Utilization of salts of volatile fatty acids by growing sheep

4*. Effects of type of fermentation of the basal diet on the utilization of salts of volatile fatty acids for nitrogen retention and body gains

By E. R. ØRSKOV† and D. M. ALLEN

Department of Agriculture, University of Reading

(*Received* 21 *September* 1965—*Accepted* 15 *March* 1966)

1. Two experiments are reported in which sodium and calcium salts of volatile fatty acids (VFA) were given to lambs as additions to two basal diets of hay and concentrate. The two diets induced distinctly different types of rumen fermentation. A low-concentrate diet gave higher acetic and lower butyric acid proportions in the rumen contents than a high-concentrate diet. The two diets were given to lambs to provide equal intake of digestible organic matter. In one change-over experiment with twelve sheep the nitrogen retention was determined, and in a second experiment involving sixty-four lambs the efficiency of the VFA salt to promote body-weight gain was estimated. 2. The estimated metabolizable energy (ME) from the high-concentrate diet was utilized significantly more efficiently than that from the low-concentrate diet to promote positive nitrogen retention and to increase empty body-weight and carcass weight. These differences in final live weight were not significant owing to the greater content of the alimentary tract of lambs given the low-concentrate diet. 3. On both diets the lambs receiving the additions of VFA salts grew faster and produced significantly greater empty body and carcass weights than the lambs receiving only the basal diets. 4. For promoting carcass gain the energy derived from the VFA salts appeared to be utilized more efficiently than the calculated ME above maintenance of the two basal diets. 5. There were no significant differences between the efficiency of utilization of the energy from acetate, propionate and butyrate to promote carcass gains. There was, however, a tendency for the response to acetate to be greater on the high-concentrate diet than on the low-concentrate diet. 6. With a low level of feeding but with positive N balance, addition of acetate resulted in a greater N retention than that of propionate with both diets. 7. It is concluded that if the reported low utilization of the ME of diets high in roughage is related to the proportion of acetic acid in the rumen contents, then the implied losses of energy may be associated with the formation of acetic acid rather than its utilization.

It has been well established that the mixture of volatile fatty acids (VFA) occurring as end-products of the rumen fermentation varies in composition according to the nature of the diet. It has been shown that the molar proportion of acetic acid is positively correlated with the fibre content of many diets (Balch, Balch, Bartlett & Rowland, 1953; Elliot & Loosli, 1959; Bath & Rook, 1963), and Ensor, Shaw & Tellechea (1959) showed that heat treatment of cereals such as flaking the maize resulted in a decrease in the molar proportion of acetic acid. As a consequence of the findings of Armstrong & Blaxter (1957) and Armstrong, Blaxter, Graham & Wainman (1958) that acetic acid was utilized very inefficiently for lipogenesis, several authors have examined the relationship between the efficiency of rations for producing weight gains and supporting lactation and the molar proportion of acetic acid in the rumen liquor.

Close negative relationships were established between the proportion of acetic acid

* Paper no. 3: *Br. J. Nutr.* (1966), **20**, 509.

† Present address, Animal Husbandry Research Division, USDA, Beltsville, Md, USA.

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

in the rumen and the efficiency of utilization, above maintenance, for lactation of digestible energy (Elliot & Lossli, 1959) and metabolizable energy (ME) (Coppock, Flatt, Moore & Stewart, 1964). Using two rations with similar digestibility, but giving rise to different proportions of VFA in the rumen, Thomson (1965) found no significant differences in the energy retained by lambs. The large differences in nitrogen intake and lack of statistical precision in this work, however, prevent an interpretation in terms of differences attributable to differences in the proportions of VFA. Blaxter & Wainman (1964) established a highly significant relationship between the net availability of ME for lipogenesis and the proportion of acetic acid in the rumen. Using VFA salts Ørskov & Allen (1966*a*, *b*) and Ørskov, Hovell & Allen (1966) found that acetate was not utilized any less efficiently than propionate and butyrate for promoting gains in body tissues by fattening lambs. The observations of Armstrong, Blaxter & Graham (1957) that, for maintenance, acetic acid was utilized with an efficiency equal to that of propionic and butyric acids when infused into the rumen as a component of VFA mixtures led us to suggest that the utilization of additional VFA might vary according to the proportion of VFA being produced when the basal diet was being fermented in the rumen, as the VFA could be utilized in different proportions for maintenance and production. This would result in an interaction between the individual VFA added and the proportions of VFA produced by rumen fermentation of the basal diet.

This communication reports a N balance experiment and a feeding trial in which salts of the different VFA were included in two basal diets which induced distinctly different types of rumen fermentation.

EXPERIMENTAL

Animals and facilities

For Expt 1, twelve wether sheep (Suffolk × Scottish half-bred) were purchased; they were approximately 8 months old and weighed from 87 to 116 lb. They were harnessed and fitted with faecal collection bags and kept in digestibility crates which allowed for the collection of urine. This experiment was carried out during the winter of 1963–4.

For Expt 2, thirty-two wether lambs and thirty-two ewe lambs, Dorset Horn or Dorset Horn cross, were used. When selected for experiment they weighed from 44 to 66 lb and were 2·5 to 3 months of age. They were all artificially reared on milk substitutes and were housed under cover in individual pens bedded with sawdust. This experiment began on 15 July 1964.

Design and treatments

After 1 month in which the twelve animals for Expt 1 became accustomed to the experimental diets and conditions, they were paired according to live weight and allocated within pairs to receive a high- or a low-concentrate ration. The animals on each ration were then randomized to pairs of 3 × 3 Latin squares balanced for carry-over effects. Also an additional period was included in which the last treatment was

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

repeated for each lamb, the periods being designated 1, 2*a* and 2*b*. The design and method of statistical analysis have been described by Cochran & Cox (1957). The periods were of 20 days, during the last 10 days of which faeces and urine were collected.

After 3 weeks in which the animals became accustomed to eating hay and concentrate the animals for Expt 2 were grouped according to sex, and allocated to eight blocks and randomized to eight dietary treatments.

The treatments in Expts 1 and 2 were similar except that butyrate was not added to the diet in Expt 1.

The diets were: basal, low-concentrate (Lc) 6:4 ratio by weight of hay to concentrate; basal (Lc) + acetate; basal (Lc) + propionate; basal (Lc) + butyrate; basal, high-concentrate (Hc) 3:7 ratio by weight of hay to concentrate; basal (Hc) + acetate; basal (Hc) + propionate; basal (Hc) + butyrate.

Composition and preparation of the food

The composition of the hay and concentrate used in Expts 1 and 2 is shown in Table 1. The ingredients of the concentrate in the low-concentrate diet were ground maize 45%, barley meal 15%, decorticated groundnut meal 20%, molassine meal 13%, white fish meal 5% and dicalcium phosphate 2%. The ingredients of the concentrate used in the high-concentrate diet were similar except that the maize was flaked and there was 20% barley meal and 15% decorticated groundnut meal.

The hay was chopped into 1 in lengths and the concentrates, including those containing VFA salts, were made into cubes of length $\frac{3}{8}$ in.

Table 1. *Percentage chemical composition of the hay and concentrate used in Expts 1 and 2*

Food	Expt no.	Dry matter	Crude protein	Crude fibre	Ether extract	Ash	Nitrogen-free extract
Hay	1	83·22	7·83	33·76	1·65	6·02	33·96
Hay	2	84·53	7·91	29·54	1·35	5·91	39·82
Concentrate Hc	1	85·77	14·91	3·26	1·58	10·79	55·23
Concentrate Hc	2	85·00	16·31	2·61	2·41	4·96	58·71
Concentrate Lc	1	85·65	16·10	3·66	2·49	8·29	55·11
Concentrate Lc	2	85·50	19·19	3·37	3·62	7·06	52·26

Hc, high-concentrate diet; Lc, low-concentrate diet.

Digestibility trial

Before each experiment, digestibility trials were carried out to enable the content of digestible organic matter in the two diets to be calculated. Six lambs were fed on each diet in Expt 1 and four animals in Expt 2. Periods were 20 days, during the last 10 days of which the faeces were collected and dried to constant weight at 100°. The organic matter was determined as for food according to the Fertilizer and Feedingstuffs Regulations (Great Britain, Parliament, 1960). The digestibility of the N was also determined in Expt 1. The results are shown in Table 2.

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

Calculation of rations

From Table 2 it was calculated that in order to ensure an equal intake of digestible organic matter, the high- and the low-concentrate diets must be given in the weight ratio of 1:1·1. Having established this ratio, the amounts of VFA salts to be added were calculated so as to achieve approximately isocaloric intakes depending on variation in dry-matter content and digestibility of the diets during the experimental period. The amounts of VFA salts added were based on a 30% addition of acetate in the concentrate given to lambs receiving the low-concentrate diet, the amounts of the other VFA salts being calculated so that all diets were isocaloric.

Table 2. *Digestible organic matter* (%) *in the food dry matter of the two diets used in Expts* 1 *and* 2*; also digestible nitrogen in food dry matter in Expt* 1

Diet	Digestible organic matter		Digestible nitrogen
	Expt 1	Expt 1	Expt 1
Low-concentrate	64·00	65·44	1·85
High-concentrate	69·93	72·28	2·21
Standard error of differences between means	±0·38	±0·46	±0·08

When the lambs received the control ration in Expt 1 they were given 1·88 and 2·09 lb daily of the high- and the low-concentrate diets respectively. The VFA were given as outlined above.

The lambs on the basal low-concentrate diet in Expt 2 received 1·66 lb of food daily when they weighed 50 lb and for each additional 10 lb live weight they received an extra 0·16 lb daily. The remainder of the lambs in each block were offered the same quantity of digestible organic matter in the form of basal diet plus the added VFA salts.

Management of the lambs, slaughter procedure and carcass analysis

The animals were fed twice daily at 08.30 and 17.00 h. In Expt 1 the daily food for the period of 20 days was weighed and stored in plastic bags. In Expt 2 the food was weighed once daily, half being offered in the afternoon and the remainder the following morning. The occasional small quantities of hay left uneaten in Expt 2 were dried to constant weight at 100° and recorded as dry matter. The amounts of concentrate occasionally left uneaten were given to the same lambs at a later stage in the experiment. The lambs in Expt 2 were weighed once every fortnight and food offered was adjusted accordingly; water was offered *ad lib.* The health of the animals in both experiments was excellent; no illness or deaths occurred. The lambs in Expt 2 were slaughtered on 2 consecutive days after 103 or 104 days on experiment. The final live weight was taken as the average of the live weights on the day of slaughter and 2 days before slaughter. The weight of the alimentary canal full and empty, and the mesenteric fat and fat in the omentum was recorded at slaughter; otherwise the slaughter procedure was as described by Ørskov & Allen (1966*a*).

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

Collection and analysis of food, faeces and urine in Expt 1

Samples of food were collected for each period. The faeces were collected once daily and, of the total quantity, 2·5 % was retained for the determination of N content; the remainder was dried at 100° to constant weight for determination of dry-matter and ash contents. The samples for N determination were bulked during the collection period and stored at −15°. The urine was also collected daily and, to prevent loss of ammonia from the alkaline urine and to terminate microbial activity, 40 ml glacial acetic acid and 40 ml 20 % formaldehyde solution were added to the collection bottles. Of the total volume of urine 10 % was retained. These samples were bulked over the collection period and stored at 0°. N was determined as for food.

Analysis of rumen liquor for VFA

Before the experiments reported here, the patterns of rumen fermentation of the two basal diets were determined. Two wether sheep with rumen cannulas were used; each weighed approximately 150 lb. A 2 × 2 Latin square design was used with the treatment in the second period repeated, the three periods being designated period 1, 2*a* and 2*b*. The animals were fed on the diets over periods of 10 days, during the last 24 h of which rumen liquor samples were obtained. The sheep received daily 2·19 lb of the high-concentrate diet or 2·50 lb of the low-concentrate diet given in two feeds.

The rumen samples were withdrawn by means of a sampling device developed at the Grassland Research Institute, Hurley, which automatically obtained a 20 ml sample at 1 h intervals. Samples were collected in test-tubes to which 0·2 ml of 5 % (w/v) mercuric chloride was added to terminate microbial activity. The twelve samples obtained between 08.00 and 20.00 h were bulked in pairs; those obtained between 20.00 and 08.00 h were incorporated into three bulk samples. The preparation of the rumen samples and determination of the VFA were as described by Ørskov & Allen (1966*b*).

RESULTS

VFA in rumen contents

The mean molar proportions of VFA in the rumen with the two basal diets are given in Table 3. The differences between the mean values for the proportions of acetic, propionic, butyric and valeric acid when animals were fed on the low- or high-concentrate diets were highly significant ($P < 0{\cdot}001$). The differences between periods approached significance. There was a significant negative relationship between the proportions of acetic and butyric acid ($r = -0{\cdot}83$), but there was no relationship between the proportions of acetic and propionic acid.

A significant diurnal variation was recorded in the proportion of acetic acid ($P < 0{\cdot}01$) and of propionic and butyric acids ($P < 0{\cdot}05$). On pooling the results from the three periods this variation was found to be associated with feeding times. In Table 4 the results from the period 2*b* have been summarized. There was a small decrease in the

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

proportion of acetic acid and small increases in the proportions of propionic and butyric acids subsequent to feeding. After approximately 4 h these changes were reversed and the levels returned to the values before feeding.

Expt 1. *Nitrogen balance*

On the assumption that 1 lb of digestible organic matter is equal to 1650 kcal ME (Langlands, Corbett, McDonald & Pullar, 1963), the mean daily intakes of ME with the basal ration were 1887 and 1833 kcal with the low- and the high-concentrate diets

Table 3. *Expt* 1. *Molar percentages of VFA in rumen liquor from two sheep given high-concentrate* (*Hc*) *and low-concentrate* (*Lc*) *diets* (*mean values for* 24 *h*)

Period (see p. 521)	Acetic acid		Propionic acid		Butyric acid*		Valeric acid†	
	Hc	Lc	Hc	Lc	Hc	Lc	Hc	Lc
1	64·4	67·6	12·8	16·1	20·0	14·2	2·8	2·1
2*a*	61·7	66·2	15·3	18·5	19·9	12·7	3·1	2·6
2*b*	56·3	66·0	16·0	20·4	23·6	11·8	4·1	1·8
Mean	60·8	66·6	14·7	18·3	21·2	12·9	3·3	2·2

* Including isobutyric acid. † Including isovaleric acid.

Table 4. *Expt* 1. *Molar percentages of VFA in rumen contents from the two sheep in period* 2*b* (*see Table* 3) *over a* 24 *h feeding cycle with a high-concentrate* (*Hc*) *or a low-concentrate* (*Lc*) *diet; feeding times were* 09.00 *and* 17.00 *h*

Time	Acetic acid		Propionic acid		Butyric acid*		Valeric acid†	
	Hc	Lc	Hc	Lc	Hc	Lc	Hc	Lc
24.00–04.00	56·2	68·1	16·6	19·5	22·3	11·3	4·9	1·1
04.00–08.00	56·6	67·4	18·3	18·8	20·9	11·6	4·2	2·2
08.00–10.00	58·4	67·4	16·3	20·2	22·1	11·3	3·2	1·1
10.00–12.00	55·6	65·2	15·3	21·3	24·7	11·9	4·4	1·6
12.00–14.00	56·4	67·5	15·4	19·6	25·0	11·5	3·2	1·4
14.00–16.00	55·0	66·3	16·0	19·3	24·8	11·9	4·2	2·5
16.00–18.00	55·4	63·6	15·6	21·8	24·8	12·5	4·2	2·1
18.00–20.00	56·5	63·2	15·2	22·0	24·6	12·8	3·6	2·0
20.00–24.00	56·2	65·5	15·7	20·6	23·5	11·9	4·6	2·0

* Including isobutyric acid. † Including isovaleric acid.

respectively. Differences were not statistically significant. The daily intakes of acetate and propionate provided 336 and 334 kcal combustible energy (Hodgman, 1962) respectively.

The daily intake of digestible N varied from 10 to 13 g with the low-concentrate diet and from 14 to 16 g with the high-concentrate diet. The intakes of N were highest on diets containing VFA salts. The reasons for this are not clear, but it was not due to nitrogenous impurities in the VFA salts and must have been a result of small differences in N content of the basal ingredients which were mixed in different batches. It is unlikely that these differences affected N retention values very much, as with both diets the N intakes were well in excess of requirements ((USA) National Research Council, 1957).

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

The total N retention, percentage N retention and intake of digestible N and the volume of urine excreted daily are given in Table 5. There were no carry-over effects and consequently residual sums of squares were pooled into the error sums of squares. The means in Table 5 are the means adjusted for sheep and period effect according to the method of Cochran & Cox (1957).

Table 5. *Expt* 1. *Digestible nitrogen intake, N retention, percentage retention of digestible N and volume of urine excreted by lambs receiving a high-concentrate* (*Hc*) *or a low-concentrate* (*Lc*) *diet with or without a supplement of VFA salts. Values are means adjusted for sheep and periods according to Cochran & Cox* (1957)

	Intake of digestible N (g/day)		N retention (g/day)		Retention of digestible N (%)		Urine excretion (ml/day)	
Diet	Hc	Lc	Hc	Lc	Hc	Lc	Hc	Lc
Basal (hay + concentrate)	13·69	10·13	2·56	1·22	18·70	12·04	1832	1330
Basal + acetate	16·41	13·15	4·37	3·42	26·62	26·00	2427	1742
Basal + propionate	13·88	12·56	2·99	2·20	21·54	17·51	1907	1680
Standard error of differences between means	—	—	±0·242	±0·456	±1·95	±3·22	±150	±122

With the low-concentrate diet the N retention was significantly increased when acetate ($P < 0{\cdot}001$) or propionate ($P < 0{\cdot}05$) was given, the effect due to acetate being greater than that obtained with propionate ($P < 0{\cdot}05$). With the high-concentrate diet the increase in N retention when propionate was given only approached significance ($P < 0{\cdot}05$); when acetate was given it was significantly greater than with the basal or propionate diet ($P < 0{\cdot}001$). Differences between the basal diet and the propionate diet in the percentage retention of digestible N only approached significance ($P < 0{\cdot}1$), and the percentage retention with the acetate diet was significantly greater than with the basal ($P < 0{\cdot}001$) or propionate diets ($P < 0{\cdot}05$).

With the low-concentrate diet, the volume of urine excreted was significantly greater ($P < 0{\cdot}01$) when acetate or propionate was given than with the basal diet. With the high-concentrate diet the difference in urine excretion when the lambs received the basal or propionate diet was not significant; when the lambs received acetate they excreted more urine than when they received the basal or propionate diet ($P < 0{\cdot}01$).

Expt 2. *Feeding trial*

The daily intakes of ME provided by the basal diets and the VFA salts are shown in Table 6. The small discrepancy in ME intake between the two diets was a result of the occasional small quantities of hay left uneaten by lambs receiving the low-concentrate diet.

The main results of Expt 2 are shown in Table 7. In the statistical analysis the standard errors of final live weight, empty body weight and carcass weight were reduced by analysis of covariance to eliminate the effect of differences in initial weight. With both diets the addition of VFA salts resulted in significantly increased ($P < 0{\cdot}001$)

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

final live weights, empty body weights and carcass weights. With the low-concentrate diet the lambs receiving butyrate had lower final weights than those receiving acetate ($P = 0{\cdot}1$) or propionate ($P = 0{\cdot}05$). In empty body and carcass weights these differences did not approach significance. With the high-concentrate diet the lambs receiving the acetate supplement were heavier in final live weight than those receiving propionate or butyrate ($P < 0{\cdot}01$). These differences were less apparent in empty body weight ($P < 0{\cdot}05$) and were not significant in carcass weight.

The difference in final live weight between the two control groups was not significant but the differences in empty body and carcass weights were highly significant ($P < 0{\cdot}001$).

Table 6. *Expt 2. Calculated mean daily intake of metabolizable energy (kcal/day) by groups of eight lambs receiving a high-concentrate (Hc) or a low-concentrate (Lc) diet with or without supplements of VFA salts*

Diet	Basal diet	VFA	Total
Basal Lc (hay + concentrate)	1784	—	1784
Basal Lc + acetate	1774	317	2091
Basal Lc + proprionate	1784	317	2101
Basal Lc + butyrate	1777	317	2094
Basal Hc (hay + concentrate)	1827	—	1827
Basal Hc + acetate	1827	317	2144
Basal Hc + propionate	1830	317	2147
Basal Hc + butyrate	1827	315	2142

Table 7. *Expt 2. Treatment means of initial and final live weights, empty body weights, carcass weights and number of carcasses placed in each grade by an official grader of the Ministry of Agriculture, Fisheries and Food, in groups of eight lambs receiving a high-concentrate (Hc) or a low-concentrate (Lc) diet with or without supplements of VFA salts*

Diet	Initial live weight (lb)	Final live weight (lb)	Empty body weight (lb)	Carcass weight (lb)	Number of carcasses in each grade		
					A	B	C
Basal Lc (hay + concentrate)	55·0	73·9	58·7	30·9	1	5	2
Basal Lc + acetate	55·0	80·3	64·9	35·2	2	6	0
Basal Lc + propionate	54·9	80·7	65·2	36·6	4	4	0
Basal Lc + butyrate	55·2	78·0	63·6	35·4	4	4	0
Basal Hc (hay + concentrate)	55·0	75·3	62·7	34·8	3	5	0
Basal Hc + acetate	54·9	86·3	73·2	41·3	6	2	0
Basal Hc + propionate	55·0	81·8	70·2	40·4	6	2	0
Basal Hc + butyrate	54·8	83·0	70·6	40·2	4	4	0
Standard error of difference between means for eight animals	±0·99	±1·33	±1·30	±0·98	—	—	—

There was a significant interaction ($P < 0{\cdot}05$) between the individual VFA and the basal diets which accounted for some of the differences between treatments in final live weight. The effect of interactions on empty body-weight only approached significance ($P = 0{\cdot}1$) and interactions exerted no effect on carcass weight.

The differences in carcass grades correspond to differences in carcass weight, the heavier carcasses generally grading better.

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

DISCUSSION

Differences in type of rumen fermentation with the high- and low-concentrate diets

With the two ratios of hay to concentrate and the two methods of preparing the maize ingredients, the attempt to establish two rations giving distinctly different types of rumen fermentation was successful; indeed the differences in the acetic acid proportions on a molecular basis in period 2*b* approached 10%. The changes in the proportion of acetic acid in the rumen contents were mainly associated with changes in the butyric acid proportions, substantiated by the negative correlation between these two acids. The large changes in the butyric acid proportions were unexpected, as the changes associated with variation in the acetic acid proportions have usually been found to be mainly in the propionic acid proportions (see Barnett & Reid, 1961; Blaxter, 1962).

The changes in butyric acid proportions with changes in diet observed in the present work stress the importance of butyric acid as a component of the rumen VFA. This is even more apparent if the butyric acid is calculated as a proportion of the energy of the VFA mixture; indeed on the high-concentrate diet used here this amounted to as much as 35%.

Although a significant diurnal variation in the proportions of VFA was detected, it is apparent that the changes were small, which is in agreement with reports by Shaw (1961). The direction of the small changes is in agreement with those observed by Gray & Pilgrim (1951) and Reid, Hogan & Briggs (1957) who found a small increase in the propionic acid proportions and a decrease in the acetic acid proportions subsequent to feeding. As in the present work, they found that after a few hours the changes were reversed and the proportions returned to the original values. With the feeding and management used here, the results might suggest that samples obtained 3–5 h after feeding would give a reasonably accurate estimate of the average proportions of the VFA during the 24 h feeding cycle.

VFA salts as a source of energy for growth

In agreement with results obtained in previous work, the additional energy from VFA salts was utilized very efficiently for carcass gains and there were no significant differences in utilization between the individual VFA salts despite differences in basal rations. Butyrate was utilized as efficiently as propionate and acetate, which confirms the result obtained by Ørskov & Allen (1966*a*) and suggests that the lower efficiency of utilization of butyrate observed in one experiment by Ørskov *et al.* (1966) is not typical.

The utilization of the VFA is best illustrated graphically as in Fig. 1, where the carcass gain (initial carcass weight estimated from lambs of similar weight, age and breeding) is plotted against estimated ME above maintenance. If lines are drawn from the origin to the basal control groups the difference in utilization of the basal ration is illustrated, but if the lines are drawn between the basal control groups and the average of the VFA treatment groups on each ration these lines are almost parallel.

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

In order to assess the consistency of the results in the present work and those obtained earlier (Ørskov & Allen, 1966*a*, *b*) and Ørskov *et al.* (1966), analysis of variance was carried out on the mean carcass weights of the animals in the basal control, acetate and propionate groups, a total of seven experiments. No adjustments were made for the smaller supplement of acetate given in the previous experiments. The variation between treatments was almost entirely accounted for in the comparison of the basal control with the VFA treatments. The overall means from the means of carcass weights were 38·46, 42·91 and 43·03 lb respectively for the basal control, acetate and propionate treatments, and differences between the treatments of 0·89 lb would be significant at $P = 0{\cdot}05$ calculated on means of carcass weights.

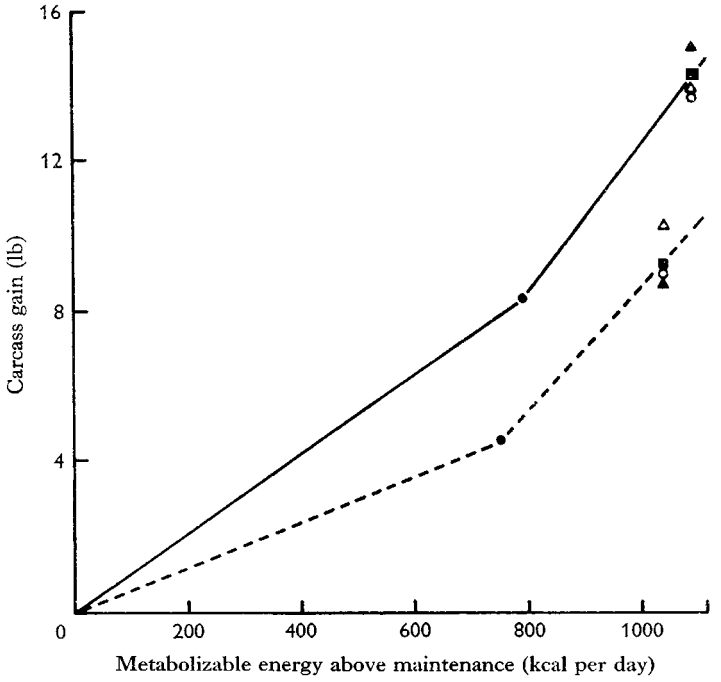

Fig. 1. Utilization by sheep of energy from acetate, ▲, propionate, △, and butyrate, ○, in relation to utilization of the metabolizable energy of the basal diet, ●. ■, mean values for acetate, propionate and butyrate treatments; —— high-concentrate diet; – – – low-concentrate diet.

Effects of type of rumen fermentation on the utilization of supplements of VFA salts for nitrogen retention and body-weight gain

To assess the utilization of the VFA it must be assumed that the response in growth rate was limited by energy and that N was available in excess of requirement. The large increases in N retention when VFA salts were given indicates that this condition was achieved.

The finding that acetate was more effective than propionate in promoting N retention is difficult to explain. The observations agree with results of Rook, Balch, Campling & Fisher (1963) who, on infusing different VFA into the rumen of young growing heifers, found in one experiment that acetic acid promoted a higher N reten-

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

tion than propionic or butyric acid, though not significantly so. Further, as supplements to basal rations for rabbits, Schneeberger (1962) found that acetate increased the N retention significantly more than butyrate or glucose.

Although the label from ^{14}C-labelled acetate has been found in amino acids of milk (Black, Kleiber, Smith & Stewart, 1957), our experiments and those of Rook *et al.* (1963) provide no information as to whether the increased N retention was an effect of a stimulation of protein synthesis, a sparing effect on protein catabolism, a sparing effect on substances participating in protein synthesis or an effect of the incorporation of acetic acid into amino acids. The level of feeding was low in both the present work and the experiments of Rook *et al.* (1963), which might suggest that this effect of acetic acid is only apparent when the animals are fed slightly above their maintenance requirement; at such levels a specific effect of any end-product might be most easily detected. With moderate levels of feeding, our previous work did not indicate a higher protein content in the carcasses of animals given acetate.

N retention was evidently not influenced by interactions between individual VFA and although there was a significant interaction between diet and individual VFA in final live weight, the interaction only approached significance in empty body weight and there were no interactions in carcass weight. This interaction was mainly an effect on the greater weight of lambs receiving the acetate supplement with the high-concentrate diet. This was less apparent in empty body weight and disappeared in carcass weight. It is possible that this effect can be explained by observations in Expt 1 in which lambs receiving acetate with the high-concentrate diet excreted about 500 ml more urine daily than the animals receiving propionate, which might be expected to affect both the weights of gut contents and viscera.

The lack of significance between the difference in final live weights of the two basal groups and the highly significant differences in empty body and carcass weights show clearly the effect of gut content and stress the caution that must be applied in using live-weight gain as the only criterion for treatment effects.

Fig. 1 illustrates the very small differences in utilization of the VFA salts regardless of basal diet. It is interesting, however, that whereas the utilization of acetate was best with the high-concentrate diet it was least with the low-concentrate diet; indeed it might help to explain the lack of agreement between results obtained by us in the present and previous work and those of Armstrong & Blaxter (1957) and Armstrong *et al.* (1958), who found the utilization of acetic acid for lipogenesis inferior to that of propionate or butyrate. When acids were infused singly (Armstrong & Blaxter, 1957) the type of fermentation of the basal diet was not determined; however, when mixtures were infused (Armstrong *et al.* 1958) the type of fermentation was determined and was similar to the VFA proportions found with the low-concentrate diet in the present work. The level of feeding used by us was higher, however, and the amounts of VFA added were smaller than those used in the work of Armstrong (1957, 1958). This is important, for in the absence of interactions the extent to which the proportions of the absorbed end-products are affected by the supplements depends on the amount of the basal diet given and on the amount of the supplements added. The results here and those of Armstrong & Blaxter (1957) and Armstrong *et al.* (1958) might then suggest that

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

the acetic acid concentration under certain circumstances can be so high as to cause a wasteful utilization. This could probably be due to lack of glucogenic materials such as propionic acid, to provide oxaloacetic acid for oxidation in the tricarboxylic acid cycle, as discussed by Annison & Lewis (1959) or to provide nicotinamide-adenine dinucleotide phosphate for fatty acid synthesis as suggested by Armstrong (1965).

Although in the present work the molar proportions of VFA in the rumen were not determined when the VFA salts were added, the results obtained by Ørskov & Allen (1966*a*) suggest that, when acetate was included in the low-concentrate diet, the molar proportions of acetic acid must have substantially exceeded 70%. The results of Bath & Rook (1963) and the review by Barnett & Reid (1961) show that only with a few diets for ruminants does the molar proportion of acetic acid slightly exceed 70%. The findings here then suggest that the utilization of diets by ruminants is seldom, if ever, impaired by an inefficient utilization of acetic acid.

Heat increment of VFA

The heat increment for lipogenesis of a mixture low in acetic acid was found by Armstrong *et al.* (1958) to be about 42 kcal/100 kcal, which was similar to that found when propionic and butyric acids were infused alone, namely 44 and 38 kcal/100 kcal respectively (Armstrong & Blaxter, 1957). Using a concentrate ration of flaked maize, Blaxter & Wainman (1964) found a net availability (ME minus heat increment) of the ME for fattening of 59%. The results of Ørskov & Allen (1966*a*) showed that the energy from VFA salts was utilized with an efficiency equal to that of the calculated ME of concentrate, and the results of Ørskov *et al.* (1966), Ørskov & Allen (1966*b*) and the present work suggest that the energy from VFA salts was utilized more efficiently than the calculated ME of hay and concentrate above maintenance. This suggests that the energy from each of the VFA salts used here was utilized with an efficiency approximately equal to that of propionic or butyric acid or of a mixture with a low concentration of acetic acid in the work of Armstrong & Blaxter (1957) and Armstrong *et al.* (1958).

Utilization of VFA salts in relation to utilization of the ME of the basal ration

In Fig. 1 it is shown that the estimated MEs of the two diets were being utilized by the lambs with different efficiency to promote gains in carcass weight. The higher molar proportion of acetic acid in the rumen content of sheep given the low-concentrate diet and the inefficient utilization of acetic acid for lipogenesis (Armstrong & Blaxter, 1957; Armstrong *et al.* 1958) offered an explanation for the lower efficiency. This was substantiated by the close negative relationships between the proportion of acetic acid in the rumen and the utilization of digestible energy or ME above maintenance (Elliot & Loosli, 1959; Coppock *et al.* 1964; Blaxter & Wainman, 1964). The interpretations of the experiments referred to must be challenged, however, since in Fig. 1 it is shown that the supplement of acetate was being utilized more efficiently to promote carcass gain than the ME of either basal ration. This observation in our experiments then suggests that, if there is a causal relationship between the acetic acid proportions and

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

the utilization of ME, the losses occur in the formation of acetic acid and not in its utilization.

It has been found that there is a close relationship between the fibre content of a ration and the proportion of acetic acid in the rumen (Elliot & Loosli, 1959; Bath & Rook, 1963) and also between the fibre content and the utilization of ME above maintenance as shown by Blaxter & Wainman (1964), who also reviewed earlier literature confirming their observation. These results indicate that, if a causal mechanism operates, the heat losses could occur in the fermentation of the crude fibre fraction giving rise to a high proportion of acetic acid rather than in the metabolism of the acetic acid. Although, in calculating the ME, an estimate was made of the heat of fermentation (Blaxter, 1962), this fraction has been very difficult to separate from the overall heat increment of feeding, and evidence as to its magnitude is most unsatisfactory. Marston (1948) attempted to calculate an energy balance sheet for in vitro fermentation of cellulose and estimated that about 15% of the heat increment of feeding was free heat dissipated during the fermentation. These determinations were made under well-controlled conditions, but the fermentation end-products were different from those which are normally associated with the fermentation of fibrous material, the propionic acid proportion being twice that of acetic acid. The difference in utilization of the high- and low-concentrate diets could also occur as a result of differences in the ratio of food fermented in the rumen to that digested in the lower alimentary tract. The effect of such differences was convincingly shown by Armstrong, Blaxter & Graham (1960), who demonstrated that the heat increment of glucose was much greater after ruminal infusion than after abomasal infusion.

Many thanks are due to Dr C. C. Balch for his help and encouragement throughout this work. We also wish to thank Dr J. D. Sutton for use of his equipment for VFA analysis at the National Institute for Research in Dairying and Messrs W. F. Raymond and R. A. Terry for use of their fistulated sheep and laboratory at the Grassland Research Institute, Hurley. The analysis of the food, faeces and urine were carried out by D. A. Balch and his staff. Dr R. N. Curnow gave valuable advice on statistical analysis. One of us (E.R.Ø.) was in receipt of a NATO Science Scholarship.

REFERENCES

Annison, E. F. & Lewis, D. (1959). *Metabolism in the Rumen.* London: Methuen.
Armstrong, D. G. (1965). *Physiology of Digestion in the Ruminant*, p. 272. [R. W. Dougherty, editor.] Washington: Butterworths.
Armstrong, D. G. & Blaxter, K. L. (1957). *Br. J. Nutr.* **11**, 413.
Armstrong, D. G., Blaxter, K. L. & Graham, N. McC. (1957). *Br. J. Nutr.* **11**, 392.
Armstrong, D. G., Blaxter, K. L. & Graham, N. McC. (1960). *Proc. Nutr. Soc.* **19**, xxxi.
Armstrong, D. G., Blaxter, K. L., Graham, N. McC. & Wainman, F. W. (1958). *Br. J. Nutr.* **12**, 177.
Balch, C. C., Balch, D. A., Bartlett, S. & Rowland, S. J. (1953). *Int. Dairy Congr.* XIII. *The Hague.* Vol. 1, p. 33.
Barnett, A. J. G. & Reid, R. L. (1961). *Reactions in the Rumen.* London: Edward Arnold Ltd.
Bath, I. H. & Rook, J. A. F. (1963). *J. agric., Sci., Camb.* **61**, 341.
Black, A. L., Kleiber, M., Smith, A. H. & Stewart, D. N. (1957). *Biochim. biophys. Acta* **23**, 54.
Blaxter, K. L. (1962). *The Energy Metabolism of Ruminants.* London: Hutchinson, Scientific and Technical.
Blaxter, K. L. & Wainman, F. W. (1964). *J. agric. Sci., Camb.* **63**, 113.

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press

Cochran, W. G. & Cox, G. M. (1957). *Experimental Designs*, 2nd ed. New York: John Wiley and Sons Ltd.
Coppock, C. E., Flatt, W. P., Moore, L. A. & Stewart, W. E. (1964). *J. Dairy Sci.* **47**, 1359.
Elliot, J. M. & Loosli, J. K. (1959). *J. Dairy Sci.* **42**, 843.
Ensor, W. L., Shaw, J. C. & Tellechea, H. F. (1959). *J. Dairy Sci.* **42**, 189.
Gray, F. V. & Pilgrim, A. F. (1951). *J. exp. Biol.* **28**, 83.
Great Britain, Parliament (1960). *Fertilizer 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.
Langlands, J. P., Corbett, J. L., McDonald, I. & Pullar, J. D. (1963). *Anim. Prod.* **5**, 1.
Marston, H. R. (1948). *Biochem. J.* **42**, 564.
National Research Council (1957). *Publs natn. Res. Coun., Wash.* no. 504.
Ørskov, E. R. & Allen, D. M. (1966*a*). *Br. J. Nutr.* **20**, 295.
Ørskov, E. R. & Allen, D. M. (1966*b*). *Br. J. Nutr.* **20**, 509.
Ørskov, E. R., Hovell, F. D. & Allen, D. M. (1966). *Br. J. Nutr.* **20**, 307.
Reid, R. L., Hogan, J. P. & Briggs, P. K. (1957). *Aust. J. agric. Res.* **8**, 691.
Rook, J. A. F., Balch, C. C., Campling, R. C. & Fisher, L. J. (1963). *Br. J. Nutr.* **17**, 399.
Schneeberger, H. (1962). Über die energetische Wirkung von Na-Azetat, Na-Butyrat und Glukose beim ausgewachsenen Kaninchen. Thesis, University of Zürich.
Shaw, J. C. (1961). *Int. Congr. Anim. Husb.* VIII. *Hamburg*, p. 29.
Thomson, D. J. (1965). *Proc. Nutr. Soc.* **24**, xxvi.

Printed in Great Britain

https://doi.org/10.1079/BJN19660051 Published online by Cambridge University Press