Utilization of salts of volatile fatty acids by growing sheep

3*. Effect of frequency of feeding on the utilization of acetate and propionate by young growing lambs

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1. The effect, on the concentration of volatile fatty acids (VFA) in the rumen, of increasing the frequency of feeding from twice daily to four or six times daily was investigated in sheep given isocaloric rations containing VFA salts. In a second experiment isocaloric rations containing acetate and propionate were given to groups of lambs in two or six equal feeds daily. The efficiency with which the lambs utilized the VFA salts was measured as gain in live weight, empty body weight and carcass weight and was compared to that of lambs receiving only the basal ration. The composition of the carcasses was estimated from that of the loin. 2. Increasing the frequency of feeding from twice daily to four or six times daily significantly reduced the marked changes in rumen VFA composition associated with giving rations containing VFA salts twice daily. In lambs receiving acetate and propionate, final live weight, empty body weight and carcass weight were significantly greater than in lambs receiving only the basal ration ($P < 0{\cdot}001$). The combustible energy from added VFA was apparently utilized more efficiently than the calculated metabolizable energy of the basal rations given above maintenance level, and there was no difference between the utilization of acetate and propionate. 3. There were no differences approaching significance between feeding twice daily and six times daily either between groups of lambs receiving only the basal diet or between those given the diets supplemented with VFA salts. Nor were there any interactions between VFA and frequency of feeding. 4. It is postulated that in ruminants the effect of frequency of feeding depends on the environment and level of feeding.

Previous experiments showed that additions of salts of volatile fatty acids (VFA) in two daily feeds caused marked changes in the concentration of VFA in the rumen content. These changes were associated with time of feeding and indicated a very rapid absorption of the VFA (Ørskov & Allen, 1966). Balch & Rowland (1957) suggested that conditions might occur in which, owing to rapid absorption, the concentration of acetic acid in the blood might be so high as to cause wasteful oxidations. This led Ørskov & Allen (1966) to suggest that the fate of VFA salts given in large quantities twice daily and that of VFA continuously infused might be different. They also suggested that the differences in results between their work and that of Armstrong & Blaxter (1957) and Armstrong, Blaxter, Graham & Wainman (1958), who applied continuous infusion, might be a result of such differences in techniques. Armstrong & Blaxter (1957) and Armstrong *et al.* (1958) found wide differences between the efficiency of the different VFA for lipogenesis whereas Ørskov & Allen (1966) found no differences in the efficiency with which the energy from different VFA promoted gains in body tissues in lambs.

The experiments described here were a preliminary trial, in which it was confirmed

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that by increasing the frequency of feeding the marked changes in the rumen concentration of VFA associated with time of feeding could be reduced, and a feeding trial in which isocaloric quantities of acetate and propionate were given daily to groups of lambs in two or six feeds as additions to a basal ration of hay and concentrate. There were two control groups receiving the basal ration in two or six daily feeds. Gordon & Tribe (1952) and Rakes, Hardison, Albert, Moore & Graf (1957) showed that ruminants utilized their food with increased efficiency when the frequency of feeding was increased.

EXPERIMENTAL

Animals and facilities

Three mature wether sheep (Suffolk × Scottish halfbred) with rumen cannulas were used in Expt 1. They weighed approximately 130 lb each and were kept in digestibility crates.

In Expt 2, fifty-six Dorset Horn or Dorset Horn cross wether lambs were used. At the beginning of the experiment they were 10–14 weeks of age and weighed from 40 to 69 lb. The lambs were housed in a covered yard in individual pens bedded with sawdust.

Design and treatments

In Expt 1 the sheep were fed twice, four times or six times daily, according to a latin square design, on a diet consisting of hay and concentrate. The feeding periods were 10 days, during the last day of which rumen liquor samples were obtained. The feeding and sampling times are shown in Table 1.

A randomized block design was used in Expt 2. At the start of the experiment, lambs were allocated to blocks according to mean live weight on 3 consecutive days. The treatments were: treatment 1, initial slaughter group; treatment 2, basal diet (hay + concentrate) given twice daily to produce a gain of approximately 0·25 lb/lamb daily; treatment 3, basal (hay + concentrate) given six times daily; treatment 4, basal + acetate given twice daily; treatment 5, basal + acetate given six times daily; treatment 6, basal + propionate given twice daily; treatment 7, basal + propionate given six times daily.

The experiment was carried out during the summer of 1963. The initial slaughter group and the group fed twice daily were treated identically to the initial slaughter group and unimplanted groups used in a previous experiment (Ørskov, Hovell & Allen, 1966, Expt 2). The composition of the hay and concentrate mixture and amounts of VFA salts added were as described by Ørskov *et al.* (1966, Expt 2).

Management of the lambs

The food was weighed once daily and divided into the appropriate number of rations according to frequency of feeding. The feeding times in Expt 1 are shown in Table 1. The fistulated sheep ate all their rations in a few minutes except for one sheep which when receiving its food twice daily consumed the food in small quantities throughout the day. Results for this sheep at this time have been excluded from consideration.

The lambs fed twice daily in Expt 2 were given their rations at 08.30 and 17.00 h, those fed six times daily were given food at 07.00 h, and every 2 h until 17.00 h. Uneaten food, if any, was weighed and removed daily. The weight of the concentrates and part of the hay left uneaten in the initial part of the experiment were given to the same lambs later in the experimental period so that the required amount of concentrates and VFA salts was consumed by each animal. The animals fed six times daily frequently left small quantities of hay uneaten.

Table 1. *Expt* 1. *Times of feeding sheep receiving their daily allowance of food in two, four or six feeds daily, and times of removal of rumen samples*

Feeding time			
Six feeds daily	Four feeds daily	Two feeds daily	Sampling time
08.45	08.30	08.30	09.00
10.30	11.30	17.30	11.00
12.15	14.30	—	13.00
14.00	17.30	—	15.00
15.45	—	—	17.00
17.30	—	—	19.00

The lambs were dosed with an anthelmintic methyridine compound (Mintic; ICI) for intestinal worms and no clinical signs of infestation occurred. Footrot was controlled by paring infected feet and by treatment with a chloromycetin tincture. Two lambs died as a result of urinary calculi. Apart from these incidences, the health of the animals was excellent.

Feeding levels

The sheep with rumen fistulas in Expt 1 received daily 1·88 lb hay and 2·63 lb concentrate, of which sodium and calcium acetates made up 20%. It was considered valid to use acetate to study the effect of frequency of feeding on the VFA concentrations in the rumen liquor as the three VFA salts were found to have similar effects on the changes of the respective acid in the rumen (Ørskov & Allen, 1966).

To achieve the required growth rate, the animals receiving the basal control diet in Expt 2 were given 0·19 lb concentrate and 0·13 lb hay per 10 lb live weight until they weighed 50 lb. Above this weight, the daily ration was increased by 0·13 lb concentrate and 0·09 lb hay per 10 lb live weight. Those animals receiving the VFA salts were given the same basal diet with the addition of acetate or propionate. The lambs were weighed once every 2 weeks and food intake was adjusted accordingly.

Slaughter procedure and analysis

The initial slaughter group was killed before the experiment began. The lambs receiving the experimental diets were slaughtered over a 4-day period after 103–106 days on experiment.

At slaughter the weights of mesenteric fat and fat in the omentum, of the pelt and of the alimentary tract, full and empty, were recorded. The carcasses were weighed between 1 and 2 h after slaughter. After 24 h chilling, the loins of the left sides were

removed as described earlier (Ørskov *et al.* 1966). The final live weight was taken as the mean of the live weights at slaughter and 2 days before slaughter. Empty body weight was calculated as live weight at slaughter minus gut contents. The carcasses were graded by an official grader of the Ministry of Agriculture, Fisheries and Food.

Analysis of rumen liquor for VFA

The rumen liquor was collected into an Erlenmeyer suction flask using reduced pressure. The samples of rumen liquor were strained through muslin and centrifuged; 2 ml of the supernatant fluid were then acidified with 0·5 ml concentrated orthophosphoric acid in a glass-stoppered test tube. The subsequent procedure was similar to that described by Ørskov & Allen (1966).

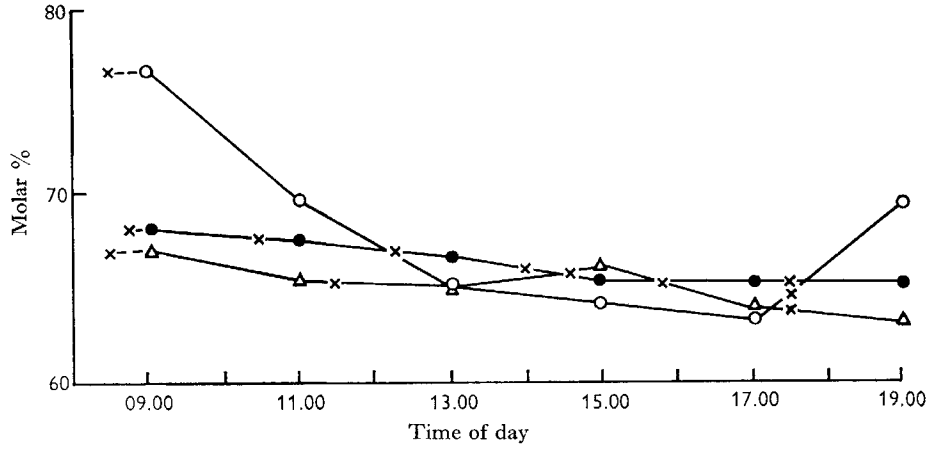

Fig. 1. Expt 1. Molar percentage of acetic acid in the rumen of sheep given a diet supplement with acetate twice daily, o—o, four times daily, △—△, or six times daily, ●—●. × shows feeding time.

Table 2. *Expt* 1. *Treatment means of log error mean square* (S^2) *and quadratic effect of the daily variation in the proportion of acetic acid resulting from a diet containing acetate and given to lambs in two, four or six feeds daily*

No. of feeds/day	Log S^2	Quadratic effect
6	0·4039	4·3
4	0·4548	3·5
2	1·2420	80·2
Standard error of difference between two means	±0·3192	±13·7

RESULTS

The results of Expt 1 are illustrated graphically in Fig. 1. The marked changes in the proportion of acetic acid associated with twice daily feeding with acetate were greatly reduced by increasing the frequency of feeding. The effects illustrated in Fig. 1 were substantiated by analysis of variance of the logarithms of the error mean

Table 3. *Expt 2. Mean daily intake of energy (calculated as metabolizable energy*) in hay, concentrate and VFA salts by groups of eight lambs receiving one of three diets in two or six feeds daily*

Diet	No. of feeds daily	Hay (kcal)	Concentrate (kcal)	VFA (kcal)	Total (kcal)
Basal (hay + concentrate)	2	659	1520	—	2179
Basal (hay + concentrate)	6	672	1527	—	2199
Basal + acetate	2	674	1515	301	2490
Basal + acetate	6	669	1527	304	2500
Basal + propionate	2	665	1516	356	2537
Basal + propionate	6	679	1529	356	2564

* Calculated by the method of Ørskov *et al.* (1966).

Table 4. *Expt 2. Treatment means of initial and final live weight, empty body weight, carcass weight, fat and protein percentage in loin, 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 slaughtered initially or receiving one of three diets in two or six feeds daily*

Diet	No. of feeds daily	Initial weight (lb)	Final weight (lb)	Empty body weight* (lb)	Carcass weight* (lb)	Loin		No. of carcasses in each grade		
						Fat content (%)	Protein content (%)	A	B	C
Initial slaughter group	—	52·6	—	44·6	25·0	11·8	17·4	—	2	6
Basal (hay + concentrate)	2	52·7	78·3	66·6	37·2	23·3	15·6	3	2	2
Basal (hay + concentrate)	6	52·7	80·2	68·5	38·8	22·9	15·6	5	3	0
Basal + acetate	2	52·7	85·8	74·6	41·9	23·2	16·0	5	2	0
Basal + acetate	6	53·1	86·7	74·9	42·8	25·0	15·4	7	1	0
Basal + propionate	2	52·1	84·9	74·4	42·0	24·2	15·5	3	4	0
Basal + propionate	6	52·9	85·0	74·0	42·2	25·9	15·2	5	2	0
Standard error of difference between two means	—	±0·64	±1·42	±1·42	±1·18	±2·67	±0·65	—	—	—

* Values for the initial slaughter group were not included in the statistical analysis.

squares from each set of determinations of acetic acid (Table 2). The difference in variation between feeding twice and six times daily was significant ($P < 0.05$) and that between feeding twice and four times approached significance ($P < 0.1$). On investigating the linear and quadratic effects of the values from which the curves in Fig. 1 were constructed, it was found that the quadratic effect of feeding four or six times daily did not differ significantly from zero, whereas there was a highly significant quadratic effect when the animals were fed twice daily ($P < 0.001$).

The mean daily intake of hay and concentrate and VFA salts in Expt 2 calculated as metabolizable energy (ME) is given in Table 3.

The most relevant results of Expt 2 are summarized in Table 4, where gains in body tissues reflected in live weight, empty body weight and carcass weight are given, together with the composition of the loins and number of carcasses in each grade. Missing values were calculated for the two lambs which died and a third which at slaughter was found to be a cryptorchid.

There were no significant differences between the two frequencies of feeding nor were there any significant interactions between feeding frequency and VFA treatment.

The lambs receiving acetate and propionate grew faster and had a greater empty body weight at slaughter than those receiving the basal diet ($P < 0.001$) and, if the results are pooled according to diet, the differences between the basal control group and the VFA treatment groups combined in final live weight, empty body weight and carcass weight were significant at $P < 0.001$; there were no differences approaching significance between the acetate and propionate groups.

There were no differences that approached significance between the different groups in fat or protein content of loin. In the initial slaughter group, however, the carcasses had a lower fat percentage and a higher protein percentage than the groups receiving the feeding treatments ($P < 0.01$). The carcass gradings show that all the lambs produced satisfactory carcasses except the carcasses of the initial slaughter group which were graded lower than those of the other groups.

DISCUSSION

Influence of frequency of feeding of VFA salts on the rumen VFA composition

The results of Expt 1 demonstrated clearly that the marked changes in the composition of rumen VFA associated with giving the sheep VFA salts twice daily could be greatly reduced by increasing the frequency of feeding. Part of this reduced variation might have been an effect of less variation on the molar proportions from fermentation of the basal constituents of the food (Satter & Baumgardt, 1962; Bath & Rook, 1963). However, using a similar basal diet, Ørskov & Allen (1966) found no appreciable diurnal variation when the basal diet was given twice daily, and it seems justifiable to assume that the reduced variation was a result of the salts being added to the rumen in smaller quantities at one time.

Influence of the frequency of feeding on the utilization of VFA as sources of energy

The results of Expt 2 are in agreement with results obtained earlier (Ørskov & Allen, 1966; Ørskov *et al.* 1966). If the maintenance requirement is subtracted from the total intake, it can be calculated from data of Langlands, Corbett, McDonald & Pullar (1963) that the energy derived from acetate and propionate per kcal was utilized about 10% more efficiently to promote carcass gain than was the calculated ME of the basal diet. This estimate could be influenced by errors involved in calculating the maintenance requirements and the ME of the basal ration. However, if it is assumed that the utilization of ME increases with increasing level of concentrate in the diet (Blaxter, 1962), the results are in agreement with those of Ørskov & Allen (1966) who showed that the utilization of VFA salts was equal to that of ME in concentrates.

Increasing the frequency of feeding did not influence the utilization of the VFA. The proposition made that the effect of twice daily feeding, with the resultant large increases in the rumen VFA concentrations, might have caused blood concentrations that exceed the metabolic capacity of body tissues must therefore be rejected. The results showed that the animals were able to utilize the VFA efficiently even when large increases in concentration occurred from twice daily feeding.

There were no signs of interactions between the utilization of VFA and feeding frequency. This suggests that the differences in the findings of Ørskov & Allen (1966) and Ørskov *et al.* (1966) and those of Armstrong and his colleagues (Armstrong & Blaxter, 1957; Armstrong *et al.* 1958) regarding the efficiency of utilization of VFA are probably not a result of difference in technique, namely feeding twice daily with VFA salts or using continuous infusions of the dilute acids. The difference in results will be discussed in a later communication.

Influence of feeding frequency on the efficiency of food utilization by ruminant animals

The effect of increasing the feeding frequency from twice to six times daily had little effect on the utilization of the basal diet. This finding led to a further inquiry into the observations of other workers where the influence of feeding frequency of isocaloric daily rations has been investigated. Table 5 presents a summary of the results of experiments on the effect of feeding frequency on daily live-weight gain when isocaloric daily rations have been given.

Except in a small-scale experiment by Rakes, Lister & Reid (1961), the experiments in which improved gains were reported from increasing the frequency of feeding were all characterized by very low growth rates, and the only criterion for response was live-weight gain. In addition to the experiments in which no effects were noted from increasing the frequency of feeding, Rhodes & Woods (1962) in a series of experiments comparing different ratios of hay to concentrate and different combinations of feeding frequencies found no effect of increasing the feeding frequency beyond twice daily in animals making rapid gains. Kay & Hobson (1963), when reviewing the subject, concluded that ruminant animals benefit substantially from increased frequency of

feeding and that the results of Rhodes & Woods (1962) were an exception. This conclusion does not seem entirely justified. When moderate growth rates have been attained none of the experiments referred to has demonstrated a significant improvement in terms of live-weight gain when an isocaloric daily ration has been offered at intervals of greater frequency than twice daily. The results of the present work conform with these observations.

Table 5. *Summary of experiments in which the effects of frequency of feeding with isocaloric daily rations have been investigated*

Source of data	Type of food	Animals	No. of feeds daily (*a*)	No. of feeds daily (*b*)	Growth rate (lb/day) (*a*)	Growth rate (lb/day) (*b*)
Gordon & Tribe (1952)	Hay and concentrate	Lambs	1	8	0·03	0·18
Rakes, Hardison, Albert, Moore & Graf (1957)	Chopped hay	Heifers	2	10	0·49	0·94
Mochrie, Thomas & Lucas (1956)	Hay and concentrate	Steers	2	4	1·03	1·21
Putnam, Gutierrez & Davis (1961)	Alfalfa	Calves	2	10	0·55	0·75
Rakes, Lister & Reid (1961)	Hay and concentrate	Young wethers	1	8	0·22	0·36
Rakes *et al.* (1961)	Hay and concentrate	Mature wethers	1	8	0·17	0·18
Gibbons (1958)	Hay and concentrate	Identical twin calves	1	2	1·30	1·33
Gibbons (1958)	Hay and concentrate	Identical twin calves	1	6	1·60	1·68
Present work	Hay and concentrate	Lambs	2	6	0·25	0·26

The following observations on the thermodynamics of feeding may partly explain the differences in results from feeding frequency experiments: (*a*) the heat increment of isocaloric rations was unaffected by the frequency at which the rations were given (Blaxter, Graham & Wainman, 1956); (*b*) most of the heat associated with feeding is liberated during the first 6 h after feeding (Brody, 1945); (*c*) Richardson & Mason (1923) demonstrated, with human subjects, that the heat increments of feeds below maintenance level could be utilized in maintaining body temperature when given at intervals of 2 h as no appreciable increase occurred in the basal heat production; (*d*) part of the environmentally induced increase in basal heat production could be compensated for by the heat increment of feeding (Rubner 1902, quoted by Sørensen, 1961).

Several factors can induce increases in the maintenance requirement of animals (Blaxter, 1962). It is possible, therefore, that the differences in results of experiments on frequency of feeding (Table 5) could be an effect of the conditions in which the experiments were conducted and that the effects of frequency of feeding might be most sensitive at or about the maintenance level of feeding. This is substantiated by the experiments referred to in Table 5.

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