The utilization of diets containing acetate salts by growing lambs as measured by comparative slaughter and respiration calorimetry, together with rumen fermentation

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1. In a comparative slaughter experiment, growing lambs were given concentrate diets in which 14 or 19% metabolizable energy (ME) provided by barley was replaced by sodium, calcium and potassium salts of acetic acid. As the proportion of ME as acetate was increased, energy retention decreased. ME intake was 9271, 9430 and 9217 ± 67 kJ/d and energy retention was 2698, 2422 and 2280 ± 71 kJ/d for the diets containing 0, 14 or 19% ME as acetate respectively. There were no differences in protein deposition. The efficiency of utilization of acetate for energy retention (k_f) was calculated by difference to be 3 and 10 ± 13% respectively for the diets containing 14 and 19% ME as acetate.

2. In a second experiment, growing lambs were given concentrate diets in which 4 or 16% ME provided by barley was replaced by salts of acetic acid, and utilization was measured by indirect calorimetry. There were no significant differences in the utilization of the diets for maintenance (k_m) or energy retention (k_f). The k_m values were 82·4 ± 2·3 and 81·2 ± 0·7%, and k_f values were 67·4 ± 4·5 and 65·8 ± 2·7% respectively for the diets providing 4 and 16% ME as acetate. The k_f of the additional acetate in the diet providing 16% ME as acetate was calculated by difference to be 54%.

3. The acetate and Ca concentrations of the rumen digesta were increased by including acetate salts in the diet, but Na and K concentrations were not affected.

4. It is concluded that the best explanation for the poor utilization of acetate in the comparative slaughter experiment is that acetate was poorly utilized for lipogenesis. The calorimetry experiment contained relatively large errors, but the results suggest that acetate may be utilized efficiently in some circumstances. It is suggested that these results and apparently conflicting results in the literature may be explained by the concept that the efficient utilization of acetate is dependent upon the supply of glucose or glucose precursor.

The efficiency with which acetic, propionic and *n*-butyric acids are utilized by ruminants in positive energy balance was first reported by Armstrong & Blaxter (1957), who measured the heat increments resulting from the infusion of dilute solutions of the acids into the rumens of sheep confined in respiration chambers. Additional energy retention was promoted with an efficiency of 33% by acetic acid, 56% by propionic acid and 62% by butyric acid. When two mixtures of the three acids containing 59 or 14% energy as acetic acid were infused, they were utilized with an efficiency of 32 and 58% respectively (Armstrong, Blaxter, Graham & Wainman, 1958). These findings provided a very satisfactory explanation of the fact that the metabolizable energy (ME) of diets which promote a high-acetate fermentation is less efficiently utilized by fattening ruminants than is that from diets which promote a high-propionate fermentation (Blaxter & Wainman, 1964).

In subsequent experiments in which an assessment of growth has been the main criterion, no difference could be found between the three acids when these were given by infusion (Rook, Balch, Campling & Fisher, 1963), or as sodium and calcium salts mixed into the diet (Ørskov & Allen, 1966*a*, *b*, *c*; Ørskov, Hovell & Allen, 1966),

although Ørskov & Allen (1966*c*) and Poole & Allen (1970) found that acetate, when included in a high-concentrate diet, was more efficiently utilized than when included in a low-concentrate diet. In a comparative slaughter experiment, Bull, Reid & Johnson (1970) supplemented diets of hay alone or hay and maize with triacetin and were unable to find differences in utilization by sheep of the two basal diets, or of the supplemented and unsupplemented diets.

In the experiments reported here, salts of acetic acid were substituted for the fermentable carbohydrate of a concentrate diet given to growing lambs.

EXPERIMENTAL

In Expt 1 utilization of diets containing salts of acetic acid was measured by comparative slaughter. A preliminary report of this work has already been given (Hovell & Greenhalgh, 1970). In Expt 2 utilization of diets containing salts of acetic acid was measured by indirect calorimetry. In Expt 3 rumen volatile fatty acids (VFA) and cation concentrations were measured in sheep given the diets used in Expt 1.

Animals

Expt 1. Twenty-nine male Suffolk × Greyface lambs were weaned in May 1968 at weights between 8 and 13 kg. They were given a diet based on rolled barley and extracted soya-bean meal, and they were treated with an anthelmintic ('Nilverm', based on levamisole; ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire). No measurements of worm burden were made. At an age of about 10 weeks, five animals were allocated to an 'initial-slaughter' group and were killed at weights between 18 and 23 kg, and the remaining twenty-four animals were started on experiment at weights between 18 and 20 kg.

Expt 2. Four Suffolk × Romney wether lambs, weighing initially 35–40 kg and aged about 6 months, were accustomed to metabolism crates and then to a respiration chamber. When selected for tests they were treated with an anthelmintic as described previously.

Expt 3. Rumen samples were provided by three 2-year-old Suffolk × Greyface wether sheep which had been fitted with permanent rumen cannulas.

Treatment and design

Expt 1. The lambs were given one of three diets, 0, 14 or 19% ME as acetate (control, acetate-14 and acetate-19 respectively), at one of two levels (753 or 837 kJ assumed ME/$kg^{0.73}$ per d) and slaughtered at one of two weights (40 or 45 kg). The experiment was thus of $3 \times 2 \times 2$ factorial design and it was replicated.

Expt 2. The lambs were given each of two diets (4 or 16% ME as acetate (control and acetate respectively)) at each of two levels (335 and 670 kJ assumed ME/$kg^{0.75}$ per d), according to a 4×4 Latin-square design, for periods of 4 weeks. The design was such that the two periods on each diet and at each feeding level were contiguous (e.g. control–low, control–high, acetate–high, acetate–low). The lambs were fasted at the beginning and end of the experiment.

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

	Expts 1 and 3			Expt 2	
Diet	Control	Acetate-14	Acetate-19	Control	Acetate
Constituents (g DM/kg DM)					
Barley*	782	613	530	727	501
Extracted soya-bean meal	149	166	176	141	165
White fish meal	37	42	44	35	41
Chopped barley straw	—	—	—	50	40
Sodium acetate	—	79	119	—	109
Calcium acetate	—	61	91	—	83
Potassium acetate	—	11	17	—	15
Calcined magnesite	1	1	1	1	1
Steamed bone-flour	20	12	—	—	—
Sodium orthophosphate	—	7	15	—	—
Sodium chloride	5	2	2	5	5
Adisco†	6	6	6	6	6
Molasses	—	—	—	10	6
Dicalcium phosphate	—	—	—	14	14
Limestone	—	—	—	8	—
Composition (/kg DM)					
Crude protein (nitrogen × 6·25) (g)	194	185	182	194	189
Organic matter (g)	942	867	832	916	844
Gross energy (MJ)	18·68	17·84	17·20	18·16	17·54
Acetate energy (MJ)	0	1·67	2·16	0·39	1·79
Assumed ME‡ (MJ)	12·22	11·84	11·72	11·86	11·55
Corrected ME§ (MJ)	11·50	11·67	11·17	11·13	11·10
Acetate energy (% corrected ME)	0	14·3	19·3	3·6	15·7
Na (g)‖	3	30	51	6	35
K (g)‖	7	11	13	8	12
Ca (g)‖	10	22	27	11	30
Phosphorus (g)‖	8	8	8	8	8

All diets contained in addition (/kg DM): $ZnCO_3.2ZnO.3H_2O$ 50 mg, $MnSO_4.4H_2O$ 120 mg, $FeSO_4.7H_2O$ 100 mg, $CoSO_4.7H_2O$ 2 mg, KIO_3 0·2 mg.

DM, dry matter; ME, metabolizable energy.

* Rolled in Expt 1, ground in Expt 2.

† Proprietary supplement containing (/g): 300 μg retinol equivalent (as retinyl palmitate), 5 μg cholecalciferol.

‡ Calculated as (MJ/kg DM): barley 12·93, soya-bean meal 11·55, fish meal 9·96, molasses 11·97, barley straw 7·36; acetate 874 kJ/mol acetic acid.

§ See Tables 3 and 7 for details of correction.

‖ Calculated from reported values of constituents (Evans, 1960), and chemical composition of salts.

Expt 3. The cannulated sheep each received each of the diets used in Expt 1 for a period of 4 weeks according to a 3 × 3 Latin-square design.

Diets

Expts 1 *and* 3. The composition of the diets used is given in Table 1. The acetates were added as their anhydrous salts, and were assumed to have a ME value equal to the heat of combustion of their acid equivalent (874 kJ/mol acid). The crude protein (nitrogen × 6·25):assumed ME ratio was kept constant by adjusting the proportionality of soya-bean meal–fish meal (80:20 w/w) with barley. All the diets were pelleted; the control and acetate-14 diets were extruded through a 9·5 mm die. Considerable difficulties were experienced in pelleting the acetate-19 diet, which was eventually

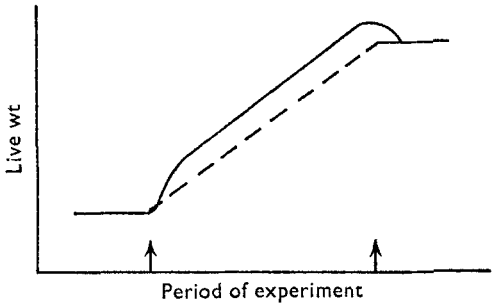

Fig. 1. Expt 2. Diagrammatic representation of the correction of live weight of the lambs for differences in gut fill when they were given the diets containing 4 (control) or 16 (acetate) % metabolizable energy (ME) as acetate at two levels (670 and 335 kJ assumed ME/$kg^{0.75}$ per d). ↑, The level of feeding was changed; (——), determined live weight; (– – –), corrected live weight.

extruded through a special short-bore mineral die (13 mm). This diet was intended to contain 21 % ME as acetate, but some acetate was lost by repeated handling. The actual acetate levels given were determined by Markham distillation (see p. 348). Two values for ME are given: (1) assumed ME, which was calculated from assumed values for the constituents, and (2) corrected ME, which was calculated from the measured digestibility of the diets multiplied by a factor derived from Expt 2 to give ME corrected for heat of fermentation.

Expt 2. The composition of the diets is given in Table 1. The diets were prepared as in Expt 1 except that all diets were pelleted (9·5 mm die) complete with minerals and vitamins, but the barley straw was excluded and given separately, and ground barley was used instead of the rolled barley as in Expt 1. Considerable difficulties were again experienced in pelleting the acetate diet, and the diets which should have contained 0 and 20 % ME as acetate were found on analysis to contain 3·6 or 15·7 % ME as acetate. This was probably because the acetate diet was processed first and some salts remained in the machinery. In this experiment corrected ME was calculated by deducting from the determined ME an amount of energy equal to 80 % of the heat of combustion of the evolved methane (Blaxter, 1967*a*). The pellets were thoroughly mixed before the experiment began.

Management

Expt 1. All animals were housed indoors in individual pens with concrete floors and bedded on sawdust. They were fed at 08.30 and 16.30 hours, water was always available, and they were weighed three times weekly before the morning meal. All animals were individually rationed and levels were adjusted weekly to live weights, which were estimated from individual cumulative growth curves. Any food not eaten, a situation which did not occur regularly after the 1st week of the experiment, was returned to the lambs and eaten during subsequent weeks.

Expt 2. The animals were housed in individual stalls with slatted floors which were of similar dimensions to the cage used in the chamber. They were given their daily ration as two equal meals at 08.30 and 16.30 hours (09.30 and 17.00 hours when in the chamber). Water was available at all times, and the animals were weighed three

times weekly before their morning meal. Since the animals grew from about 40 kg to nearly 60 kg while on experiment, and there were differences in gut fill between the two feeding levels, the live weights were plotted as a cumulative growth curve and the values used for subsequent calculations were estimated by eye from the curve corrected for fill as shown in Fig. 1. Food intakes were kept constant after the 10th day of each period, any small adjustments at this time being based on weight changes during the first 10 d of the period.

Expt 3. The cannulated sheep were individually penned, were bedded on sawdust and were offered 16·7 MJ assumed ME/d, which was equivalent to about 753 kJ/$kg^{0·75}$ per d. They were fed at 08.30 and 16.30 hours.

Health

No problems attributable to the experimental treatments were encountered.

Experimental techniques

Digestibility of diets. In Expt 1 the digestibility of the diet was measured using the animals of one replicate, towards the end of the experiment. The pens were cleared of sawdust 2 weeks before a 10 d total faeces collection was made. Faeces were stored at 1° until the collection was complete. In Expt 2 faeces were collected during the 3 d each animal was in the chamber and for the next 4 d, and were stored at −10°.

Slaughter and processing (*Expt* 1). The 'initial-slaughter' group was killed at the Institute, and the rest of the animals at a commercial abattoir. Killing was by captive-bolt gun, and the throat was then cut for bleeding. All components were weighed immediately or sealed into polyethylene bags and weighed on return to the Institute. The head was severed from the neck at the anterior face of the atlas vertebra. The feet were severed from the carcass at the proximal end of the metacarpals–tarsals. The carcass was split down the centre of the spinal column, the right side being retained for chemical analysis. The animals were processed and analysed as four components: (*a*) right half of the carcass, (*b*) skin, (*c*) wool, (*d*) all the remaining components (the non-carcass remainder). The skin and wool were separated by keeping the pelt at room temperature for 2 or 3 d, after which time the wool could easily be pulled away from the skin.

The half-carcass, non-carcass remainder and skin were minced twice using a Wolfking B 200 LF mincer (St Lawrence Mink Farm Supply Ltd, Wolfcastle, Dyfed, Wales) with a 5 mm end-plate, mixed in a bakery-type mixer (Baker-Perkins Engineering, Peterborough), sampled into open aluminium-foil trays, and stored at −20° until analysed. The wool was chopped using the mincer with a 13 mm end-plate, mixed, and stored in a sealed polyethylene bag at 1°. The composition of the half-carcass was assumed to be representative of the whole carcass.

Calorimetry (*Expt* 2). The chamber used was one of the Institute sheep chambers (Wainman & Blaxter, 1969). Urine was continuously evacuated through a funnel strapped under the animal (Wainman & Paterson, 1963). Sulphuric acid was used as a preservative, sufficient being added to neutralize the considerable quantities of

carbonate excreted in the urine when the lambs were given the acetate diet. Heat production was calculated from oxygen consumption, carbon dioxide and methane production, and urinary N using the equation of Brouwer (1965). When the lambs were given the acetate diet, calcium carbonate was excreted in the faeces, therefore the CO_2 excreted by this process was estimated and included in the final calculation of heat production, although the correction made never exceeded 1% of total heat production. A correction was applied for the metabolism of acetate present in the diets, as Brouwer's (1965) equation over-estimated (by 75 kJ/mol acetate) the heat evolved from the oxidation of acetate. It was assumed that the amount of acetate oxidized was in direct proportion to its contribution to ME.

The initial plan was to place the animals in the chamber for the 4th week of each period and to measure gaseous exchange over the last 5 d. However, it was found that the animals frequently refused food from about the 3rd or 4th day in the chamber. The problem was solved by placing the animals in the chamber for two periods of 3 d separated by 4 d in their stalls. Values for each 3 d period for gaseous exchange and urine excretion were analysed separately; thus two measurements (period 1 and period 2) for each diet at each level were made with each animal. Of the thirty-two measurements possible, twenty-nine were completed, although on two occasions a third period was necessary to secure two satisfactory measurements.

Rumen sampling (*Expt* 3). The rumens of the cannulated animals were sampled on the 26th and 28th days of the 28 d periods six times during the day (08.00, 09.30, 11.30, 13.30, 15.30 and 17.30 hours). The pH of each sample was taken immediately and the sample was then poisoned with mercuric chloride and stored at $-20°$. Equal amounts of the day-26 and day-28 samples were bulked for each sampling time, giving six samples/sheep per period, for chemical analysis.

Chemical analysis

Faecal and tissue samples were freeze-dried before analysis, apart from the faecal samples analysed for N and CO_2. Urine energy was determined on freeze-dried material, the urine being dried in a dish lined with polyethylene, and polyethylene and urine being burnt together in the bomb calorimeter. Rumen digesta were prepared for VFA analysis by centrifugation. N was determined by the automated Kjeldahl method of Davidson, Mathieson & Boyne (1970); fat as diethyl ether extract (Association of Official Agricultural Chemists, 1965); Ca by the automated method of Gitelman (1967); Na and potassium by flame photometry, the samples being ashed at 450° and taken up in concentrated hydrochloric acid; total VFA by steam distillation in a Markham still and titration against 0·1 M-sodium hydroxide using phenol red as the indicator; individual VFA by gas–liquid chromatography (Pye Unicam Ltd, Cambridge; with a Pye 104 ionization detector); and energy using an adiabatic bomb calorimeter (A. Gallenkamp & Co. Ltd, London EC2P 2ER), corrections were made for carbon only. Ash in tissue samples was determined by ashing at 600°, but for samples of diet and faeces the modification of Hovell & Ørskov (1972) was used which corrects for carbonate converted into oxide by the ashing process. Wool fibre was washed in hot water and detergent, dried and extracted with diethyl ether. Urine

samples were checked for ketones with Labstix (Ames Chemical Co. Ltd, Stoke Poges, Slough, Bucks.). Carbonate was determined in the faeces from Expt 2 by manometric measurement of the CO_2 evolved when the faeces were boiled with excess HCl (Hovell, 1972).

Statistical analysis

Expt 1 was analysed as a replicated $3 \times 2 \times 2$ factorial design. The interactions were always non-significant and they were included in the estimate of residual variation when standard errors were calculated. Expt 2 was analysed as two (period 1 and period 2) 4×4 Latin squares. Residual variation was that remaining after removal of variation attributable to period (squared), animal, diet, period and the square of the period × animal, diet and period interactions. Expt 3 was analysed as a 3×3 Latin square on the mean value of the six samples taken.

RESULTS

Digestibility and ME *content of diets*

Expt 1. The digestibility values are given in Table 2. The basal coefficients were calculated on the assumption that the energy and organic matter of the salts were completely digestible. The 'observed' digestibility of the acetate-19 diet was slightly lower than the predicted value, which suggests either that acetate given at high levels was not completely digested, or (and this is the more likely alternative) that the digestibility of the basal constituents was slightly reduced.

Expt 2. At the high level of feeding there was again a suggestion that the digestibility of the basal constituents was slightly reduced by acetate. This was not statistically significant for energy, but was significant ($P < 0{\cdot}01$) for N. The agreement between period 1 and period 2 was very good (0·843 and 0·848 ± 0·0045 respectively, for energy).

Utilization of the diets

Expt 1. *Comparative slaughter*

Effect of feeding level and slaughter weight. Slaughter weight had no significant effect when the results were expressed as rates of gain of body or body component. The only statistically significant effects of level of feeding were on the rates of gain (mean ± SE of difference for the 753 and 837 kJ/$kg^{0{\cdot}73}$ per d levels respectively) of the non-carcass remainder (40 and 44 ± 1·6 g/d; $P < 0{\cdot}05$), whole-body fat (40·3 and 49·7 ± 1·9 g/d; $P < 0{\cdot}001$) and whole-body energy (2293 and 2644 ± 84kJ/ d; $P < 0{\cdot}01$). Protein gains (excluding wool) were the same (25·2 g/d). None of the interactions with diet was significant and therefore the results are given as the mean of all the values for each diet.

Effect of diet. The final weights of lambs and their body components, their estimated initial weights, daily food intakes and period on experiment, are given in Table 3. The small but significant differences in assumed ME intake disappeared when these were expressed as corrected ME. There were no differences between dietary treatments in period on experiment, final empty-body-weight or hot-carcass weight. There was a progressive and significant decrease in the weight of the non-carcass remainder as the

Table 2. *Expts* 1 *and* 2. *Digestibility coefficients and corrected metabolizable energy* (ME) *content of the diets given to lambs*

	Expt 1‡				Expt 2				
					Low level of feeding (335 kJ assumed ME/kg$^{0.75}$ per d)		High level of feeding (670 kJ assumed ME/kg$^{0.75}$ per d)		
Diet†	Control	Acetate-14	Acetate-19	SE of difference between means	Control	Acetate	Control	Acetate	SE of difference between means
Digestibility coefficient									
Nitrogen									
'Observed'	0·784	0·806	0·789	0·0221	0·853	0·852	0·862	0·822**	0·0085
Organic matter									
'Observed'	0·849	0·860	0·837	0·0169	0·855	0·872*	0·845	0·846	0·0072
Basal§	0·849	0·851	0·822		0·852	0·862	0·842	0·834	
Energy									
'Observed'	0·832	0·851*	0·834	0·0090	0·838	0·860	0·841	0·844	0·0063
Basal§	0·832	0·836	0·810		0·834	0·844	0·838	0·826	
Energy (MJ/kg dry matter)									
Gross	18·68	17·84	17·20		18·16	17·54	18·16	17·54	
From basal	18·68	16·18	15·05		—	—	—	—	
Digested basal	16·54	13·52	12·19		—	—	—	—	
Corrected ME (see Table 6)									
From basal	10·88‖	9·46‖	8·53‖		10·47	9·08	11·08	9·54	
From acetate	0	1·67	2·15		0·39	1·79	0·39	1·79	
Total	10·88	11·13	10·68		10·86	10·87	11·47	11·33	

Statistical significance of the difference between acetate diets and the control diet: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

† For details of diets, see Table 1 and pp. 345–6.

‡ Mean of measurements at two levels of feeding (753 and 837 kJ/kg$^{0{\cdot}73}$ per d).

§ Basal digestibility $= \frac{A-S}{1-S}$, where A is 'observed' digestibility, S is proportion of organic matter or energy from acetate salt.

‖ Corrected ME from basal, 74% of digested energy from basal (Table 6).

Table 3. *Expt* 1. *Effect of diets containing* 14 (*acetate*-14) *or* 19 (*acetate*-19) % *of their metabolizable energy* (ME) *as acetate on the weight of male lambs initially* 10 *weeks old, and of their body components*

	Initial wt†	Diet‡			SE of difference between dietary treatments (*n* 8)
		Control	Acetate-14	Acetate-19§	
Period on experiment (d)		120	123	122	5
ME (kJ/d)					
Assumed	—	9849	9565*	9661 NS	100
Corrected	—	9272	9431	9217	96
From acetate	—	0	1318	1987	—
Wt of body and components (kg)					
Initial live wt	18·3	18·3	18·3	18·3	—
Final live wt	—	42·8	42·9	42·7	—
Empty-body-wt‖	14·1	34·0	33·6	32·8	0·70
Hot-carcass	8·1	21·0	21·2	20·4	0·48
Non-carcass remainder¶	5·7	10·9	10·4*	10·1**	0·21
Skin	0·6	2·2	2·0	2·3	0·19
Wool fibre	0·24	1·02	0·93	1·02	0·09
Wool grease	0·03	0·14	0·12	0·10*	0·02
Blood	0·82	1·76	1·59	1·74	0·09
Head + feet	1·80	2·90	2·94	2·95	0·08
Gastrointestinal tract††	1·94	4·45	4·31	3·95*	0·18
Pluck‡‡	0·83	1·74	1·55**	1·46***	0·04

NS, $P < 0{\cdot}1$; * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.
† Calculated for an animal of 18·3 kg.
‡ For details of diets, see Table 1 and pp. 345–6.
§ Includes values for casualty killed at 43·9 kg instead of 45 kg.
‖ Wool-free.
¶ For details, see p. 347.
†† Included alimentary tract, blood, abdominal fat, pancreas, spleen, sex organs.
‡‡ Included liver, heart, lungs, trachea.

proportion of dietary acetate was increased. When the non-carcass remainder was analysed as its four components (head + feet, pluck (liver, heart, lungs, trachea), blood and gastrointestinal tract), about two-thirds of this decrease could be accounted for by the gastrointestinal tract component (alimentary tract, blood, abdominal fat, pancreas, spleen, sex organs), and one-third by the pluck. There was a significant decrease in the weight of wool grease as the level of acetate was increased, although it should be emphasized that this merely represents the difference between secretion into, and loss from, the pelt. The pelts (skin + wool) of the animals given the acetate-14 diet contained about 0·5 kg, and of those given the acetate-19 diet about 1 kg more moisture and dirt than did those of the control animals. This was probably because the animals on the salt diets drank more water and voided more urine, with the result that their pens quickly became fouled.

The rates of gain of empty-body-weight, and of hot-carcass and non-carcass remainder weights (Table 4) were all reduced by increasing the level of acetate, although the differences were significant only for the gain in weight of non-carcass remainder. When the composition of the gains was analysed, clear differences became apparent. As the amount of dietary energy contributed by acetate increased, the rate of fat

Table 4. *Expt* 1. *Effect of diets containing* 14 (*acetate-14*) *or* 19 (*acetate-19*) % *of their metabolizable energy* (ME) *as acetate on growth rate of* 10-*week-old male lambs, and the composition of the gains made*

	Diet†			SE of difference between dietary treatments (*n* 8)
	Control	Acetate-14	Acetate-19	
Corrected ME (kJ/d)	9272	9431	9217	96
Rate of gain (g/d)				
Empty body	169	160	154	8·7
Hot-carcass	109	107	101	5·5
Non-carcass remainder‡	46	41*	39**	2·0
Empty body				
Water	86·6	84·9	82·7	6·6
Protein§	25·9	24·7	25·0	1·8
Ash	6·5	7·6	7·3	0·7
Fat	50·3	44·5*	40·2**	2·3
Energy (kJ/d)‖	2699	2423*	2280**	100

* $P < 0.05$, ** $P < 0.01$.
† For details of diets, see Table 1 and pp. 345–6.
‡ For details, see p. 347.
§ Nitrogen × 6·25, excludes wool protein.
‖ Calculated from values of Paladines, Reid, Bensadoun & Van Niekerk (1964); includes wool protein, but not wool grease.

deposition decreased; the difference between the fat deposition with control and acetate-19 treatments (10 g/d) was highly significant. There were no significant differences between dietary treatments in the rates of gain of water, protein or ash. The group given the acetate-19 diet retained 15% less energy (418 kJ) than the controls when given an almost identical amount of ME. Acetate reduced fat deposition both in the carcass, which contained about three-quarters of the total body fat, and in the non-carcass component.

There were no differences between treatments in the Na:N and K:N ratios for the carcasses; these values were (mean ± SE of difference) 2·34, 2·40, 2·41 ± 0·08 mmol K/g N and 1·75, 1·80, 1·79 ± 0·08 mmol Na/g N for the control, acetate-14 and acetate-19 treatments respectively. The values for K:N ratio agree well with a mean value of 2·38 mmol K/g N calculated from the values of Kirton & Pearson (1963).

Utilization of dietary energy. Table 5 gives the energy balance results expressed on a metabolic body-weight basis. Median live weight was calculated as follows: the empty-body-weight of each animal at the mid-point of the experimental period was calculated and was converted into live weight (empty body + fleece + gastrointestinal tract contents) by using a factor derived from the final empty-body-weight and final live weight of the control animals. Therefore, median live weight has no bias due to differences in gut fill and wool contamination.

The efficiency with which dietary energy surplus to the requirement for maintenance was utilized for energy retention (k_f; Agricultural Research Council, 1965), was calculated on the assumption that the requirement for maintenance was 311 kJ corrected ME/$kg^{0.75}$ per d (Expt 2). All calculations have been based on corrected ME, because this corrects for bias due to differences in digestibility and differences in heat

Table 5. *Expt* 1. *Utilization of dietary energy by* 10*-week-old male lambs given the control diet* (*without acetate*) *or diets containing* 14 (*acetate*-14) *or* 19 (*acetate*-19) % *of their metabolizable energy* (ME) *as acetate*

	Diet†			SE of difference between dietary treatments (*n* 8)
	Control	Acetate-14	Acetate-19	
Median live wt ($kg^{0.75}$)‡	12·94	12·71	12·65	0·14
Energy ($kJ/kg^{0.75}$ per d)				
Corrected ME intake	719	743 NS	729	12
ME used for maintenance	311	311	311	—
ME used for gain (A)	408	432	418	—
ME retained (B)	210	190*	180***	6
Efficiency, k_f (100B/A)	51·5	44·5*	43·4**	2·4
Energy ($kJ/kg^{0.75}$ per d)				
ME used for gain from basal	408	370	337	13
ME used for gain from acetate	—	62	81	2
ME retained from basal	210	191	174	7
ME retained from acetate	—	−1	6	11
k_f of acetate§	—	3·0	10·1	18·1

k_f, Units of energy retained per 100 units of energy given above the maintenance requirement (Agricultural Research Council, 1965).

NS, $P < 0·1$; * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

† For details of diets, see Table 1 and pp. 345–6.

‡ For details of calculation, see p. 352.

§ These values represent the means of individually calculated k_f values, therefore do not correspond exactly with the other values given.

of fermentation. There was a clear and significant decrease in k_f as the level of dietary acetate was increased, and an attempt was made to calculate the k_f of acetate. The method of calculation used was to estimate the energy retention to be expected from the basal constituents of each diet and to attribute any difference to energy retention from the acetate salts. The calculated values were then analysed by a normal analysis of variance. The k_f of the basal constituents of the acetate diets was assumed to be that of the control (51·5), and it was also assumed that the partition of nutrients between maintenance and production was the same for the basal constituents and acetate. The k_f values for acetate given in Table 5 indicate that the utilization of acetate calculated by this method was very poor, and that there were no significant differences between results with the two acetate diets. It should be emphasized that the errors implicit in this type of calculation are very large, and the individual k_f values for acetate ranged from −66 to +52.

Expt 2. *Indirect calorimetry*

Metabolizability of diets. Results are given in Table 6. All ME values were corrected for heat of fermentation, thus:

ME = gross energy − faecal energy − urine energy − 1·8 × methane energy (Blaxter, 1967*a*).

There were no significant differences between diets in their metabolizability when this was expressed as a proportion of the basal (barley + soya-bean meal + fish meal +

Table 6. *Expt 2. Utilization of dietary energy by 6-month-old wether lambs given the diets containing 4 (control) or 16 (acetate) % of their metabolizable energy (ME) as acetate, at two levels of feeding (low and high)*

	Low level of feeding (335 kJ assumed ME/kg$^{0.75}$ per d)		High level of feeding (670 kJ assumed ME/kg$^{0.75}$ per d)		SE of difference between means (n 8)
Diet†	Control	Acetate	Control	Acetate	
Live wt (kg$^{0.75}$)	18·4	18·3	18·8	18·7	0·5
GE (kJ/kg$^{0.75}$ per d)	122·1	122·4	245·7	239·6*	2·5
Energy (kJ/100 kJ GE)					
Total digested (DE)	83·8	85·2	82·8	84·6	1·1
From acetates	2·1	8·7	2·1	8·7	—
Methane	10·4	9·5	9·0	7·4	0·9
Urine	5·9	8·0**	5·2	6·4 NS	0·6
Corrected ME‡	59·3	61·4	62·1	64·8	2·0
Basal ME (% basal DE)§	70·2	68·3	74·5	73·3	2·8
Methane energy (% basal DE)§	12·7	12·6	11·1	10·0	1·2
Urine VFA (mmol/100 kJ GE)‖	0·26	0·24	0·30	0·19	0·08
Diets (kJ/kg DM)					
GE	4341	4191	4341	4191	
Corrected ME	2579	2599	2742	2709	
From acetates	94	427	94	427	
From acetates (% ME)	3·6	16·4	3·4	15·8	

VFA, volatile fatty acids; GE, gross energy; DE, digestible energy.
NS, $P < 0.1$; * $P < 0.05$; ** $P < 0.01$.
† For details of diets, see Table 1 and pp. 345–6.
‡ ME corrected for heat of fermentation (80% methane energy (Blaxter, 1967*a*)).
§ Calculated for basal constituents assuming acetates 100% absorbed.
‖ By Markham distillation of urine (see p. 348); all VFA calculated as acetic acid.

straw) component. Corrected ME amounted to 74·5 and 73·3% digestible energy (DE) of the basal component of the control and acetate diets respectively at the high level of feeding. An average value of 74% DE was used in the calculation of corrected ME for Expt 1 (Table 2). The acetate diet produced significantly ($P < 0.05$) greater losses of energy in the urine, although these were quantitatively small (1–2% DE). The difference was not due to differences in the amount of VFA in the urine or of urinary N (Table 6). Nor were the differences due to ketones, for the difference calculated as acetone would have been equivalent to a concentration of 2·7–3·4 mmol/l urine, which is within the stated sensitivity of the method used to analyse the urine. No further analyses were undertaken; therefore the reason for the greater loss of urine energy from the acetate diet is not known.

Fasting catabolism. Fasting catabolism (heat production + energy lost in the urine) was used as the measure of basal metabolism in all the calculations made here, and was defined as the energy catabolism which occurred during the period between 88 and 136 h after the last meal. During this period methane production was less than 2 l/24 h. The lambs were fed at the maintenance level for at least 1 week before being fasted. The results for the two fasting periods are given in Table 7.

Utilization of ME. Values for the efficiency with which ME was utilized to spare the

Table 7. *Expt 2. Fasting catabolism (kJ/kg$^{0.75}$ per d) of 6-month-old wether lambs fasted at the beginning (fasting period 1) and end (fasting period 2) of the experiment**

	Fasting period 1				Fasting period 2				
Lamb	Wt (kg$^{0.75}$)	Heat	Urine	Total	Wt (kg$^{0.75}$)	Heat	Urine	Total	Mean
A	16·36	240	22	262	21·35	231	16	247	255
B	16·44	247	23	270	20·70	235	13	249	259
C	14·95	216	15	231	18·95	232	17	249	240
D	15·67	249	31	279	20·37	230	21	249	265
Mean	15·86	238	23	260	20·34	232	17	248	255

* For details of experimental procedure, see p. 347.

catabolism of body energy reserves when the animal was at or below energy equilibrium (k_m; Agricultural Research Council, 1965) and k_f (the efficiency of ME utilization for energy retention) are given in Table 8.

Maintenance. The k_m values given in Table 8 were calculated for individual determinations as:

$$k_m = \frac{\text{fasting catabolism} - \text{energy loss}}{\text{corrected ME intake}} \times 100.$$

Fasting catabolism was corrected for time with the assumption that the change between fasting periods 1 and 2 was linear. The k_f and k_m values given in Table 8 are the means of the average value (treatment periods 1 and 2) calculated for each lamb, with the exception of the k_m for the acetate diet, which is the mean of three lambs only. Only one measurement was made on one of the lambs with the acetate diet at the low level of feeding and the k_m calculated was 59·7%. This value was excluded from the mean, since it was twenty units below the values obtained with the other three lambs.

There was no significant difference between diets, and the results were combined to give one value (k_m 81·9 ± 1·7%). This value when combined with the mean fasting catabolism gave a maintenance requirement of 311 kJ corrected ME/kg$^{0.75}$ per d; this value was used in all calculations in Expt 1.

Fattening. The k_f values were calculated for individual determinations as:

$$k_f = \frac{\text{energy retained}}{\text{total ME} - \left(\frac{\text{fasting catabolism}}{0{\cdot}01 \times k_m}\right)} \times 100,$$

and mean values were obtained as described previously for k_m. Fasting catabolism was again adjusted for time, and the k_m value used was that obtained with the same animal with the same diet for the same treatment period (1 or 2). The differences between diets were small and not statistically significant.

N balance. There were no differences between diets in the amount of N retained (Table 9). Values for treatment periods 1 and 2 compared very well. The amounts of N retained corresponded to about 11–12 and 38–40 g protein/d at the low and high levels of feeding respectively (equivalent to about 21% energy retained). These values compared with about 6 g wool fibre/d and 25 g body protein/d by the control animals in Expt 1 (26% energy retained).

Table 8. *Expt 2. Utilization of corrected metabolizable energy* (ME) *by 6-month-old wether lambs given the diets containing* 4 (*control*) *or* 16 (*acetate*) % *of their metabolizable energy* (ME) *as acetate, at two levels of feeding* (*low and high*)

	Low level of feeding (335 kJ assumed ME/kg$^{0.75}$ per d)		High level of feeding (670 kJ assumed ME/kg$^{0.75}$ per d)			Treatment period		
Diet*	Control	Acetate	Control	Acetate	SE	1	2	SE
Live wt (kg$^{0.75}$)	18·37	18·27	18·80	18·67	0·49	18·38	18·68	0·35
Energy (kJ/kg$^{0.75}$ per d)								
Corrected ME intake†	303	316	638	648	17	474	478	11
Heat production (corrected)†	308	325	420	442	12	379	369	9
Balance	−5	−9	218	206	10	95	109 NS	7
Efficiency (%)								
Maintenance‡	82·4±2·3	81·2±0·7	—	—	—	—	—	—
Fattening‡	—	—	67·4±4·5	65·8±2·7	—	—	—	—

NS, $P < 0{\cdot}1$.
* For details of diets, see Table 1 and pp. 345–6.
† Corrected for heat of fermentation (Blaxter, 1967*a*).
‡ Mean values with their standard errors for treatment periods 1 and 2 calculated for individual lambs (n 4, except acetate diet at low level of feeding, n 3).

Table 9. *Expt 2. Nitrogen balance of 6-month-old wether lambs given the diets containing* 4 (*control*) *or* 16 (*acetate*) % *of their metabolizable energy* (ME) *as acetate, at two levels of feeding* (*low and high*)

	Low level of feeding (335 kJ assumed ME/kg$^{0.75}$ per d)		High level of feeding (670 kJ assumed ME/kg$^{0.75}$ per d)		SE of difference between means	Treatment period		SE of difference between means
Diet* ...	Control	Acetate	Control	Acetate		1	2	
N (mg/100 kJ ME)								
Ingested	281	276	259	265	9	272	269	6
Digested	239	234	226	212	7	228	228	5
Urine	196	202	179	157	—	184	182	—
Retained	43	33	47	54	11	44	44	8
Retained N (mg/kg$^{0.75}$ per d)	119	72	348	342	48	221	220	34

* For details of diets, see Table 1 and pp. 345–6.

Expt 3. Rumen VFA and cations

The pH levels of the digesta are shown in Fig. 2. The differences between diets were clear, the reduction in pH after feeding became progressively less marked as the proportion of acetate in the diet was increased. Total VFA concentrations are also shown in Fig. 2. There were large diurnal fluctuations when the acetate diets were fed, which tended to reflect the pattern of eating. The control and acetate-14 diets were usually completely consumed by the time the fourth sample was taken, whereas the acetate-19 diet was only about two-thirds eaten by this time, the balance being eaten before the evening meal. This pattern was similar to that obtained (subjectively) with the lambs used in Expt 1. The mean values for pH and VFA concentration as well as

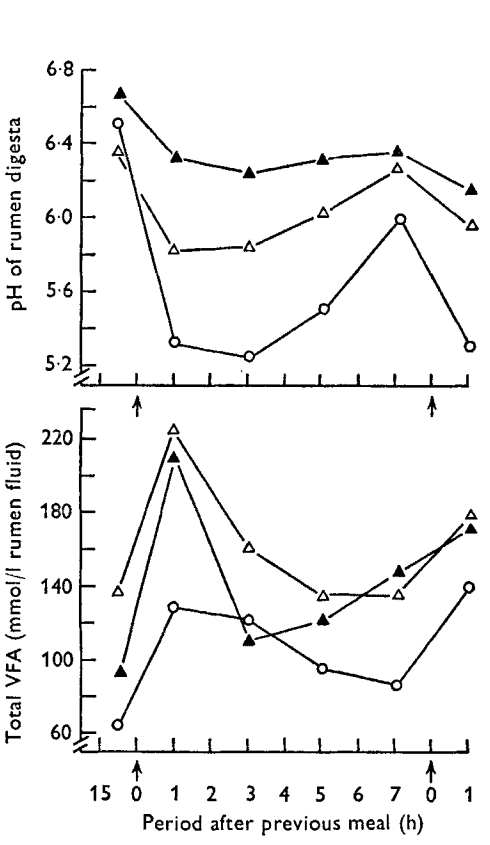

Fig. 2

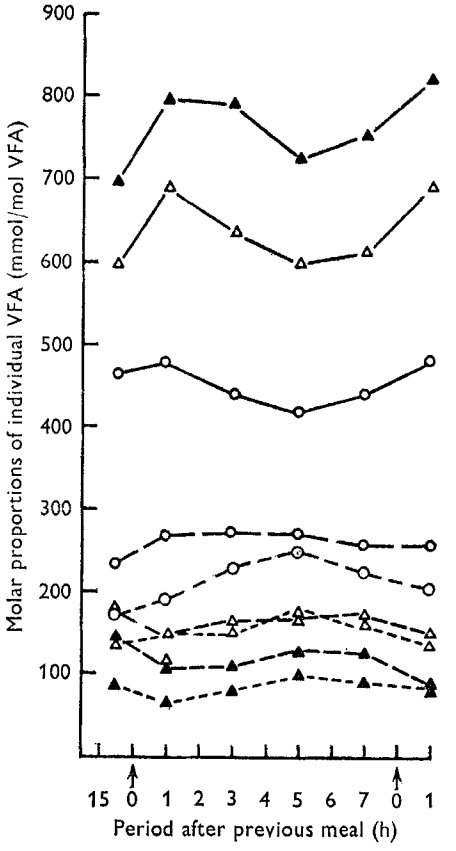

Fig. 3

Fig. 2. Expt 3. Rumen pH and total volatile fatty acids (VFA) (mmol/l) in rumen fluid from 2-year-old wether sheep given a control diet (without acetate) (○) or diets containing 14 (△) or 19 (▲) % of their metabolizable energy as acetate (for details of diets, see Table 1 and pp. 345–6). Each point is the mean value for three sheep. ↑, Daily ration fed at two equal meals (08.30 and 16.30 hours).

Fig. 3. Expt 3. Molar proportions (mmol/mol volatile fatty acids (VFA)) of acetic (——), propionic (– – –) and *n*-butyric (-----) acids in rumen fluid of 2-year-old wether sheep given a control diet (without acetate) (○) or diets containing 14 (△) or 19 (▲) % of their metabolizable energy as acetate (for details of diets, see Table 1 and pp. 345–6). Each point is the mean value for three sheep. ↑, Daily ration fed at two equal meals (08.30 and 16.30 hours).

molar proportions of rumen VFA are given in Table 10; diurnal fluctuations of the latter are shown in Fig. 3. The addition of acetate to the diet resulted in large increases in rumen acetate with concomitant decreases in the other VFA. In an attempt to determine whether the fermentation pattern was altered, the *n*-butyrate:propionate ratio was calculated (Table 10); no significant differences were apparent.

Rumen cation concentrations are shown in Fig. 4. Na and K concentrations remained very constant; the increase in Na after feeding was rapidly eliminated by dilution (drinking) and absorption. Ca concentrations in the rumen were increased considerably by the acetate diets, although the difference between the two acetate diets was not statistically significant.

Table 10. *Expt 3. Volatile fatty acid (VFA) and cation concentrations in the rumen of 2-year-old wether sheep given the control diet (without acetate) or diets containing 14 (acetate-14) or 19 (acetate-19) % of their metabolizable energy as acetate*

	Diet†			SE of difference between means (*n* 3)
	Control	Acetate-14	Acetate-19	
Rumen pH	5·65	6·06	6·32	0·21
VFA (mmol/l)	106	163**	142**	3·2
Molar composition (mmol/mol VFA):				
Acetic	449	634	762*	56·6
Propionic	253	162	115	67·2
n-Butyric	208	144**	79**	8·3
Isobutyric	21	15	12*	2·2
n-Valeric	32	15**	10**	2·1
Isovaleric	24	25	16	6·2
Caproic	14	6	4	4·8
n-Butyric:propionic	9·2	10·4	8·1	4·6
Digesta:				
DM (g/kg)	159	102*	75*	13·8
Ash (g/kg DM)	113	212**	238**	9·7
Cations (mmol/kg digesta):				
Sodium	103	114	113	7
Potassium	48	47	49	6
Calcium	44	102**	80**	3·5

DM, dry matter.
* $P < 0.05$, ** $P < 0.01$.
† For details of diets, see Table 1 and pp. 345–6.

DISCUSSION

Metabolizability of diets. There was no evidence from any of the experiments that the metabolizability of the diets was significantly altered by the substitution of salts of acetic acid for fermentable carbohydrate if the energy of the salts was assumed to be completely absorbed. High levels of Na have been found to inhibit fermentation in vitro (Walker & Forest, 1964). However, the rapid rate at which rumen Na was equilibrated suggests that, at the levels of Na supplementation used here, inhibition in vivo would have been minimal. Differences in the digestibility of the basal constituents of the diets were small and not statistically significant and there were no differences in methane production in Expt 2. The fact that the *n*-butyric:propionic acid ratio remained relatively constant also suggests that the fermentation of the basal constituents was not drastically altered by the addition of the salts. There was some evidence of very slightly higher losses of energy in the urine when diets containing acetates were given, although the energy lost did not appear to be in the form of VFA or ketones. At the high level of feeding in Expt 2 corrected ME was 73·9 ± 1·5 % DE (basal constituents) or 82·3 % DE when not corrected for heat of fermentation, which agrees well with the value of 82 % DE estimated from the values of Blaxter (1967*b*).

N retention. There was no effect of acetate on N retention, contrary to the reports of Rook *et al.* (1963), Ørskov & Allen (1966*c*) and Vermorel (1968).

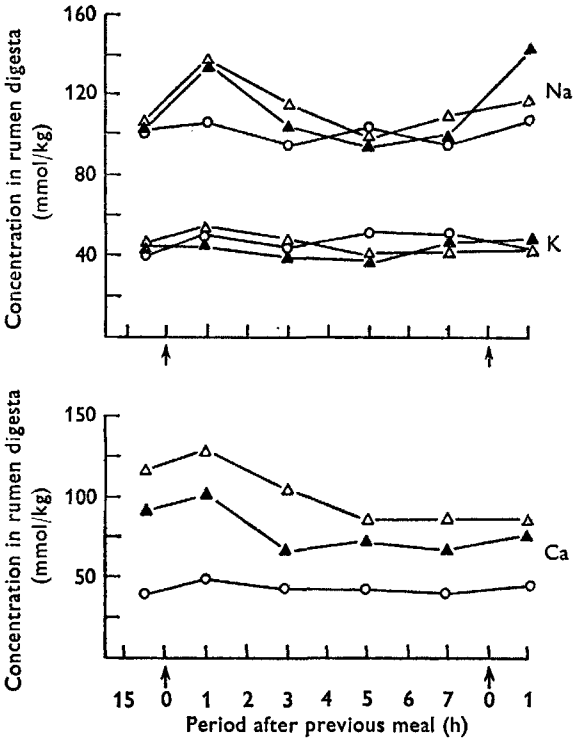

Fig. 4. Expt 3. Concentrations of sodium, potassium and calcium in rumen digesta of 2-year-old sheep given a control diet (without acetate) (○) or diets containing 14 (△) or 19 (▲) % of their metabolizable energy as acetate (for details of diets, see Table 1 and pp. 345–6). Each point is the mean value for three sheep. ↑, Daily ration fed at two equal meals (08.30 and 16.30 hours).

Utilization of ME *for maintenance.* The combined value of k_m for the two diets used in Expt 2 was $81{\cdot}9 \pm 1{\cdot}7$% and is in good agreement with that of 82·3 % given by Blaxter (1967*a*). Fasting catabolism at 255 ± 11 kJ/kg$^{0{\cdot}73}$ per d (heat production plus 20 kJ lost in urine) was not significantly lower than the value of 255 kJ/kg$^{0{\cdot}75}$ per d for the fasting metabolism (heat production only) of a 6-month-old lamb calculated from the preferred value of 264 kJ/kg$^{0{\cdot}73}$ per d given by the Agricultural Research Council (1965) for a lamb of 40 kg live weight.

Utilization of ME *for energy retention.* There are several possible reasons for the difference between experiments in the k_f of the control diets, the most likely of which is that the lambs confined within the respiration chamber had a lower maintenance requirement than that of the less closely confined and young lambs of Expt 1, which were also exposed to the draughts and other vicissitudes of the sheep house. Ørskov & McDonald (1970) estimated the maintenance requirement of such lambs to be 418 kJ ME/kg$^{0{\cdot}75}$ per d which is greater than the value of 348 kJ ME (311 kJ corrected ME)/kg per d used here. The lambs used in Expt 2 were 3–4 months older and might be expected to have had a slightly lower maintenance requirement (Agricultural Research Council, 1965). Furthermore, balance experiments and slaughter experiments have errors

which tend to accumulate to values of the same order as the differences reported here. Although the use of a different value for maintenance will alter the k_f value of the diets, it has less effect on k_f of the acetate as this is calculated by difference.

The poor utilization of ME found in Expt 1 with the acetate diets is in agreement with the conclusion of Armstrong & Blaxter (1957) that acetate is poorly utilized for lipogenesis. It is not in agreement with the findings of Ørskov & Allen (1966*a*, *b*, *c*), Ørskov *et al.* (1966), Poole & Allen (1970) and Bull *et al.* (1970). However, in Expt 2 no differences were found between the k_f values for the two diets. A recurrent problem in experiments in which the utilization of dietary energy is determined is one of precision. In the comparative slaughter experiment reported here no statistically significant differences were found until chemical analysis was used as a means of assessment (apart from the gain in weight of the non-carcass remainder). The control group gained 15 g empty-body-weight/d more than the acetate-19 group, a difference which was not statistically significant. However, two-thirds of this difference was due to the differences in fat deposition, which was statistically highly significant. There were also large errors in the calorimetry experiment. The k_f of the acetate in the acetate diet was calculated, by the difference method used in Table 6, to be 54%. However, statistically this value could have been 11 or 96%. Similar calculations made from the values of Bull *et al.* (1970) suggest that their experiment was not sensitive enough to detect differences between acetate utilized with an efficiency of 67% (the value obtained), and about 44%. A final indication of the problems of experimental precision and interpretation is provided by the fact that the k_f values obtained in Expt 1 were calculated on the assumption that the maintenance requirement was 311 kJ ME/$kg^{0.75}$ per d, which was estimated from the mean value of k_m for the two diets used in Expt 2. If this mean value is used to recalculate the k_f value for the two diets used in Expt 2, then that of the control diet is estimated as 66·7% and that of the acetate diet as 61·1%, and although these values do not differ significantly (given the same errors), the k_f value for the additional acetate of the acetate diet then becomes 21%.

Bull *et al.* (1970) suggested that a possible reason for the differences between the original infusion experiments of Armstrong and his colleagues (Armstrong & Blaxter, 1957; Armstrong *et al.* 1958), and the various comparative slaughter experiments reported, was that there was adaptation by the animals to high levels of acetate, the adaptation taking longer than the 5–7 d duration of the original infusion experiments. The comparative slaughter experiment reported here lasted over 14 weeks, which is considerably longer than the 8–15 d suggested by Bull *et al.* (1970) as being the time necessary for adaptation. A small difference in energy retention which approached statistical significance was found between treatment periods 1 and 2 in Expt 2. However, this was removed if an adjustment was made for maintenance, which suggests that the differences in heat production were associated with the activity of the animals rather than with metabolic adaptation, and that treatment period 1 acted as a retraining period for treatment period 2.

Armstrong *et al.* (1958) calculated expected values for the k_f of VFA mixtures from the values they obtained for the individual acids. These expected values were

based on the molar proportions of the mixtures, but Ørskov (1965) recalculated them from the energy proportions. The recalculated value he obtained for an acetate–propionate–butyrate mixture containing molar proportions of 75:15:10 (corresponding energy proportions 59:21:20) was $43{\cdot}6 \pm 2{\cdot}7\%$; the value obtained with this mixture ($31{\cdot}8 \pm 1{\cdot}8\%$) was significantly lower. However, the expected and 'observed' k_f values for a low-acetate mixture, containing 14% energy as acetate, were $55{\cdot}5 \pm 3{\cdot}2$ and $58{\cdot}2 \pm 1{\cdot}3\%$ respectively, which did not differ significantly. The implication is that the utilization of acetate for lipogenesis is related to its contribution to the energy available. An attractive explanation of this is that the utilization of acetate for lipogenesis is dependent upon a supply of glucose sufficient to provide (by the pentose phosphate pathway) the NADPH necessary for fatty acid synthesis (see Armstrong, 1965; Blaxter, 1967*a*; Ballard, Hanson & Kronfeld, 1969), although Ballard *et al.* (1969) comment 'Since acetate is utilized for lipogenesis in ruminant tissues in the absence of added glucose (in vitro) the source of reducing equivalents to support lipogenesis is in question'.

There is evidence that the ruminant can obtain NADPH from acetate by means of cytosolic isocitrate dehydrogenase (NADP) (*EC* 1.1.1.42) in mammary tissue (Bauman, Mellenberger & Derrig, 1973; Gumaa, Greenbaum & McLean, 1973) and in adipose tissue (Ingle, Bauman & Garrigus, 1972). Bauman *et al.* (1973) estimated that more than 50% of the NADPH required for fatty acid synthesis in ruminant mammary tissue might be produced from acetate in this way. The relative importance of this pathway and the pentose phosphate pathway for the provision of NADPH for fat synthesis by ruminant adipose tissue in vivo is difficult to evaluate. Ingle *et al.* (1972) indicated the difference between the ruminant and non-ruminant by calculating the activity ratio, glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49):isocitrate dehydrogenase (NADP) in calf, lamb and rat adipose tissue to be 1:4, 1:2 and 2:1 respectively, and suggested that these ratios might be indicative of the relative importance in the tissues of the pentose phosphate pathway and metabolism of acetate via isocitrate. A similar calculation from the results of Bauman, Brown & Davis (1970) and those of Gumaa *et al.* (1973) respectively, gives the activity ratio, glucose-6-phosphate dehydrogenase:isocitrate dehydrogenase (NADP) to be 9:1, 4:1 and 1:13 for rat, sow and cow mammary tissue respectively, and 20:1, 1:1 and 1:6 for rat mammary, rat adipose and sheep mammary tissue respectively. Although there were inherent difficulties in relating different experiments, these values suggest that the disparity between ruminants and non-ruminants is greatest in mammary tissue. Balmain, Folley & Glascock (1954) found that whereas rat mammary tissue responded to insulin by increasing the incorporation of both acetate- and glucose-C into fatty acid, sheep mammary tissue had no response, and suggested that this represented a true species difference. Ruminant adipose tissue, however, does respond to insulin; Škarda & Bartoš (1969) found that the incorporation of acetate-C into fatty acid, and the oxidation of glucose by goat adipose tissue in vitro were both increased by insulin, and suggested that this was due to stimulation of the pentose phosphate pathway. It is therefore possible that a real difference exists between ruminant adipose tissue and ruminant mammary tissue in the relative importance of

the pentose phosphate pathway and cytosolic isocitrate dehydrogenase (NADP) for the generation of NADPH. The metabolism of acetate for the generation of NADPH by the pathway proposed by Bauman *et al.* (1973) would also generate other ATP equivalents. Presumably this energy could be usefully utilized by the mammary gland for the many other processes associated with milk synthesis and secretion, but might prove an embarrassment to adipose tissue if relatively large amounts of NADPH were generated from acetate. If this is the situation, then the efficient utilization of large amounts of acetate for lipogenesis by ruminant adipose tissue will require sufficient glucose for the provision of most of the necessary NADPH. Another possibility is that the glucose:acetate ratio may be important in initiating lipogenesis or lipolysis (see Sutherland, 1967). Both these hypotheses imply that as the contribution of acetate to ME available for lipogenesis increases, an increasing proportion of acetate is wastefully oxidized, and the average efficiency of utilization decreases. This relationship between the utilization of acetate and the relative amount available provides a very satisfactory explanation for many of the published results discussed earlier (see p. 360). The use of triacetin by Bull *et al.* (1970) ensured that one C_3 molecule was provided for every three C_2 molecules, more than sufficient glucose precursor for the NADPH and glycerol synthesis necessary to utilize the acetate given; therefore the efficient utilization of triacetin-acetate was to be expected. The finding of Ørskov & Allen (1966*c*) and Poole & Allen (1970) that acetate included in a high-concentrate diet was better utilized than when included in a high-fibre diet is also relevant, since the high-concentrate diets should have provided more glucose precursor in the form of propionate.

Notwithstanding the problems of precision discussed earlier, if the difference between the experiments reported here was real, then a possible explanation might be that in Expt 2 the relationship between acetate and glucose was nearer to the optimum for efficient lipogenesis. There are no obvious reasons why this should have occurred. The diets were practically identical except for the fact that in Expt 2 the barley was ground, and a small proportion of chopped straw was included. Whether this resulted in a different fermentation pattern, or an increase in the proportion of α-linked glucose which reached the small intestine is not known. There may have been differences in the pattern of eating, for although no records were taken of eating behaviour, it was observed that the lambs in Expt 2 tended to nibble their ration over most of the day, whereas those in Expt 1 tended to consume their ration as discrete meals. If the utilization of acetate is related to the availability of glucose or glucose precursor and if, as suggested by McClymont (1952), the utilization of acetate at any moment in time is as a partition between lipogenesis and oxidation, then a large meal of rapidly absorbed acetate would be inefficiently utilized, and a situation of glucose shortage aggravated.

It must be emphasized that there is no direct evidence that there is any interaction between acetate and other metabolites, apart from the infusion results of Armstrong *et al.* (1958) already discussed, and the disagreement between Expts 1 and 2 may have been due to the errors already described, or to there having been large losses of energy in the urine, or disproportionate losses as methane by the lambs given the acetate

diets in Expt 1. However, some experimental evidence of a non-linear relationship between the utilization of acetate and its contribution to ME will be provided in another paper.

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