separately (Table 6).

Rumen pH was higher and more stable when the salt diets were given, and tended to be more stable in the control-1 period than the control-2 period. Total rumen VFA and molar

proportions reflected the type of diet given, and the pattern of eating by the animals. The control diet was eaten fairly rapidly (all food eaten by 3 h after feeding). Of the diets containing salts of VFA, the butyrate diet was eaten most rapidly (within 1–2 h of feeding) for the bulk was low, and butyrate appears to be very palatable to sheep. The acetate diet was eaten within approximately 5 h of feeding, and the propionate diet was eaten most slowly, nibbling continuing throughout most of the day. With the diets containing VFA salts peak VFA concentrations were observed in the sample taken 1 h after feeding, whereas with the control diet, the peak concentration was in the sample taken 3 h after feeding. The propionate diet gave the highest concentration (approximately 180 and 220 mmol/l for the samples taken 1 h after feeding in the morning and afternoon respectively). The principal difference between the two periods when the control diet was given was that in control-2 period, the fermentation produced less propionate and more acetate.

DISCUSSION

Digestibility and metabolizability of the diets. The increase in the apparent digestibility of N as the inclusion of VFA was increased may be partly or wholly explained by two factors. First, as the level of VFA salt was increased, so was the proportion of protein contributed by fish and soya-bean meals. Secondly, as the level of barley (and hence fermentable carbohydrate) was decreased, so the amount of microbial protein synthesized probably decreased. If barley or microbial protein or both were less digestible than the fish or soya-bean protein, then the observed trend was to be anticipated.

The increase in the digestibility of the energy of the basal constituents caused corrected ME intake to be greater for some of the VFA salt treatments than for the control. Corrected ME was calculated from digestible energy (DE) with the assumption that the metabolizability of the digested energy was unchanged. It is possible that this assumption may have led to a slight over-estimation of the metabolizability of the VFA salt diets. (For example, the control diet contained approximately 0·20 MJ as digestible protein per MJ DE, whereas the acetate-22, propionate and butyrate diets contained approximately 0·26 MJ digestible protein/MJ DE.) Notwithstanding the shortcomings of corrected ME, it is preferred to assumed ME as the basis for discussion.

Growth of body and components. Although there were no significant effects of diet on the growth of the 'non-carcass remainder' as a whole, there were clear effects on some of its components. The effect on liver weight may be sensibly related to the expected level of metabolic activity and glycogen storage (see Bassett, 1975). Ørskov, Fraser & Gordon (1974) noted that lambs given a diet producing a high propionate fermentation had heavier livers. The heavier kidneys of the lambs given VFA salts were better related to the cation content of the diets than to a specific VFA inclusion. Practically all the ingested sodium would have been excreted in the urine (Hovell, 1972), and this additional load resulted in heavier kidneys. The heavier abomasums also appear to be associated with higher cation intakes. The heavier feet of the lambs given the VFA-salt diets were probably due to an increased weight of bone, since there was a clear effect of diet on ash deposition (Table 4).

Composition of growth. The greater ash deposition of lambs given the VFA-salt diets was clearly associated with their greater cation intakes; it was not due simply to a greater increase in lean-body mass because the value for ash:protein was changed (Table 4). Further analysis showed that it was associated with an increase in calcium and phosphorus deposition (Hovell & Davidson, unpublished observations), and therefore must reflect bone mineralization.

Differences in the deposition of protein were confounded with differences in the apparent digestibility of N (Table 4). However, the correlation between apparently-digested N intake

and protein deposition (body N × 6·25 + wool fibre) was poor (r 0·06) and non-significant. There were also the differences in protein source referred to earlier. For these reasons it is not possible to draw any conclusions as to the effect of treatment on protein deposition, although Eskland, Pfander & Preston (1973) reported that propionate, and possibly butyrate, may stimulate more protein synthesis than acetate.

Fat deposition and energy retention were closely related since fat deposition is the main contributor to energy retention. This will be discussed later in relation to VFA utilization.

Utilization of VFA salts for energy retention. There were significant differences between treatments in corrected ME intake owing to differences in the digestibility of the diets (Table 2). Covariance analysis was not suitable to correct for this since the range in energy intakes was induced by small differences in digestibility and the slopes of the regressions were not significant. However, a correction is effectively made when utilization is expressed as utilization above maintenance (k_f) as shown by Table 5. The differences in k_f were largely due to differences in fat deposition, and cannot be explained by differences in protein deposition even if the efficiency of protein deposition was as low as 0·33 (Ørskov & McDonald, 1976).

One interpretation of the results is that salts of VFA are utilized inefficiently *per se*, possibly because of their cation content, and it must be emphasized that the design of this experiment was such that this possibility cannot entirely be excluded. However, there is no immediately obvious reason why the cations should have been the cause of the less efficient utilization. Examination of Tables 1 and 5 shows that whereas the propionate and butyrate diets contained less Ca and Na than the acetate-15 diet, they were in fact utilized slightly less efficiently; although the difference was significant statistically only for the propionate diet. The cation concentration of the butyrate diet was similar to that of the acetate-7 diet (138 *v.* 124 mmol/MJ ME) and the former diet was utilized significantly less efficiently ($P < 0{\cdot}05$). Regression analysis showed there to be no significant relationship between the cation content of the diets, and their k_f value. Furthermore, we found earlier (Hovell *et al.* 1976) that a diet which contained 16% of ME as a mixture of Ca, Na and K salts was utilized with a high efficiency (k_f 65·8 ± 2·7). Experiments with sheep in which high Na intakes have been associated with a reduction in fat deposition (e.g. Jackson, Kromann & Ray, 1971) may be explained by differences in energy intake.

If the ingested salts were absorbed rapidly, the high flux of VFA made available to the animals might have contributed to the poor utilization of the salt diets; in the current experiment, the diets (particularly that containing butyrate) were eaten very rapidly, whereas in the previous calorimetry experiment (Hovell *et al.* 1976) they were nibbled over a longer period. Whether a high flux associated with rapid ingestion was also associated with high urine losses, is not known. In the previous experiment there were slightly greater losses of energy (not as VFA) in the urine of sheep given acetate, amounting to approximately 1% of DE. However, if a true k_f of 60% is assumed for the VFA salts, then urine losses of 17–25% of the total VFA salts given (i.e. approximately 5% of ME) from the acetate-22, propionate and butyrate diets would be necessary in order to explain the lower energy retention observed.

Therefore the following discussion will be in terms of the utilization of the VFA *per se*.

Utilization of acetate. Calculated on corrected ME, the acetate-22 diet was utilized significantly less efficiently than the control. There was the suggestion that the acetate-7 diet was utilized slightly more efficiently than the control (not statistically significant), which agrees with the results of Johnson (1972) who found that the k_f of a ground-maize diet was increased by the addition of acetate.

The utilization of acetate itself is clearly demonstrated by the partial k_f (Table 5), which decreased significantly as the contribution of acetate to ME was increased. This agrees with

the concept that the efficient utilization of acetate for lipogenesis requires sufficient glucose or glucose precursor, as discussed by Bauman & Davis (1975) and Hovell *et al.* (1976). Tyrrell, Reynolds & Moe (1976) also found the efficiency of utilization of acetate to be dependent upon its contribution to ME.

Utilization of propionate. The poor utilization of the propionate diet and the low partial k_f of propionate (calculated on corrected ME) was at first sight surprising, and conflicted with the original observations of Armstrong & Blaxter (1957); Armstrong, Blaxter, Graham & Wainman (1958); Ørskov & Allen (1966*a*, *b*, *c*) and Ørskov, Hovell & Allen (1966). However, the level of feeding used here was higher and the level of salt inclusion greater. There is evidence that large amounts of propionate can affect the over-all metabolism of sheep. Fat from lambs on the propionate diet contained large amounts of branched-chain and odd-chain fatty acids (Garton *et al.* 1972). The branched-chain acids were probably produced by the intrusion of methylmalonate, originating from propionate, into the acetate pool used for fatty acid synthesis. The odd-chain acids would have originated from propionyl-CoA being used to initiate a fatty acid chain.

The reasons for the accumulation of propionate metabolites are not clear. The propionate given was equivalent to an average of 1·08 mmol/min, and Judson & Leng (1973) reported that 31% of a propionate infusion of 3·2 mmol/min was converted to glucose by 3–5-year-old ewes (equivalent to 0·99 mmol/min). Vitamin B_{12} deficiency has been shown to be associated with unusual propionate metabolism in sheep (Smith & Marston, 1971), although the sheep on our experiment were given supplementary cobalt. In our experiment energy lost in urine was not measured, and may have been increased by propionate (Garton, 1975). It is also possible that by basing the calculation of k_f on corrected ME, we underestimated slightly the efficiency of utilization of propionate, owing to the over-estimation of corrected ME as discussed previously. However, the high proportion of odd-chain and branched-chain fatty acids synthesized, and the steady nibbling pattern of eating (which differed from all the other treatments), suggest that the propionate diet induced some form of metabolic stress, although the reason for the inefficiency is not clear.

Utilization of butyrate. The poor utilization of butyrate is also contrary to the observations of Armstrong & Blaxter (1957) although in one experiment of Ørskov *et al.* (1966), butyrate was inefficiently utilized.

The utilization of butyrate for lipogenesis other than for the provision of the initial primer molecule may depend on the citrate cleavage pathway for the transport of two-carbon units derived from butyrate, from the mitochondria into the cytosol (Bauman & Davis, 1975). There is evidence (Ballard, Filsell & Jarrett, 1972) that sheep adipose tissue will increase the activity of the enzymes associated with citrate cleavage when the amount of glucose available for lipogenesis is increased. Whether such an adaptation to butyrate occurs is not known. The poor utilization of butyrate in our experiment suggests that the ability of the sheep to utilize butyrate efficiently may be limited.

There are many difficulties in interpreting comparative slaughter experiments, and there were obvious shortcomings in our experiment, in that values for energy balance were incomplete or lacking. For the calculation of partial k_f it was assumed that there were no interactions between dietary ingredients, but the results suggest strongly that in the instance of acetate, such interactions existed. The k_f values are therefore useful only as indexes of relative utilization.

However, our results do suggest that the efficiency with which acetate is utilized for energy retention by growing-fattening lambs varies with its contribution to ME, and that in some circumstances propionate and butyrate may also be inefficiently utilized. Confirmation of these results must await more detailed experimentation, for the poor utilization of VFA salts *per se* cannot be excluded as a contributory factor.

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