Utilization of salts of volatile fatty acids by growing sheep

I. Acetate, propionate and butyrate as sources of energy for young growing lambs

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1. An experiment was conducted in which sodium and calcium salts of acetic, propionic and butyric acids were given to groups of eight lambs as additions to basal diets of hay and concentrate. Two control groups were included, one group which received only the basal ration and one high-level control group which received sufficient additional concentrate to achieve growth rates greater than those of the groups receiving volatile fatty acid (VFA) salts. 2. With rumen-fistulated sheep, the effect on the rumen VFA composition and the pH of the rumen liquor, of diets supplemented with VFA salts given twice daily, was also investigated and compared with the effect of the basal diet alone. 3. The lambs receiving the VFA salts grew faster and achieved significantly greater empty body and carcass weights than those receiving only the basal rations; the high-level control group had significantly greater empty body and carcass weights than groups receiving salts of VFA. 4. There were no differences approaching significance in the efficiency of the three VFA in promoting gains in live weight, empty body weight and carcass weight. There appeared to be equal efficiency of utilization of the gross energy of the VFA and of the calculated metabolizable energy of the concentrates. 5. The effect of twice-daily feeding on the rumen VFA composition was that the concentration of the supplementary acid was greatest shortly after feeding, and about 5–6 h after feeding the effect was difficult to detect. There were no differences in the pH of the rumen liquor between animals given diets containing VFA salts and those given the basal ration.

Acetic, propionic and butyric acids, occurring as end-products of the rumen fermentation of food carbohydrate, represent a major source of energy for the ruminant. It has been demonstrated that these volatile fatty acids (VFA) have slightly different pathways of intermediary metabolism and that there are quantitative differences in the extent to which they are metabolized at different sites in the body (Annison & Lewis, 1959; Annison, Leng, Lindsay & White, 1963; Leng & Annison, 1963).

The efficiency with which sheep utilized the VFA as energy sources for maintenance and lipogenesis was studied by Armstrong & Blaxter (1957*a*, *b*), Armstrong, Blaxter & Graham (1957) and Armstrong, Blaxter, Graham & Wainman (1958). In those experiments when dilute acids were infused into the rumen of fasting sheep it was found that mixtures containing various proportions of VFA were utilized with equal efficiencies for maintenance; but in similar experiments with sheep fed above maintenance acetic acid was utilized less efficiently than either propionic or butyric acids.

Mixtures of VFA salts have been given to lambs as supplements to basal rations of hay and concentrate (Bentley, Johnson, Royal, Deatherage, Kunkle, Tyznik & Bell, 1956) and as ingredients of purified diets (Matrone, Ramsey & Wise, 1957, 1959; Essig, Hatfield & Johnson, 1959 and Essig, Garrigus & Johnson, 1962). This paper

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reports the first of a series of experiments in which the efficiency of utilization of VFA by growing sheep was studied by adding mixtures of the sodium and calcium salts of individual acids to basal diets of hay and concentrate. The influence of twice-daily feeding with the diets on the composition and concentration of the rumen VFA is also reported. A preliminary report on these experiments has been published (Ørskov, 1963).

EXPERIMENTAL

Animals and facilities. Forty wether lambs aged 10–14 weeks and weighing from 46 to 57 lb live weight at the beginning of the experiment were used. These were either pure Dorset Horn or Dorset Horn crosses. The sheep were housed in a barn in individual pens and bedded on sawdust.

Experimental design and treatments. After an initial period of 3 weeks during which the animals became accustomed to eating hay and concentrate, they were allocated on 9 June 1962 to five treatments using a randomized block design. The criterion for allocating lambs to blocks was the mean of live weights measured on 3 consecutive days. The treatments were as follows: treatment 1, basal control (given a basal diet of hay and concentrate sufficient to achieve a daily gain of 0·25 lb); treatment 2, basal + acetate; treatment 3, basal + propionate; treatment 4, basal + butyrate; treatment 5, high-level control (basal + 40% additional concentrate).

Table 1. *Chemical composition of hay and concentrate and their calculated content of metabolizable energy*

Foodstuff	Dry matter (%)	Crude protein (%)	Ether extract (%)	Crude fibre (%)	Ash (%)	Nitrogen-free extract (%)	Metabolizable energy (kcal/lb)
Hay	83·2	14·5	1·69	24·6	7·64	34·8	896
Concentrate	86·2	17·0	1·99	3·27	5·48	58·5	1194

Treatment 5 (high-level control) was included to demonstrate a potential for growth in the lambs greater than that realized by the diets supplemented with VFA.

Lambs receiving the basal control diet were fed so as to gain 0·25 lb live weight daily. This was achieved with a daily ration of 0·19 lb basal concentrate and 0·16 lb hay per 10 lb live weight for the first 60 lb of live weight, and for lambs of greater weight a further 0·06 lb basal concentrate and 0·16 lb hay per 10 lb live weight. Lambs on other treatments were given the same basal diet and, in addition, the VFA salts or additional concentrate. The lambs were weighed once weekly to the nearest 0·5 lb and feeding levels adjusted accordingly.

Composition and preparation of foods. The hay was made from a predominantly rye-grass sward cut at the flowering stage; it was chopped before feeding. The percentage composition by weight of the basal concentrate was barley 45, flaked maize 20, molassine meal 13, decorticated groundnut meal 15, white fish meal 5 and dicalcium phosphate 2; the concentrate was supplemented with vitamins A and D and prepared as $\frac{3}{8}$ in pellets. The chemical composition and calculated metabolizable energy (ME) content of the hay and basal concentrate are given in Table 1.

Using data collected by Evans (1960) and Wood (1924) the digestible energy per lb was calculated; the ME was calculated using the method suggested by Blaxter (1962).

A mixture of equal parts of the sodium and calcium salts of the VFA was used to minimize any adverse effect of a single cation. To prevent rejection by the lambs of these rather unpalatable salts, they were incorporated into the concentrate before pelleting. The levels of inclusion were such that 20% (by weight) of the acetate diet was in the form of acetate salts; propionate and butyrate were included at levels calculated to be isocaloric with acetate. The energy from VFA salts was calculated as combustible energy according to Hodgman (1962).

Management of lambs. The lambs were given hay and concentrate twice daily at 08·00 and 17·00 h. The rations were weighed once daily, half the daily ration being given in the morning and the remainder in the afternoon. Uneaten food, if any, was weighed daily. Water was offered *ad lib.* It was necessary to dose lambs with an anthelmintic (Mintic; ICI Ltd) during the experimental period to eliminate intestinal parasites. One lamb on the high-level control treatment died from unknown causes after five days on experiment. These incidences apart, the health of the lambs was good.

Slaughter procedure and carcass analysis. The lambs were slaughtered between 73 and 122 days after the experiment began. The mean experimental period was 101 days. A block of lambs was slaughtered when the lamb receiving the basal control treatment reached 80 lb live weight. The final live weight was the mean of three weighings on the day of slaughter and the 2 days previous to slaughter. At slaughter the weights of feet, pelt, pluck, liver, mesenteric and omental fat and the alimentary canal with and without its content were recorded. The carcasses were weighed between 1 and 2 h after slaughter. Empty body weight was calculated as live weight at slaughter minus the weight of the content of the alimentary canal. The carcasses were graded by an official grader of the Ministry of Agriculture, Fisheries and Food.

The specific gravity of the carcasses was determined, by the method of Kirton & Barton (1958) and Meyer (1962), as an indication of carcass composition. After 48 h of chilling, the carcasses were immersed in water held at 7° and weighed to the nearest 0·5 g.

Rumen liquor samples and VFA analysis. Two mature wether sheep weighing 140 lb each were fitted with rumen cannulas. These sheep were fed on the experimental rations in amounts sufficient to provide the same intake per unit of metabolic body size ($W^{0.73}$) as that of the lambs in the main experiment. Each ration was given for 13 days and rumen liquor samples were collected on the 11th and 13th days. Six samples were obtained from each sheep; the first sample was collected $\frac{1}{2}$ h before the first feed, the second sample $\frac{1}{2}$ h after it and then every 2 h. The last sample was obtained 1 h after the second feeding. This is further illustrated in Fig. 1. On one of the days of sampling the samples of rumen liquor were bulked for each sheep; on the other day they were individually analysed.

The rumen samples were filtered through muslin into a beaker for pH determination. Rumen liquor (20 ml) was acidified with 5 ml conc. orthophosphoric acid and filtered. Of the filtrate, 2·5 ml were transferred to a 50 ml glass-stoppered test tube and 20 ml

of diethyl ether were added. The tube was shaken, and 15 g anhydrous sodium sulphate were added to absorb water. The samples were stored at 0°. Gas–liquid chromatographic partition analysis was carried out using a modification of the apparatus described by James & Martin (1952). The method of McAnally (1943–4) was used to determine total VFA, the final distillations being carried out in a Hoskins apparatus.

RESULTS

In Table 2 the mean calculated daily intakes of ME are given, on the assumption that the ME of VFA salts is equal to the combustible energy. The sodium acetate used, which was assumed to be anhydrous, was found at a later stage to be crystalline; this accounts for the slightly lower calorific intake of acetate.

Table 2. *Mean daily intake of energy (kcal calculated metabolizable energy*) by groups of eight lambs receiving one of five diets*

Diet	Hay	Concentrate	VFA salts	Total
Basal (hay + concentrate)	915	1360		2275
Basal + acetate	913	1356	270	2539
Basal + propionate	916	1360	317	2593
Basal + butyrate	914	1356	320	2590
Basal + additional concentrate	914	1907		2821

* See p. 297.

Table 3. *Treatment means of initial and final live weight, empty body weight and carcass weight, specific gravity of the carcasses and number of carcasses graded A–C* in groups of eight lambs receiving one of five diets*

Diet	Initial live weight (lb)	Final live weight (lb)	Empty body weight (lb)	Carcass weight (lb)	Specific gravity	No. of carcasses in grade A	B	C
Basal (hay + concentrate)	53·5	79·8	64·0	35·7	1·0416	3	4	1
Basal + acetate	53·3	81·9	67·1	37·8	1·0459	3	5	0
Basal + propionate	53·7	83·0	69·1	39·1	1·0450	4	4	0
Basal + butyrate	52·9	83·3	68·4	38·8	1·0411	4	4	0
Basal + additional concentrate	53·5	88·9	73·5	41·7	1·0393	6	1	0
Standard error of the mean	±0·38	±1·36	±1·20	±0·78	±0·0028			

* As judged by an official grader of the Ministry of Agriculture, Fisheries and Food.

In Table 3 the results are given as final live weight, empty body weight and carcass weight. The specific gravity of the carcasses and the number of carcasses in each carcass grade are also given. The weights of the other components of the animals that were recorded at slaughter have not been included as they did not appear to provide additional useful information.

In the statistical analysis the missing plot technique was used to calculate values for two lambs, of which one died after 5 days on experiment and the other was found at slaughter to be a cryptorchid. No appreciable refinements of treatment differences or reduction in error could be gained by analysis of covariance to initial live weight.

The final live weights, empty body weights, and carcass weights were significantly higher for the groups receiving VFA salts than for the control group, but did not differ significantly according to which salt was employed. The weights were significantly higher still for the high-level control group.

Table 4. *Molar percentages of volatile fatty acids in bulk samples of rumen contents of groups of eight lambs and the total VFA concentration in the rumen with one of four diets*

Diet	Acetic acid	Propionic acid	Butyric acid	Total VFA (m-equiv./100 ml)
	(molar % of total VFA)			
Basal (hay + concentrate)	57·0	25·0	18·0	11·1
Basal + acetate	66·9	15·2	17·9	13·5
Basal + propionate	53·4	34·1	12·5	12·7
Basal + butyrate	51·0	22·4	26·6	12·3

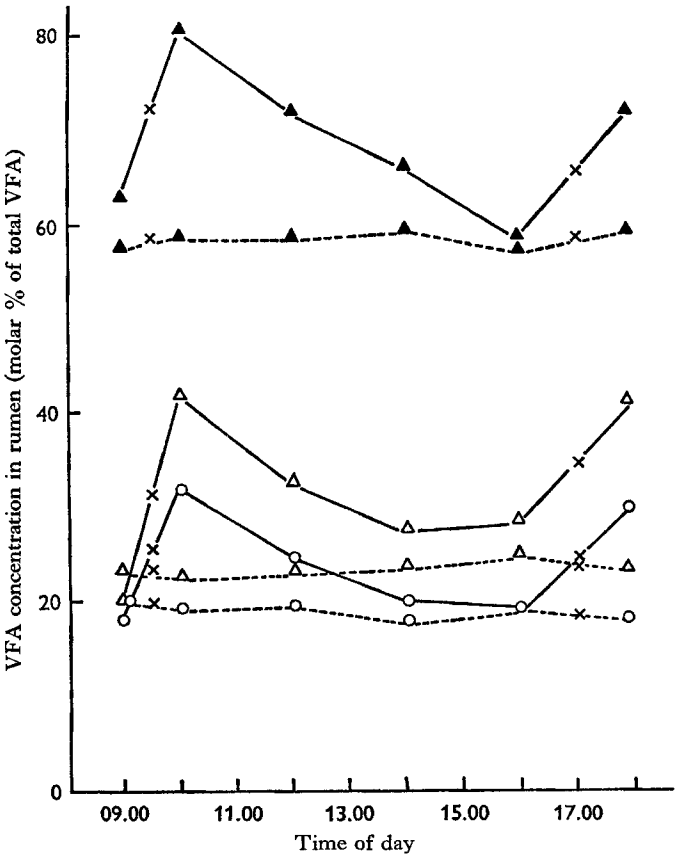

Fig. 1. Effect on the concentration in the rumen of lambs of acetic (▲—▲), propionic (△—△) and butyric (○—○) acids of the addition of acetate, propionate or butyrate respectively, to a basal diet. - - - - , basal diet; ————, supplemented diet; ×, feeding time.

The errors associated with the specific gravity determinations were large, and the variance ratio revealed that there was no significant treatment effect. No attempts were made to calculate the energy content of the carcasses although formulas have

been given from which this could be derived from the specific gravity (Meyer, 1962). The results suggest that the animals receiving the high-level control treatment produced the fattest carcasses. Lambs receiving that treatment also produced carcasses of higher grades than those of lambs receiving the other treatments.

The general effect of the VFA salt on the proportions of the individual acids and the content of total VFA in the rumen are shown in Table 4.

It can be seen that the dietary addition of a VFA salt greatly increased the molar proportion of that VFA in the rumen liquor. The effect can also be seen in the total

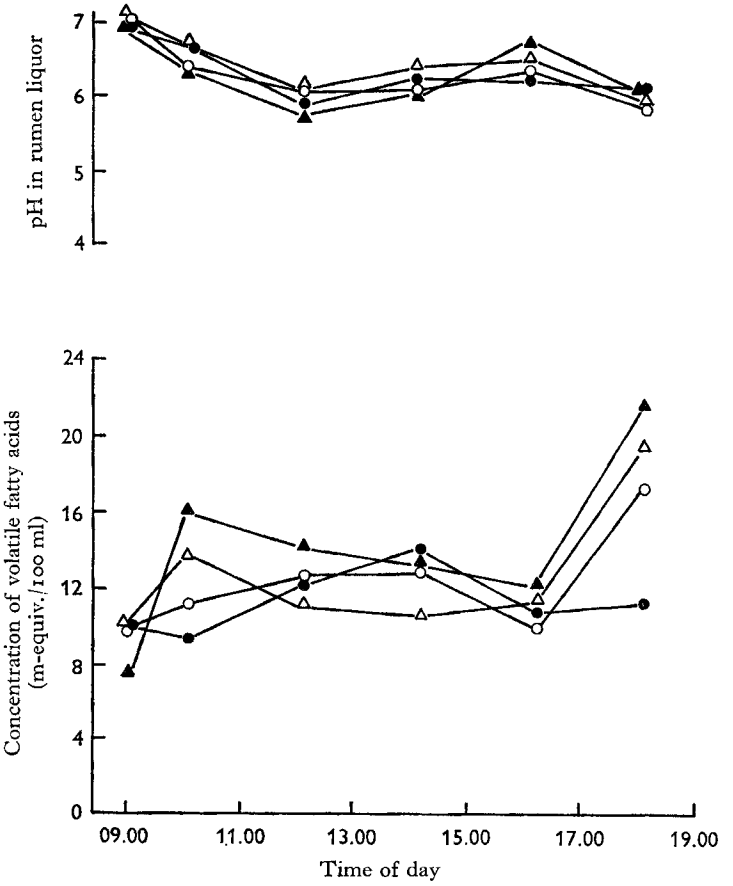

Fig. 2. Effect of supplementing a basal diet (●—●) with acetate (▲—▲), propionate (△—△) or butyrate (○—○) on the pH of the rumen liquid and the concentrations of VFA in the rumen of lambs.

VFA, descending in the order acetate to butyrate. This was expected as the molar inclusions descended in that order. The inclusion of acetate did not appear to affect the molar percentage of butyric acid and the inclusion of butyrate did not affect that of propionic acid.

Fig. 1 illustrates the effect of the different salts on the molar percentage of the relevant acid in the rumen liquor at intervals during and after feeding time. There was a very marked response in molar proportion of the included acid immediately

after feeding, but the effect could not be detected 6 h after feeding. The diurnal variation in VFA proportions with the basal diet was very small (Fig. 1).

The mean pH values in individual samples of rumen content are shown in Fig. 2. It is apparent that the supplements of VFA salts did not affect pH. The highest pH was recorded before the morning feed after which there was at first a gradual decline and then an increase towards the time of the second feed.

Concentrations of total VFA in the rumen are also given in Fig. 2 and show trends similar to those in Fig. 1.

DISCUSSION

VFA as sources of energy for growing lambs

The results show that the VFA salts were being utilized by the lambs to promote additional body gain. This was not only apparent in live-weight gain; the increases in empty body weight and carcass weight showed that there had been a real gain in body tissues. Indeed the level of statistical significance was higher for differences between the control group and the VFA treatment groups in empty body and carcass weights than for differences in live weights.

In previous experiments in which VFA salts have been given to lambs it was not possible to assess energetic efficiency, either because the addition of VFA salts or the number of replicates was too small to achieve significant increases (Bentley *et al.* 1956) or because there were no control groups and differences in intake (Matrone *et al.* 1957, 1959; Essig *et al.* 1959, 1962). In the experiment presented now the efficiency of the VFA salts is best illustrated graphically, as in Fig. 3, by plotting carcass weights on each treatment against the calculated intake of ME above that necessary for maintenance.

The mean daily ME required for maintenance was calculated to be approximately 1000 kcal for the basal control group, 1020 kcal for the VFA treatment groups and 1050 kcal for the high-level control group using the values given by Langlands, Corbett, McDonald & Pullar (1963). There was a highly significant linear relationship ($P < 0{\cdot}001$) between energy intake above the maintenance level and carcass weight with each of the five treatments, described by the equation $Y = 21{\cdot}461 + 0{\cdot}011047X$, where Y = carcass weight (lb) and X = daily ME intake (kcal) above the maintenance level. The equation shows that the energetic efficiency of the VFA was equal to that of the calculated ME of the concentrate.

Fig. 3 also shows that there were no differences in the efficiency of acetate, propionate and butyrate to promote gains in body tissues in growing lambs. This is in agreement with the results of Rook, Balch, Campling & Fisher (1963). They conducted two experiments, infusing VFA into the rumen of young growing heifers already receiving a basal diet of hay and concentrate. The interpretation of the results is difficult, however, as the increased nitrogen retention resulting from the VFA infusion was in no instance significantly greater than when only water was infused.

Our results do not, however, agree with those of Armstrong & Blaxter (1957*b*) and Armstrong *et al.* (1958), as these authors observed large differences in the energetic efficiency for lipogenesis of the different VFA which could not be confirmed in the

experiment reported here. On examining the two sets of experiments, however, it is apparent that there were differences in animals and techniques which might account for the differences in the results. Differences which might have influenced the results include: (*a*) in their experiment VFA were infused into the rumen whereas in our work salts of VFA were added to the diet; (*b*) the ratio of fat to protein synthesis in

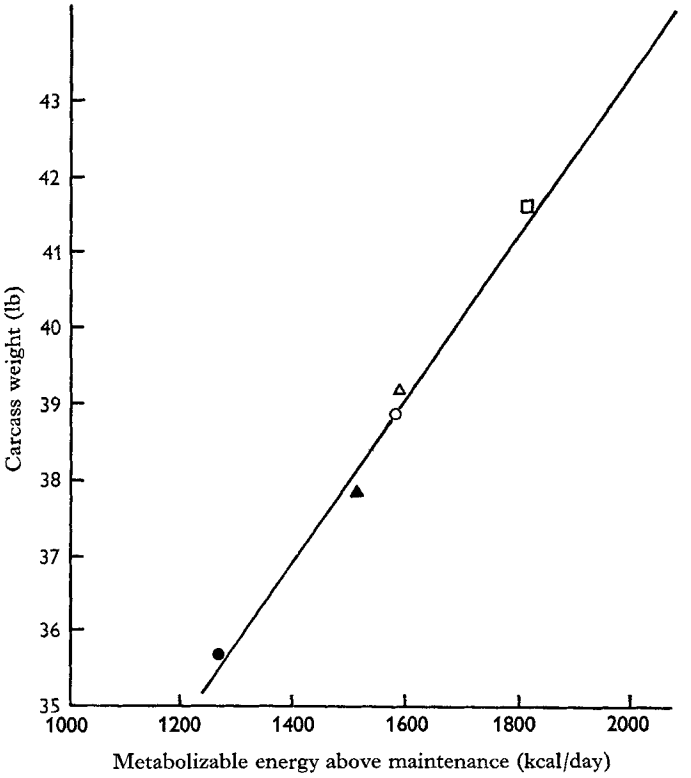

Fig. 3. Utilization by lambs for gaining carcass weight of energy from a basal control diet (●), and from the addition of acetate (▲), propionate (△), butyrate (○), or concentrate (□). Relationship: $Y = 21{\cdot}461 + 0{\cdot}011047X$.

the experimental animals, this being partly a reflection of the stage of maturity of the sheep, and (*c*) in our experiments the animals were given VFA salts twice daily, whereas in their experiments they were given free acids as a continuous infusion. Points *a*–*c* are discussed below.

Administration of salts of VFA compared to administration of VFA in solution

When the utilization of the VFA was assessed in our experiment the acids were accompanied by sodium and calcium cations which were as a consequence given far in excess of requirement. Comparison of the effects of the acids is only valid if it may be assumed that the sodium and calcium did not interfere with the digestibility or fermentation of the basal constituents with which they were included.

The additional sodium given as sodium acetate amounted to about 600 m-equiv./

day. Meyer & Weir (1954) showed, however, that sheep have a very high tolerance for sodium. They gave sodium chloride to growing ewe lambs and found no effect on live-weight gains after including 4500 m-equiv. sodium/day and Phillipson (1955) showed that sodium can be rapidly absorbed from the rumen even against a concentration gradient.

In our work it can be calculated that about 20 g of additional calcium were given daily as calcium acetate; although this addition must have substantially increased the concentration of calcium in the rumen liquor, there is no evidence to suggest an adverse effect. Nicholson, Cunningham & Friend (1962) found no effect on the utilization of a ration consisting of 4·5 kg concentrate when 100 g calcium was given daily to steers. They observed a decrease in dry-matter digestibility of the ration though the digestibility of the organic matter was unaffected. Further the results illustrated in Figs. 1 and 2 show that neither the fermentation of the basal constituents nor the pH of the rumen liquor was altered.

Ratio of fat to protein synthesis in the experimental animals

No facilities were available for assessing the ratio of fat to protein synthesis in the animals used here. Young growing animals might be expected to retain considerably more energy as protein than the mature wethers used by Armstrong & Blaxter (1957*b*) and Armstrong *et al.* (1958) in which 80–90% of the retained energy was stored as fat. As there are differences in the intermediary metabolism of the different VFA, differences of this nature may have influenced the results.

Twice-daily feeding as opposed to continuous infusion

It is shown in Figs. 1 and 2 that there were marked changes in the proportions of VFA in rumen contents associated with twice-daily feeding with VFA salts. This is in contrast to the findings with continuous infusion.

Balch & Rowland (1957) advanced the hypothesis that occasions might arise when the concentration of acetate in the blood due to rapid absorption might be as high as to cause wasteful oxidation. Fig. 1 suggests that the VFA were absorbed very rapidly; accordingly it is possible that the concentration of VFA in the blood may have exceeded the demands of tissues for synthesis, leading to oxidation or excretion which might be manifested differently by the different VFA owing to differences in sites and pathways of their metabolism.

Carcass composition

The endeavour to estimate the carcass composition indirectly using the specific gravity method provided no reliable information additional to that already available from the carcass weights. Measurement of the specific gravity was found to be difficult. Air traps under the fascia, in the front and rear flank and under the kidney fat were difficult to eliminate completely. From data given by Kirton, Barton & Rae (1962) and Meyer (1962), it can be calculated that, with the group size here employed, differences of approximately 5% fat between means could be detected statistically

using the specific gravity method. Calculated from the formula derived by Meyer (1962) the differences between treatment means hardly exceeded 3% fat in this experiment.

Addition of acetate, propionate or butyrate each had a similar effect on the proportions of the corresponding VFA in the rumen liquor (Fig. 3). The effects $\frac{1}{2}$ h after feeding were very marked—this might be expected as the acids would be liberated from their salts on the dissolution of the concentrate pencils in which they were included. About 6 h after feeding the salts were difficult to detect as the proportions of VFA were similar to those when the basal diet was given. This rapid disappearance is in accordance with the early observations made by Phillipson & McAnally (1942). They observed that on introducing sodium acetate into the rumen its effect could not be detected after an interval of 5 h. On including butyrate in the diet Essig *et al.* (1959, 1962) were unable to detect changes in the butyric acid proportion in the rumen. Considering that they obtained rumen liquor samples either 14 or 7 h after feeding, the results here suggest that the butyric acid was absorbed before sampling.

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