Utilization of salts of volatile fatty acids by growing sheep

2.* Effect of stage of maturity and hormone implantation on the utilization of volatile fatty acid salts as sources of energy for growth and fattening

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1. In two experiments with growing and fattening lambs sodium and calcium salts of volatile fatty acids (VFA) were added to a basal diet of hay and concentrate. In Expt 1 the lambs were approaching maturity, and the utilization of salts of acetic, propionic and butyric acids was studied. In Expt 2 the animals were younger; the experimental groups received acetate or propionate, and half of them were implanted with hexoestrol. The chemical composition of the carcass was assessed with the loin as a representative joint. The influence of the VFA salts on the digestibility of the basal ration was also investigated. 2. In both experiments the lambs receiving supplements of VFA salts grew faster, and their empty body weights and carcass weights were significantly greater than in lambs receiving the basal diet. 3. The energy derived from acetate and propionate was utilized more efficiently to promote carcass gains than the calculated metabolizable energy above maintenance of the basal ration. There were no differences in the utilization of energy from the different VFA, except in Expt 1 in which butyrate was utilized somewhat less efficiently than acetate and propionate. 4. Hexoestrol implantation resulted in faster growth, and highly significantly greater empty body weights and carcass weights. There was no evidence of an interaction between the VFA and hexoestrol treatments. 5. There were no differences between the VFA treatments in the composition of the carcass gains as judged by the composition of the loins, except in Expt 1 in which lambs receiving acetate tended to be the fattest. The coefficient of variation in loin fat percentages was large in both experiments, but, in Expt 1 in which both ewe and wether lambs were used, the coefficient of variation among the wether lambs was four times that of the ewe lambs. 6. Additions of VFA salts to the diet resulted in a significant increase in the excretion of ash.

Ørskov & Allen (1966) found that young growing lambs utilized the energy from volatile fatty acid (VFA) salts with an efficiency equal to that of the calculated metabolizable energy (ME) of concentrates, and that there were no differences in the utilization of acetate, propionate and butyrate in promoting gains in body tissues. Armstrong & Blaxter (1957) and Armstrong, Blaxter, Graham & Wainman (1958) infused VFA into the rumen of mature wether sheep and found that for lipogenesis, acetic acid was utilized very inefficiently when compared with propionic and butyric acids.

It was postulated by Ørskov & Allen (1966) that the difference between their results and those of Armstrong & Blaxter (1957) and Armstrong *et al.* (1958) might be a consequence of differences in the ratio of protein to fat synthesis in the experimental animals. These workers used mature wethers in which 80–90 % of the energy retained was stored as fat. Although no information was available from which the relative importance of fat and protein synthesis could be calculated in the work of Ørskov &

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Allen, it might be expected that young growing animals would retain more energy as protein than the mature animals used in the experiments of Armstrong & Blaxter (1957) and Armstrong *et al.* (1958).

The utilization of VFA salts has now been studied in one experiment in which the sheep were more mature than those in our earlier work and in a second experiment an attempt was made to create differences in tissue anabolism by implanting half of the animals with hexoestrol to study the utilization of the VFA under these conditions. Gee & Preston (1957) demonstrated that lambs implanted with hexoestrol laid down more protein, moisture and bone and less fat than a similarly fed control group.

A preliminary report of the second experiment was given by Hovell (1964).

EXPERIMENTAL

Animals and facilities. All animals were housed under cover and were individually penned and fed. Sawdust was used as bedding. In Expt 1, twenty-five ewe lambs and fifteen wether lambs were used. They were Dorset Horn, or Dorset Horn crosses, were 35–40 weeks of age and weighed from 65 to 91 lb at the start of the experiment. The experiment was carried out during the winter of 1962–3. In Expt 2, fifty-six wether lambs were used. They were Dorset Horn, Dorset Horn crosses and Suffolk crosses, which were initially 10–14 weeks of age and weighed between 40 and 68 lb.

Design and treatments. In both experiments a period of 3 weeks was allowed for the lambs to become accustomed to eating hay and concentrate. Eight animals were allocated to each treatment and a randomized block design was used. Lambs were allocated to blocks according to the average live weight on 3 consecutive days and to sex. To allow an estimate of the gains in carcass weight and components, an initial slaughter group was included in both experiments.

The treatments in Expt 1 were: treatment 1, initial slaughter group; treatment 2, basal (hay + concentrate) fed to gain approximately 0·3 lb per head per day; treatment 3, basal + acetate; treatment 4, basal + propionate; treatment 5, basal + butyrate.

The treatments in Expt 2 were: treatment 1, initial slaughter group; treatment 2, basal (hay + concentrate) fed to gain approximately 0·25 lb per head per day; treatment 3, basal, implanted with 15 mg hexoestrol; treatment 4, basal + acetate; treatment 5, basal + acetate, implanted with 15 mg hexeostrol; treatment 6, basal + propionate; treatment 7, basal + propionate, implanted with 15 mg hexeostrol.

In both experiments the levels of feeding were set by the lambs receiving the basal control diet; the amounts of food given to these lambs were adjusted every 2 weeks to achieve a predetermined growth rate; in Expt 2 this was that of the unimplanted group. In Expt 1 the lambs required 0·16 lb of concentrate and 0·13 lb of hay per 10 lb weight to achieve the required growth rate. In Expt 2 they required 0·19 lb of concentrates and 0·13 lb of hay per 10 lb live weight until they reached 50 lb; per 10 lb live weight above 50 lb they received 0·13 lb of concentrate and 0·09 lb of hay. Groups of lambs receiving the supplemented diets were given the same amount of basal diet together with their respective VFA salts.

Composition and preparation of the food. The chemical composition of the hay, which was given chopped, is given in Table 1. The composition and ingredients of the concentrate mixture were similar to those of the mixture given in a previous experiment (Ørskov & Allen, 1966).

The mixtures of the sodium and calcium salts of the VFA were included to supply about 400 kcal daily in Expt 1 and about 350 kcal daily in Expt 2.

Table 1. *Expts 1 and 2. Chemical composition and calculated metabolizable energy of the hay*

Expt no.	Dry matter (%)	Crude protein (%)	Ether extract (%)	Crude fibre (%)	Ash (%)	Nitrogen-free extract (%)	Metabolizable energy (kcal/lb)
1	86·0	14·0	2·16	23·8	7·91	38·0	923
2	82·2	10·1	1·42	26·3	7·64	36·7	863

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

Management of the lambs. The lambs were given hay and concentrate twice daily at 08.30 and 17.00 h. Water was offered *ad lib.* Uneaten food was recorded daily. Initially small quantities of concentrate were left uneaten, and the same amounts by weight were added to the diet of the same lambs at a later stage in the experimental period. The lambs were weighed every 2 weeks to the nearest 0·5 lb, and feeding levels adjusted accordingly. The lambs were dosed with an anthelmintic (Mintic; ICI, Ltd) before the experiment and no clinical signs of worm infestation occurred. In the first experiment one lamb was taken off the experiment after suffering from pneumonia and one lamb died as a result of copper poisoning. The incidence of copper toxicity was observed in other sheep by Ørskov (1965) who showed that in a group of animals receiving hay and concentrate indoors, liver copper levels were eight times higher (1027 ppm in liver dry matter) than in a comparable group grazing outdoors (127 ppm) even though the mean copper content of the hay and concentrate was only 10–12 ppm. Of the lambs in Expt 2, two lambs died from unknown causes, one as a result of urinary calculi and one owing to physical injury.

Digestibility trials. In the second half of the experimental period in Expt 2, four blocks of lambs were in turn put into digestibility crates where they remained for 13 days with no alteration in food intake or management. After 3 days in the crates they were harnessed and the faeces were collected for a period of 10 days. Of the daily output of faeces, 10% was retained for determination of dry matter and ash content. The ash was determined as for food according to the Fertilizer and Feedingstuffs Regulations (Great Britain, Parliament, 1960).

Slaughter procedure and carcass analysis. The initial slaughter groups were killed when the experiment began. The lambs in Expt 2 were slaughtered after an experimental period of 99 days and those in Expt 1 were slaughtered over a period of 4 days after 103–106 days on experiment.

In the slaughterhouse the weights of omental and mesenteric fat, and of the alimentary tract full and empty were recorded. Pelt weights were recorded in Expt 2.

The final live weight was taken as the average of the live weight on day of slaughter and on 2 days previous to slaughter. The empty body weight was calculated as live weight at slaughter minus weight of gut content. The carcasses were graded by an official grader of the Ministry of Agriculture, Fisheries and Food and weighed between 1 and 2 h after slaughter.

After 24 h of chilling, the carcasses were split and the loins of the left sides removed by cutting at the joint between the penultimate and last lumbar vertebras and between last thoracic and first lumbar vertebras (see Kirton & Barton, 1962). The loins were chopped and put three times through a commercial mincer, the final mincing being through a 1 mm screen. After thorough mixing, three 50 g subsamples were taken for fat and protein analysis. The protein was determined by the Kjeldahl procedure and the fat and dry-matter contents of the samples were determined by the method of Callow (1947) except that the initial drying was done in a vacuum oven at 60°.

RESULTS

In Tables 2 and 3 the mean daily intake of hay, concentrate and VFA calculated as ME is given (ME of VFA salts was assumed to be equal to combustible energy; see Hodgman, 1962). The sodium acetate purchased as anhydrous was later found to contain water of crystallization. This resulted in a slightly lower calorific intake of acetate.

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

Diet	Hay	Concentrates	VFA salts	Total
Basal diet (hay + concentrate)	1000	1724	—	2724
Basal + acetate	1006	1722	343	3071
Basal + propionate	1005	1724	402	3131
Basal + butyrate	1005	1721	405	3131

* See p. 309.

Expt 1

In Table 4 the main results of Expt 1 are given. Missing values were calculated for the two lambs which died. A small reduction in error was achieved by analysis of covariance to initial weight for final empty body and carcass weight.

The VFA salt supplements resulted in significantly greater gains in live weight, empty body and carcass weight. The differences between the basal control group and the groups having received acetate and propionate was very highly significant ($P < 0.001$). The differences in live weight and empty body weight between the basal control group and the group receiving butyrate was highly significant ($P < 0.01$). The difference in carcass weight was significant ($P = 0.05$). Lambs receiving butyrate had gained less weight than those receiving acetate and propionate, but these differences were not significant ($P > 0.05$). The difference in empty body weight and final live weight between the propionate treatment and butyrate treatment reached significance ($P < 0.05$).

The mean values for the fat content of the loin were adjusted according to variations within sexes. The variation in fat content was significantly greater among wether lambs than among ewe lambs, the coefficients of variation being 20·6 and 5·8 % respectively. The fat percentages of lambs of each sex were therefore analysed separately and the two means weighted according to the ratio of variance about the means. This was valid as there was no sex × treatment interaction. The loins of ewe

Table 3. *Expt 2. Mean daily intake of energy (kcal calculated metabolizable energy*) with each of three diets by lambs with or without implants of 15 mg hexoestrol*

Diet	Hay	Concentrates	VFA salts	Total
Basal (hay + concentrate):				
Without implant	659	1520	—	2179
With implant	667	1520	—	2187
Basal + acetate:				
Without implant	674	1575	301	2490
With implant	672	1520	302	2494
Basal + propionate:				
Without implant	665	1516	356	2537
With implant	659	1519	357	2535

* See p. 309.

Table 4. *Expt 1. Treatment means of intial and final live weight, empty body weight and carcass weight, fat and protein percentage in the loin, and number of carcasses graded A–C* in groups of eight lambs slaughtered initially or receiving one of four diets*

Diet	Initial live weight (lb)	Final live weight (lb)	Empty body weight (lb)†	Carcass weight (lb)†	Loin fat (%)	Loin protein (%)	No. of carcasses in grade: A	B	C
Initial slaughter group	77·9	—	68·2	37·5	21·9	15·0	4	4	0
Basal (hay + concentrate)	75·8	103·8	91·1	52·3	32·0	15·0	6	2	0
Basal + acetate	77·1	112·2	100·1	57·0	33·8	14·0	6	1	0
Basal + propionate	77·0	113·9	101·1	57·3	29·8	14·4	8	0	0
Basal + butyrate	76·7	109·7	96·5	55·2	29·6	14·3	7	0	0
Standard error of difference between means‡	±0·81	±1·40	±1·50	±1·26	±1·05	±0·45			

* As judged by an official grader of the Ministry of Agriculture, Fisheries and Food.
† Initial slaughter group not included in analysis of variance.
‡ Standard errors were adjusted by a covariance procedure (see above).

lambs did, however, contain more fat (29·63 %) than those of the wether lambs (25·83 %). The initial slaughter group had lower fat contents in the loin than the groups receiving the other diets. The loins of lambs receiving acetate were significantly fatter than those of lambs receiving propionate or butyrate ($P < 0·05$).

The content of protein in the loin did not differ significantly between treatments but the protein percentage was significantly higher in the initial slaughter group than in the other four groups ($P < 0·05$). The carcasses of the initial slaughter group were graded lower than those of the treated groups. The gains in fat and protein tissues were calculated from the formulas of Kirton & Barton (1962) for calculating carcass fat and protein percentages from the composition of the loin. From the values for combustible energy in sheep protein and fat derived by Garrett, Meyer & Lofgreen (1959), it was calculated that the carcass energy gained as fat was 81·7%

Expt 2

The main results of Expt 2 are given in Table 5. Missing values were calculated for the animals which did not complete the experimental period and for one lamb which, at slaughter, was found to be cryptorchid.

Table 5. *Expt 2. Means of initial and final live weight, empty body weight and carcass weight, fat and protein percentage in the loin, and number of carcasses graded A–C* in groups of eight lambs, slaughtered initially or receiving one of three diets with or without implants of 15 g hexoestrol*

Diet	Initial live weight (lb)	Final live weight (lb)	Empty body-weight (lb)†	Carcass weight (lb)†	Loin fat (%)	Loin protein (%)	No. of carcasses in grade: A	B	C
Initial slaughter group	52·6	—	44·6	25·0	11·8	17·4	0	2	6
Basal (hay + concentrate)									
Without implant	52·7	78·3	66·6	37·2	23·2	15·6	3	2	2
With implant	51·9	80·8	68·9	39·5	22·5	15·9	2	5	0
Basal + acetate:									
Without implant	52·7	85·6	74·4	41·7	22·9	16·0	5	2	0
With implant	52·6	90·1	78·3	44·6	25·2	15·2	7	1	0
Basal + propionate:									
Without implant	52·1	84·4	74·0	41·8	24·3	15·6	3	4	0
With implant	52·5	88·5	76·5	43·8	22·5	15·6	5	2	0
Standard error of difference between means	±0·63	±1·97	±1·52	±1·22	±2·72	±0·58			

* As judged by an official grader of the Ministry of Agriculture, Fisheries and Food.
† Initial slaughter group not included in analysis of variance.

Analysis of variance showed the hormone × diet interaction to be small and in no instance significant. Pooled values were therefore examined for hormone and diet effects. Animals implanted with hexoestrol were heavier at slaughter and had greater empty body and carcass weights than the controls ($P < 0·01$). For animals receiving diets supplemented with VFA salts, final live weights, empty body weights and carcass weights were greater ($P < 0·001$) than for those receiving the basal control diet. Differences between groups receiving acetate and propionate supplements were not significant. The proportions of fat and protein in the half loins are also shown in Table 5. The loins of lambs slaughtered initially had proportionately less fat and more protein ($P < 0·001$) than any of the lambs slaughtered at the end of the experiment. No differences could be detected, however, either between diets or as a result of implantation.

The gradings of the carcasses of the lambs in the two experiments are shown in

Tables 4 and 5. The gradings of the initial slaughter groups were lower than of those receiving the experimental diets. Lambs receiving diets containing VFA salts tended to be graded higher than those receiving only the basal diet.

The results of the digestibility trial in terms of faecal excretions of dry matter and organic matter are shown in Table 6. The results are pooled according to diet as there was no effect of hormone implantation on the digestibility.

There was a significant increase in dry-matter excretion with animals receiving propionate ($P < 0.05$) and a very highly significant increase with animals receiving acetate ($P < 0.001$). The differences in faecal excretion of organic matter were not significant, although the difference between groups receiving acetate and propionate was large.

Table 6. *Expt 2. Mean daily faecal output (g) of dry matter and organic matter in groups of eight lambs receiving one of three diets*

Diet	Dry matter	Organic matter
Basal diet (hay + concentrate)	239	200
Basal + acetate	276	210
Basal + propionate	253	197
Standard error of difference between means	±6·5	±6·1

DISCUSSION

There was no indication of a lower utilization of acetate for promoting gains in live weight, empty body weight or carcass weight despite acetate being given at a slightly lower level of supplementation than propionate and butyrate. The studies of carcass composition did not show a lower energy content in the carcasses of animals given acetate. Indeed in Expt 1, loins from lambs given acetate had slightly higher fat percentages than those from lambs given propionate or butyrate. Of the energy retained in the carcasses in Expt 1, more than 80% was in the form of fat as calculated from the composition of the loin with the regression of Kirton & Barton (1962). Further use was not made of the regression by Kirton & Barton (1962) as it was felt that under the conditions of our experiment, in particular when hexoestrol was implanted, the regression might not be sufficiently precise. Nevertheless, the results and the absence of a hexoestrol × diet interaction in Expt 2, despite the growth response obtained, would suggest that propionate was not utilized more efficiently for lipogenesis than was acetate.

The lower utilization of butyrate in Expt 1 was surprising since Ørskov & Allen (1966) found butyrate to be utilized as efficiently as acetate and propionate. However, two of the animals on the butyrate treatment in Expt 1 did poorly and it is possible that this result might have happened by chance.

The utilization of the VFA salts in the present work is best illustrated graphically as in Fig. 1, in which the calculated ME intake above maintenance is related to carcass gain. The maintenance requirements were assumed to be 1235 and 1290 kcal ME/day for the basal control group and the groups given VFA respectively in Expt 1, using the data of Langlands, Corbett, McDonald & Pullar (1963). Similarly, the maintenance requirements in Expt 2 were assumed to be 960, 990 and 1010 kcal ME/day for the basal control group, the unimplanted group and the group given VFA and hexoestrol respectively. In Fig. 1 the straight lines joining values for the basal control group and the origin and joining the values for the basal control group and those for the groups given acetate or propionate have different slopes. This suggests that the ME derived from acetate and propionate was utilized more efficiently to promote carcass gain than was the calculated ME of the basal diet of hay and concentrate. Although the slopes

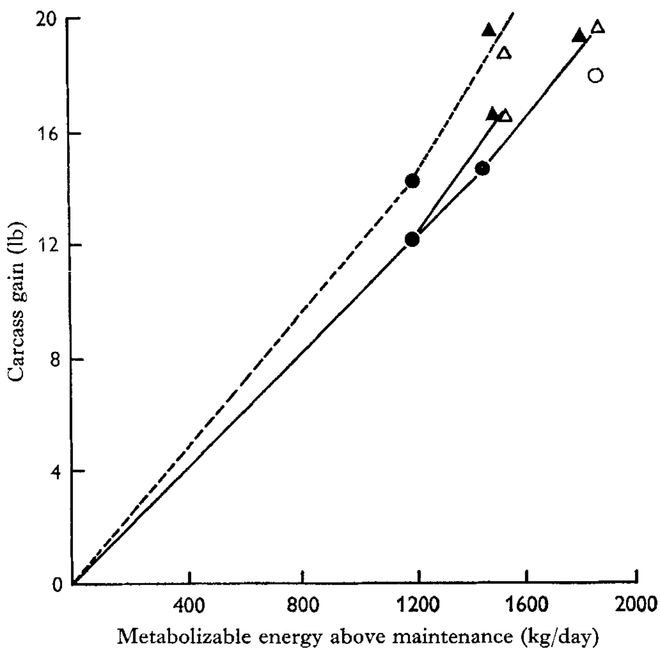

Fig. 1. Utilization by lambs of energy from acetate (▲), propionate (△) and butyrate (○) in relation to utilization of metabolizable energy of the basal diet (●). ———, unimplanted animals (Expts 1 and 2); - - - - -, hexoestrol-implanted animals (Expt 2).

of the lines depend on the accuracy of predicting the maintenance requirement and of calculating the ME, the results illustrated may confirm the results of Ørskov & Allen (1966), suggesting that the energy of the VFA was utilized with an efficiency equal to that of the calculated ME of concentrate. That the efficiencies of acetate and propionate in both experiments reported here were the same suggests that the difference between results obtained by Ørskov & Allen (1966) and by Armstrong & Blaxter (1957) and Armstrong *et al.* (1958) may not be accounted for by the differences in the maturity of the experimental animals.

The large difference observed in Expt 1 between ewe and wether lambs in the coefficient of variation of fat percentages (5·8 and 20·6 % respectively) was surprising. The coefficient of variation among the wether lambs used in Expt 2 was also large (23·4 %). If confirmed, this observation would suggest that entire males or ewe lambs might be preferable as experimental animals in nutritional experiments of this type.

The increased excretions of dry matter in the faeces when the animals were given supplements of VFA salts were found to be almost entirely an effect of increased excretions of minerals. This was probably due to an excretion of a salt of calcium since a large amount of calcium was given as cation of the VFA, and it was shown by Brine & Johnston (1955) that calcium given in excess of requirement was excreted in the faeces. An apparent increase in the excretion of organic matter when the animals received acetate can be explained by a higher content of carbonate in the faecal dry matter and a higher content of oxides in the faecal ash of sheep receiving diets containing acetate (Ørskov, 1965).

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REFERENCES

Armstrong, D. G. & Blaxter, K. L. (1957). *Br. J. Nutr.* **11**, 413.
Armstrong, D. G., Blaxter, K. L., Graham, N. McC. & Wainman, F. W. (1958). *Br. J. Nutr.* **12**, 177.
Blaxter, K. L. (1962). *The Energy Metabolism of Ruminants*. London: Hutchinson.
Brine, C. L. & Johnston, F. A. (1955). *Am. J. clin. Nutr.* **3**, 418.
Callow, E. H. (1947). *J. agric. Sci., Camb.*, **37**, 113.
Evans, R. E. (1960). *Bull. Minist. Agric. Fish. Fd, Lond.*, no. 48, 15th ed.
Garrett, W. N., Meyer, J. H. & Lofgreen, G. P. (1959). *J. Anim. Sci.* **18**, 528.
Gee, I. & Preston, T. R. (1957). *Br. J. Nutr.* **11**, 329.
Great Britain, Parliament (1960). *Fertilizers and Feedingstuffs Regulations*, 1960. *Stat. Instrum.* no. 1165.
Hodgman, C. D. (1962). *Handbook of Chemistry and Physics*, 44th ed. Ohio: The Chemical Rubber Publishing Co.
Hovell, F. D. (1964). *Anim. Prod.* **6**, 261.
Kirton, A. H. & Barton, R. A. (1962). *J. Anim. Sci.* **21**, 553.
Langlands, J. P., Corbett, J. L., McDonald, I. & Pullar, J. D. (1963). *Anim. Prod.* **5**, 1.
Ørskov, E. R. (1965). The utilization of volatile fatty acids by growing lambs. PhD Thesis, University of Reading.
Ørskov, E. R. & Allen, D. M. (1966). *Br. J. Nutr.* **20**, 295.
Wood, T. B. (1924). *Animal Nutrition*. London: University Tutorial Press Ltd.

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