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Volatile fatty acid production in the rumen of cattle given an all-concentrate diet

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1. Short-term in vitro incubations of whole rumen contents have been used to measure the rate of production of volatile fatty acids (VFA) in two heifers given a diet containing 85 % barley and 15 % protein–mineral–vitamin supplement. Daily intake of the diet was restricted to 5·1 kg which was given in three equal feeds. In one heifer (no. 794), VFA production was measured over a 24 h feeding cycle; in the other (no. 832), a single 6 h feeding period was examined.

2. The feeding schedule outlined above was found in previous work to result in a major alteration in the rumen environment and in the end-products of rumen fermentation relative to findings in animals given the same diet *ad lib.* Some of these earlier observations have been confirmed and extended in the present work.

3. In heifer 794, the rate of VFA production reached a maximum (52–62 m-moles/l. h) 15 min after each feed and then declined steadily throughout the remainder of the feeding period. Consistent changes with time were observed in rumen pH and in the concentration of VFA and dry matter in rumen contents during each feeding period.

4. Simultaneous measurements of rumen fluid volume and outflow allowed estimates to be made of VFA production and absorption in the entire rumen during each feeding period. In heifer 794, total VFA production amounted to 18·2 moles/day of which approximately 85 % was absorbed directly from the reticulo-rumen. On a caloric basis, total daily production accounted for 36·5 % of the digestible energy intake.

5. In heifer 832 there were significant differences in the rate of VFA production and in rumen pH and VFA concentration between two sets of observations made some 8 weeks apart. These differences appeared to be related to changes in the rumen microbial population and in particular to a marked alteration in the numbers of ciliate protozoa. The possible role of rumen ciliates in modifying both the rate of VFA production and the environment within the rumen is discussed.

The fattening of cattle on diets based entirely on cereals and protein supplements is now an established part of British livestock husbandry. These diets are frequently given *ad lib.* and without supplementary roughage (Preston, Aitken, Whitelaw, McDearmid, Philip & MacLeod, 1963) and are known to lead to conditions of low pH and high molar proportions of propionic acid in rumen contents (Eadie, Hobson & Mann, 1967; Topps, Kay, Goodall, Whitelaw & Reid, 1968; Fell, Kay, Whitelaw & Boyne, 1968). On these diets also the concentration of volatile fatty acids (VFA) in the rumen is high (Eadie *et al.* 1967) and this suggests either a more rapid rate of production of VFA or some impairment in the rate of VFA absorption in animals given all-cereal diets relative to those given diets based on roughage. Apart from some limited observations by Topps, Reed & Elliot (1966), no direct measurements appear to have been made of VFA production in and absorption from the rumen of cattle given all-concentrate diets.

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The experiments described here were designed to provide quantitative information on this subject. A brief account of this work has been given elsewhere (Whitelaw, Reid, Murray & Hyldgaard-Jensen, 1966).

EXPERIMENTAL

Animals

Two Hereford × Ayrshire heifers (nos. 794 and 832) were used. These had a mean age of 19 months and a mean live weight of 360 kg over the period of the experiment and each had been fitted with a permanent rumen cannula of 2 in. internal diameter at about 8 months of age. Details of the management and previous nutritional history of these animals have been given by Eadie, Hyldgaard-Jensen, Mann, Reid & Whitelaw (1970).

Diet

The diet consisted of a pelleted all-concentrate mixture containing 85% barley and 15% protein–mineral–vitamin supplement (Preston, 1963). The daily intake of both animals was restricted to 5·1 kg air-dry feed and was given in three equal feeds at 08.00 h, 14.00 h and 20.00 h.

General procedure for experiments

The periods 08.00–14.00 h, 14.00–20.00 h, 20.00–02.00 h and 02.00–08.00 h were designated periods 1, 2, 3 and 4 respectively. Period 4 differed from the others in that no food was given. In heifer 794, three replicate series of measurements were made in each of periods 1 and 2 and two replicates in each of periods 3 and 4. Only period 1 was examined in heifer 832; further experiments with this animal were prevented by the occurrence of excessively frothy rumen contents which made it impossible to obtain representative samples. In this animal replicate 1 preceded replicates 2, 3 and 4 by a period of 8 weeks and the results of replicate 1 were found to differ in many respects from those of the later replicates. Results for this animal have therefore been presented as period 1*a* (replicate 1) and period 1*b* (replicates 2, 3 and 4).

Each animal was restrained in a holding-stall during an experiment and had its rumen contents mixed by means of a rumen pump which was identical in construction with that described by Sutherland, Ellis, Reid & Murray (1962) but was approximately one-third larger. The pump was inserted in the rumen 30 min before feeding but was removed for two 45 min periods during an experiment to allow the animal to lie down.

Since each feed was invariably consumed within 10 min the food given during the experiments was offered 5 min before the nominal times of feeding given above. No water was given during the experiments.

In vitro incubations

In periods 1, 2 and 3 samples of whole rumen contents were withdrawn from the rumen pump by means of an open-ended syringe 15 min before, and 15, 30 and 60 min

and 1·5, 2·5, 4·25 and 6 h after feeding. During period 4, samples were taken 8, 10 and 12 h after the previous feed. At each sampling time six samples were taken for incubation, three samples for dry-matter determination and one sample for pH measurement. Each sample weighed about 15 g.

Immediately after withdrawal each sample for incubation was transferred to a warm, tared Erlenmeyer flask and placed in a Warburg bath maintained at $38 \pm 1°$. A gas mixture (50% CO_2, 50% N_2) was passed through the flask for approximately 2 min after which the flask was sealed and incubation continued with gentle shaking.

Pairs of samples were 'killed' after 15 or 30 min incubation by the addition of 1 ml 10 N-H_2SO_4, injected into the flask through a rubber septum. A third pair of samples had the acid added to the flask before the rumen contents. Zero-time, from which 'killing-time' was measured, was taken as the mid-point of the time taken to withdraw the samples. After killing, samples were cooled, weighed and centrifuged at 38000*g* for 20 min. The clear supernatant fluid so obtained was stored at $-20°$. Estimations of total VFA were made on each sample and individual VFA were separated by gas–liquid chromatography on one sample from each pair of duplicate incubations.

Samples for dry-matter estimation were transferred from the sampling syringe to wide-mouthed weighing bottles and immediately chilled to $-20°$. They were later freeze-dried. The pH of rumen samples was measured at 38° on a Cambridge pH meter (W. G. Pye and Co. Ltd) with a glass electrode.

Rumen fluid volume estimations

In each experiment, 20 g polyethylene glycol (PEG) as a 50% solution in water were injected into the rumen via the rumen pump 15 min before feeding. Six samples of rumen contents for PEG estimation (Hydén, 1955) were withdrawn at intervals after injection and the zero-time PEG concentration was derived from the regression equation relating log PEG concentration to time. A second injection of PEG was given 6 h after the first and further samples were taken after this injection. 'Initial' and 'final' rumen fluid volumes for each feeding period were calculated from the zero-time concentrations obtained from the first and second injections respectively. Where 'initial' and 'final' volumes differed these were plotted against time and volumes for intermediate times arrived at by interpolation. Mean outflow rate for each feeding period was derived from the regression coefficient relating to the first PEG injection.

In preliminary experiments, solutions of PEG and VFA were added separately to whole rumen contents in vitro and it was established from the final concentration of these substances in rumen fluid that the volume occupied by both PEG and VFA was equal to the volume of total rumen water.

Calculation of VFA production rates

The rate of production of total VFA at each sampling time was calculated from the increments in VFA concentration after incubation for 15 min and for 30 min. Two models were assumed: (*a*) that increments observed in vitro are curvilinear with

respect to time, or (*b*) that these increments decline exponentially with time, due to gradual exhaustion of substrate and accumulation of metabolic end-products. The working equations derived for these two models were:

$$B = 8y_1 - 2y_2, \qquad (a)$$

$$B = \frac{4\{y_1^2 \log_e[(y_2 - y_1)/y_1]\}}{y_2 - 2y_1}, \qquad (b)$$

where B is the rate of production (m-moles/l. h) at zero-time and y_1 and y_2 are the increments (m-moles/l.) observed after 15 min and 30 min incubations respectively. Equation (*a*) is identical with that used by Stewart, Stewart & Schultz (1958), except that these workers used incubation periods of 30 min and 1 h; equation (*b*) was previously used by Sutherland (1963; and unpublished results.)

Table 1. *Rates of VFA production calculated from the increments in VFA concentration observed after incubation in vitro of rumen samples from heifers for periods of* 15 *and* 30 *min,* 30 *and* 60 *min and* 120 *min*

	VFA production rate (m-moles/l. h)			
	Expt 1*		Expt 2*	
Period of incubations (min)	(*a*)†	(*b*)	(*a*)	(*b*)
15–30	48·8	49·6	42·1	42·6
30–60	46·5	48·5	36·8	36·9
60–100	37·6	38·5	36·9	38·0

* The samples were withdrawn from the rumen 0·5 h after feeding in Expt 1 and 1 h after feeding in Expt 2.

† (*a*) and (*b*) refer to the method of calculation (see above).

Table 2. *pH in vivo and in vitro of rumen contents from heifers*

		Time of incubation (min)		
Time after feeding (h)	Sample	0	15	30
1	Rumen	5·31	5·25	5·28
	Incubation flask	5·31	5·25	5·19
1	Rumen	5·85	5·80	5·74
	Incubation flask	5·85	5·78	5·64
6	Rumen	6·43	6·50	6·52
	Incubation flask	6·43	6·42	6·32

In preliminary experiments comparisons were made of samples incubated for 15 and 30 min, 30 and 60 min and 60 and 120 min. The calculated rates of VFA production are shown in Table 1. In each experiment the maximum rate of production was found with the 15–30 min incubations and these times were therefore selected as the incubation periods of the main experiment.

In other experiments, pH values were determined after incubation for 15 and 30 min and were compared with the pH values existing in the rumen of the donor animal over the same time intervals. As shown in Table 2, the pH values of incubated samples

differed by only 0·1–0·2 unit from the corresponding samples of normal rumen contents.

The rate of VFA production at each sampling time was calculated by each of the equations given above and was assumed to equal the rate of VFA production in vivo at the instant of removing samples from the rumen. These rates were then multiplied by the appropriate rumen fluid volume to obtain the rate of production in the entire rumen (m-moles/h) at each sampling time. Total production during an entire feeding period was taken as the area under the curve obtained by plotting production rate (m-moles/h) against time. Area measurements were made by planimeter and were all done by the same person to ensure uniformity. Production rates of individual VFA were calculated in the same way from the mean molar proportions of the zero, 15 and 30 min samples of each incubation.

Analytical methods

Total VFA were determined by steam-distillation in a Markham still and the individual acids separated by the chromatographic procedure of James & Martin (1952) with the column packing described by Annison & Pennington (1954). This method failed to give complete separation of butyric acid from higher acids, and these components have been reported as 'butyric plus higher acids'. In later experiments in which a different column packing was employed, higher acids were found to account for less than 2% of the total acids present (Eadie *et al.* 1970).

Microbiology of the rumen

Samples of rumen fluid were taken at frequent intervals throughout the experiments and were examined for rumen ciliate protozoa and bacteria. These observations have been reported by Eadie *et al.* (1970). In the terminology of these workers (see their Tables 1 and 3), the present experiments covered the period 20–46 'weeks on barley diet' for animal 794, and 13 (period 1*a*) and 21–26 (period 1*b*) 'weeks on barley diet' for animal 832.

Digestibility of the diet

The apparent digestibilities of dry matter and of gross energy were measured in animal 794 at the end of the experiment. Digestibility was determined both by total collection of faeces over a 7-day period and by the excretion of Cr_2O_3 administered into the rumen on impregnated paper (Corbett, Greenhalgh & MacDonald, 1958) during the same period. Gross energy of feed and faeces was determined by bomb calorimetry.

RESULTS

Conditions within the rumen

Microbiology. Details of the microbial population present in the rumen of heifers 794 and 832 have been reported by Eadie *et al.* (1970). These workers adopted a 'total unit' value based on the relative sizes of different species as an index of ciliate activity and this measure only will be considered here in discussing the ciliate populations. Heifer 794 maintained a very high and essentially stable population of ciliates

throughout the present experiments and 'total unit' values ranged from 2352×10^3 to 3381×10^3/ml. During the period 17–25 'weeks on barley diet' total bacterial numbers ranged from 8·4 to $10{\cdot}8 \times 10^9$/ml and a coccal form of *Bacteroides* spp. was the predominant type seen in Gram films (Eadie *et al.* 1970).

In contrast, heifer 832 showed highly unstable rumen conditions up to 20 weeks on the barley diet. At week 13, when the period 1*a* experiment was made, the 'total unit' value was 134×10^3/ml and the total bacterial count was $7{\cdot}6 \times 10^9$/ml. At this time a Gram-negative spirillum-type rod had displaced *Bacteroides* as the principal bacterial type. By 21 weeks the microbial population had become similar to that found in heifer 794; the ciliate population had increased markedly and during period 1*b* it had a mean 'total unit' value of 1871×10^3/ml while total bacterial numbers ranged from $7{\cdot}7$ to $12{\cdot}7 \times 10^9$/ml.

Table 3. *pH, dry matter, total VFA concentration and molar proportions of individual VFA in rumen contents of heifers during in vitro incubation experiments*

(Mean values and standard errors for replicates)

					Volatile fatty acids			
						Molar proportions (%)		
Heifer	Period	Replicates	pH	Dry matter (%)	Total (m-mole/l.)	Acetic acid	Propionic acid	Butyric + higher acids
794	1	3	6·37 ± 0·09	10·2 ± 0·2	95·8 ± 5·4	67·2 ± 1·2	9·4 ± 2·2	23·4 ± 1·0
	2	3	6·34 ± 0·01	9·9 ± 0·3	95·2 ± 3·6	57·9 ± 1·8	13·3 ± 0·5	28·8 ± 1·4
	3	2	6·23 ± 0·07	10·3 ± 0·4	97·1 ± 3·5	58·6 ± 3·5	13·0 ± 0·6	28·4 ± 3·0
	4	2	6·54 ± 0·02	7·9 ± 0·6	72·6 ± 1·0	59·9 ± 1·9	8·6 ± 0·6	31·5 ± 2·4
832	1*a*	1	6·03*	10·5	109·8*	—	—	—
	1*b*	3	6·30 ± 0·03	10·2 ± 0·8	90·9 ± 2·7	61·5 ± 1·6	14·2 ± 2·6	24·3 ± 1·3

* Values differ significantly from the corresponding values of period 1*b* ($P < 0{\cdot}05$).

Rumen pH, VFA and dry matter. Overall mean values for rumen pH, dry-matter content, total VFA concentration and molar proportions of individual VFA during each feeding period are given in Table 3.

In heifer 794 the mean values for pH, dry matter and VFA concentration were similar for periods 1–3 in which the animal was fed, but they differed from the values recorded in period 4 in which no food was given. Greater variation was found between periods in the mean molar proportions of VFA and these were characterized by a higher proportion of butyric acid and a much lower proportion of propionic acid than is usually encountered in cattle given all-concentrate diets.

In animal 832 there were significant differences ($P < 0{\cdot}05$) between periods 1*a* and 1*b* in pH and total VFA concentration but not in percentage dry matter of rumen contents. The samples relating to period 1*a* were discarded in error before VFA separations were made.

The changes with time in pH, VFA concentration and percentage dry matter in the rumen are shown for heifers 794 and 832 in Figs. 1 and 2 respectively. In 794 almost

identical patterns of change were observed after each feed and all values returned slowly to the 08.00 h, prefeeding level during the final 6 h period. Total VFA concentration in this animal never exceeded 109 m-moles/l. and reached a maximum 2·5 h after each feed. This point coincided with the minimum pH which remained

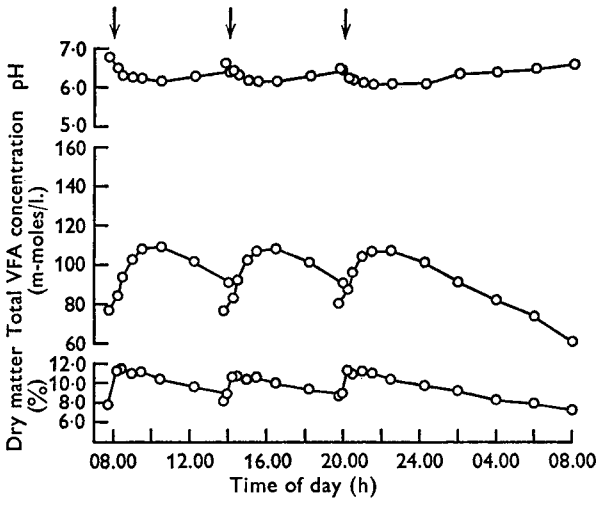

Fig. 1

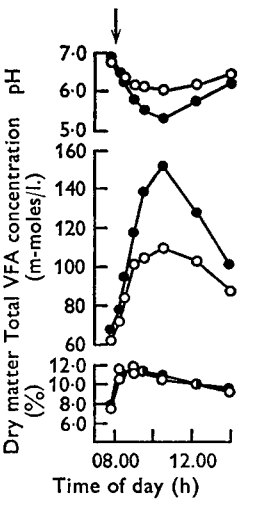

Fig. 2

Fig. 1. Rumen pH, volatile fatty acid (VFA) concentration and dry-matter content in the rumen ingesta of heifer 794 when given 1·7 kg of a barley diet in each of three feeds. Times of feeding are indicated by arrows.

Fig. 2. Rumen pH, volatile fatty acid (VFA) concentration and dry matter content in the rumen ingesta of heifer 832 when given 1·7 kg of a barley diet in period 1*a* (●) and period 1*b* (○). The time of feeding is indicated by an arrow.

Table 4. *Rumen fluid volume and outflow during in vitro incubation experiments*

(Mean values and standard errors for replicates)

Heifer	Period	Replicates	Rumen fluid volume (l.) Initial*	Rumen fluid volume (l.) Final*	Outflow (% of rumen volume/h)
794	1	3	24·3 ± 1·3	25·0 ± 1·1	5·16 ± 0·86
	2	3	27·5 ± 0·5	23·3 ± 1·6	4·84 ± 0·67
	3	2	25·5 ± 2·7	26·3 ± 1·9	4·19 ± 1·45
	4	2	26·3 ± 1·9	22·9 ± 1·8	3·72 ± 0·38
832	1*a*	1	23·9	18·1	4·11
	1*b*	3	22·5 ± 1·2	22·3 ± 0·4	4·08 ± 0·64

* 'Initial' and 'Final' refer to measurements made at the beginning and end respectively of each feeding period.

above 6·0 during the entire feeding cycle. The same pattern and similar values for pH and VFA concentration occurred in 832 during period 1*b* but these differed markedly from the findings in period 1*a*. In this period total VFA concentration reached a maximum value of 152 m-moles/l. and pH fell to 5·3; all values for pH and

VFA concentration recorded between 09.00 h and 12.15 h in this animal differed significantly between periods 1*a* and 1*b* ($P < 0.05$–0.01).

The molar proportions of individual VFA differed only slightly at the different sampling times within each feeding period. The mean standard errors of these measurements derived from the pooled sampling-time by replicate interactions were 1·5, 0·8 and 1·1% for acetic, propionic and 'butyric + higher' acids respectively.

Rumen fluid volume and outflow. Mean values for 'initial' and 'final' rumen fluid volumes and for fractional clearance rate during each feeding period are given in Table 4.

In vitro incubations

The rate of production of VFA at each sampling time was calculated by each of the equations given on p. 182. The two equations gave values which did not differ significantly although there was a tendency for the logarithmic model (equation *b*) to give slightly higher values at the times of maximum production (see Table 1). The logarithmic model was used for all subsequent calculations.

The mean rates of VFA production are given in Table 5. In heifer 794, production rates showed a similar pattern during each feeding period, being low before feeding and reaching a maximum in the sample withdrawn 15 min after feeding. The rate of production recorded before feeding increased progressively from period 1 to period 3, indicating that the fermentation of available substrate was not complete during the 6 h period following each feed.

In animal 832, production rates during period 1*b* were similar to those recorded for 794 during period 1, although the maximum rate was somewhat higher in 832. In period 1*a*, however, the maximum rate of production occurred 30 min after feeding and the values recorded were higher than those found during period 1*b* or during the corresponding feeding period in 794.

VFA production in the entire rumen

The time-course of VFA production rate (m-moles/h) over a complete 24 h period in heifer 794 is shown in Fig. 3, and for period 1*a* and 1*b* in heifer 832 in Fig. 4. In 794 the pattern of production was similar from feed to feed and values recorded at the end of each feeding period agreed well with those obtained at the start of the next period. In 832 however, there, was a considerable difference between periods 1*a* and 1*b* in the time-course of VFA production (Fig. 4). In period 1*b* the pattern observed was similar to that recorded for period 1 in 794, whereas in period 1*a* there was a much higher maximum production, a longer time-lag in reaching this maximum and a more abrupt return to the preceding values. All values for VFA production (m-moles/h) recorded between 0·25 and 1·00 h after feeding differed significantly between periods 1*a* and 1*b* ($P < 0.01$).

Total VFA production in each animal during each feeding period is given in Table 6. In 794, production ranged from 4·6 to 5·4 moles in the periods in which food was given and fell to 3·0 moles in period 4. Total production in this animal during the entire 24 h feeding cycle was 18·2 moles. In 832, total production during period 1*b*

Table 5. *Mean rates of production of VFA (m-moles/l. h) at different times after feeding*

Period	Replicates	Time after feeding (h) −0·25	0·25	0·50	1·00	1·50	2·50	4·25	6·00	8·00	10·00	12·00	SE†
		VFA production rate (m-moles/l. h)											
Heifer no. 794													
1	3	14·6	62·1	45·5	44·0	35·1	36·6	20·5	24·4	—	—	—	±5·24
2	3	21·2	52·5	43·9	44·8	45·3	33·6	29·3	26·6	—	—	—	±6·12
3	2	30·2	52·9	50·1	41·9	38·6	30·3	28·6	27·6	—	—	—	±3·52
4	2	—	—	—	—	—	—	—	—	23·7	17·9	12·2	
Heifer no. 832													
1*a*	1	6·7	81·9	97·2*	80·0*	65·0*	26·8	26·0	17·2	—	—	—	—
1*b*	3	12·8	71·8	64·1	43·4	41·8	34·4	29·6	25·2	—	—	—	±4·19

* Values differ significantly from corresponding values of period 1*b* ($P < 0{\cdot}05$).

† Standard error; mean values derived from pooled sampling − time by replicate interaction.

was comparable with that in period 1 in 794 but differed significantly ($P < 0.01$) from that recorded in period 1*a*.

The mean molar proportions in which the individual VFA were produced during each feeding period are also shown in Table 6. These values closely resemble the values for mean molar proportions of VFA present in the rumen of each animal during the same feeding period (Table 3) and confirm the suggestion first made by Shaw

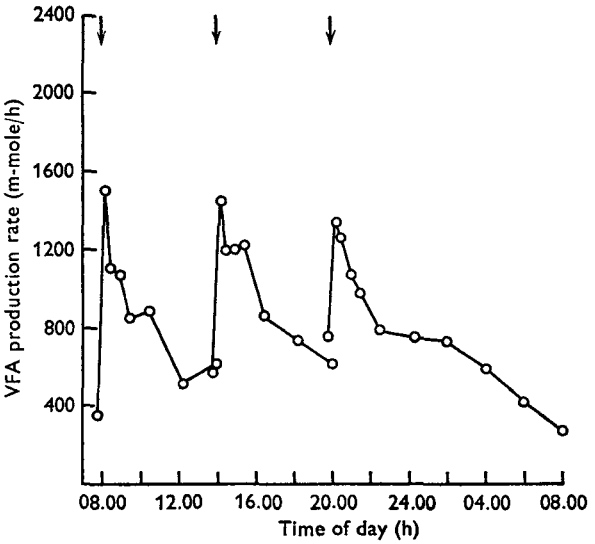

Fig. 3

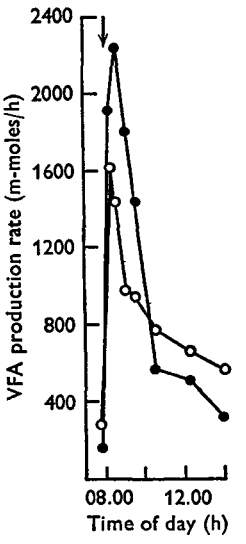

Fig. 4

Fig. 3. The rate of production of volatile fatty acids (VFA) in the rumen of heifer 794 when given 1·7 kg of a barley diet in each of three feeds. Times of feeding are indicated by arrows.

Fig. 4. The rate of production of volatile fatty acids (VFA) in the rumen of heifer 832 when given 1·7 kg of a barley diet in period 1*a* (●) and period 1*b* (○). The time of feeding is indicated by an arrow.

Table 6. *Total production of volatile fatty acids in the rumen of cattle during 6 h periods*

(Mean values and standard errors for replicates. Feed intake was 1700 g in all the periods except no. 4, in which no food was given)

			VFA production			
				Molar proportions (%)		
Heifer	Period	Replicates	Total (moles)	Acetic acid	Propionic acid	Butyric + higher acids
794	1	3	4·63 ± 0·06	66·0 ± 1·6	9·9 ± 2·6	24·1 ± 0·7
	2	3	5·40 ± 0·49	58·8 ± 0·7	13·3 ± 0·4	27·9 ± 1·0
	3	2	5·16 ± 0·37	58·4 ± 2·7	12·8 ± 0·1	28·8 ± 2·6
	4	2	3·02 ± 0·21	59·7 ± 0·4	9·5 ± 0·9	30·8 ± 1·3
832	1*a*	1	5·43*	—	—	—
	1*b*	3	4·87 ± 0·02	61·3 ± 1·6	14·9 ± 2·4	23·7 ± 1·1

* Value differs significantly from corresponding value of period 1*b* ($P < 0.01$).

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Table 7. *Mean rates of VFA absorption from the rumen (m-moles/h) at intervals after feeding*

		Interval after feeding (h)									
		−0·25–+0·25	0·25–0·50	0·50–1·00	1·00–1·50	1·50–2·50	2·50–4·25	4·25–6·00	6·00–8·00	8·00–10·00	10·00–12·00
		VFA absorption rate (m-moles/h)									
Heifer	Period										
794	1	90	278	505	562	703	670	594	—	—	—
	2	259	225	575	836	859	758	711	—	—	—
	3	192	287	652	760	753	741	775	—	—	—
	4	—	—	—	—	—	—	—	695	541	445
832	1*a*	125	549	967	658	577	703	629	—	—	—
	b	14	345	354	733	653	701	726	—	—	—

(1961) that the mean molar proportions of VFA in rumen contents might provide a good index of relative production rates.

Absorption of VFA

The difference between the rate of production on the one hand and the rate of onward passage to the omasum plus the rate of increase in the rumen on the other gives an estimate of the rate of absorption of VFA from the rumen. Overall mean values for the rate of VFA absorption during successive sampling intervals within each feeding period are shown in Table 7. Within each period the time-course of VFA

Table 8. *Total absorption, increase in the rumen and onward passage of VFA as a percentage of total VFA production in each period*

		% of total VFA production		
Heifer	Period	Absorption	Increase in rumen	Onward passage
794	1	75·9	7·5	16·6
	2	79·6	6·7	13·7
	3	81·3	6·0	12·7
	4	111·2	−25·3	14·1
	24 h total	84·4	1·4	14·2
832	1*a*	74·8	13·2	12·0
	1*b*	77·4	11·5	11·1

Table 9. *Daily energy intake and calorific value of VFA produced in the rumen of heifer* 794

	Energy (kcal/day)	Total VFA (% of energy)
Gross energy intake	18187	31·5
Digestible energy intake	15699	36·5
Metabolizable energy* intake	13226	43·4
VFA produced:		
Acetic acid	2313	—
Propionic acid	780	—
Butyric+higher acids	2641	—
Total	5734	—

* True metabolizable energy (Blaxter, 1962).

absorption followed very closely that of VFA concentration (Figs. 1 and 2). The maximum rate of absorption in any feeding period amounted at most to 50% of the maximum rate of production during the same period (Figs. 3 and 4).

The total quantities of VFA absorbed from the rumen, passing directly to the omasum and remaining in the rumen during each feeding period, each expressed as a percentage of total VFA production during that period, are shown in Table 8. During the 24 h feeding cycle in 794 approximately 85% of the total production was absorbed directly from the rumen while some 14% passed to the omasum in the flow of digesta.

Energy intake

The apparent digestibility of dry matter in heifer 794 was found to be 86·4% by total collection of faeces and 84·8% by Cr_2O_3 recovery; the mean of these values, 85·6% was used in subsequent calculations. The apparent digestibility of gross energy was 86·3%. The gross energy intake, digestible energy intake and calculated metabolizable energy intake of this animal are given in Table 9, together with the calorific value of VFA produced and the proportion of the various categories of energy intake which could be accounted for as VFA.

DISCUSSION

Since preliminary experiments had shown wide fluctuations in daily food intake and in the concentrations of various metabolites in the rumen under an *ad lib.* feeding regime, the diet in the present experiments was offered in amounts below appetite and was given in three equal feeds throughout the day. This feeding schedule was adopted purely for convenience but was found later to be largely responsible for a major alteration in the rumen microbial population and in the ratio of end-products of rumen fermentation (Eadie *et al.* 1970). In consequence, the findings presented here cannot be considered typical of cattle given the same diets *ad lib.*

In both animals, VFA production rate increased very rapidly immediately after feeding and reached a maximum value after 15 min. The maximum values recorded ranged from 52 to 97 m-moles/l. h and were considerably higher than those reported by Stewart *et al.* (1958) for cattle given diets of hay and concentrates (29–30 m-moles/l. h). However, they were probably of the same order as those of Sutherland (1963) who recorded values of 600 m-moles/h in a sheep given a diet of grass cubes. Sutherland, Reid & Murray (1963) showed that the very rapid rate of production immediately after feeding was associated with the fermentation of soluble carbohydrate, which made up about 20% of the grass cube diet. Although the barley diet used in the present work contained only about 4% of soluble carbohydrate (Topps, Kay & Goodall, 1968) it was rich in starch which would also be fermented rapidly by rumen micro-organisms.

The molar proportions of VFA found in these experiments were unlike those previously reported for cattle given diets high in starch (Balch & Rowland, 1957; Kay, Walker & McKiddie, 1967; Fell *et al.* 1968). The low propionic–high butyric acid pattern recorded here was shown by Eadie *et al.* (1970) to be a constant feature in heifers 794 and 832 when large numbers of ciliate protozoa were present in the rumen. It is unfortunate that no information is available regarding the molar proportions of VFA in heifer 832 during period 1*a*; at this time, very few ciliate protozoa were present in the rumen and this has been shown to lead to low proportions of acetic acid and high proportions of propionic acid in the fermentation mixture (Eadie *et al.* 1970).

In addition to these postulated differences in VFA proportions there were significant differences between periods 1*a* and 1*b* in rumen pH, VFA concentrations and VFA production rates. Since no changes in feeding or management took place between

Table 10. *Estimates of daily VFA production in the rumen of sheep and cattle*

Authority	Method	Species	Diet	VFA produced/24 h: Moles/kg dry matter	VFA produced/24 h: As % digested energy
Bergman *et al.* (1965)	Isotope dilution	Sheep	Dried grass cubes	6·7	62
Leng & Leonard (1965)			Lucerne chaff	6·6 (5·4)*	79 (53)*
Gray *et al.* (1966)			50% lucerne chaff + 50% wheaten hay	5·0	57
Gray *et al.* (1967)			50% lucerne chaff + 50% wheaten hay	4·7	54
Weller *et al.* (1967)			60% lucerne chaff + 40% wheaten hay	5·3	62
Bath *et al.* (1962)	Acid dilution	Cattle	Hay	5·8	60
Stewart *et al.* (1958)	In vitro incubation	Cattle	Lucerne hay and concentrates	3·6	38
Sutherland (1963)		Sheep	Dried grass cubes	4·0	38
Present work		Cattle	Barley cubes	4·2	36
Balch (1958)	Fermentation of feed in vitro	Cattle	Hay and concentrates	3·4†	38†

* Values in parentheses were recalculated by Leng, Corbett & Brett (1968) from the original values of Leng & Leonard (1965).
† One aberrant value (part 1a) omitted.

these two sets of observations it seems likely that these differences were directly related to the alteration in the rumen microbial population and in particular to the marked increase in rumen ciliates during period 1*b*. The VFA production curves shown in Fig. 4 indicate a more rapid and extensive fermentation process in period 1*a* relative to 1*b* and can be explained by the known ability of rumen ciliates to ingest starch. Such ingestion of starch by the ciliates would render this substrate unavailable to bacterial attack in the immediate post-feeding period and would result in a more flattened production rate–time curve similar to that noted in period 1*b* and in all feeding periods in 794 (Figs. 3 and 4). A consequence of this more gradual fermentation in the presence of ciliates would be a rather less rapid accumulation of VFA in the rumen and this, in turn, would lead to less violent fluctuations in rumen pH (Fig. 2). Thus one could argue that the ciliate protozoa, especially when present in large numbers, have a considerable capacity to stabilize the pH of rumen contents in cattle given all-concentrate diets. This effect, of course, is highly beneficial to the ciliates, which cannot survive under conditions of low pH (Hungate, 1966; Eadie *et al.* 1970). A relationship between ciliate protozoa and rumen pH in animals given large amounts of starch was postulated by Abou Akkada & Howard (1960) but has not previously been demonstrated.

On a caloric basis, the total daily production of VFA accounted for 36·5% of the intake of apparently digested energy. This value is similar to that found by Stewart *et al.* (1958) in in vitro incubation experiments with cows but is considerably lower than the values of 62 and 54% which have been obtained in isotope-dilution experiments with sheep (Bergman, Reid, Murray, Brockway & Whitelaw, 1965; Gray, Weller, Pilgrim & Jones, 1967). Since it seemed likely that the method of measurement rather than the species difference would be responsible for this discrepancy, the data in the literature relating to VFA production have been re-examined on this basis. In Table 10 are listed all estimates of VFA production which have been measured over a full 24 h period or which can reasonably be extrapolated over this period. For many of them, assumptions have been made regarding the digestibility of the diets when this information has not been given by the original authors. It is immediately evident that the estimates presented in Table 10 fall into two distinct categories: when measured in vivo, VFA production accounts for some 53–62% of digested energy while in vitro the corresponding values are about 36–38%. The fact that the results of Bath, Balch & Rook (1962) agree well with results from isotope-dilution studies suggests that the differences recorded here are true in vivo–in vitro differences and are not caused by some inherent defect in the isotope-dilution procedure.

Since it has been shown that little starch reaches the abomasum of sheep and cattle given barley diets (Topps, Kay & Goodall, 1968; Topps, Kay, Goodall *et al.* 1968) it must be accepted that the in vitro methods of measurement give the more likely estimates of the conversion of digested energy into VFA. To our knowledge, no direct comparison of the two methods of measurement has ever been made in the same animal, although the work of Gray and his colleagues is of relevance in this regard. These workers (Gray, Weller & Jones, 1965) added ^{14}C-labelled VFA to the rumen of a sheep and then transferred a quantity of rumen contents to an in vitro artificial

rumen. The decline in specific activity of the labelled VFA in the rumen over 2 h was found to equal that in the artificial rumen in 3 h, suggesting that the rate of VFA production was 50% greater in vivo than in vitro. This is almost exactly the extent of the discrepancy noted above. Since the artificial rumen system employed by these workers (Gray, Weller, Pilgrim & Jones, 1962) appeared to replicate faithfully the physical conditions obtaining in the rumen of the sheep, it must be concluded that the simple process of removing contents from the rumen is sufficient to cause an appreciable reduction in the activity of the micro-organisms.

The difficulty of maintaining normal conditions within the incubation medium was discussed by Sutherland (1963) who argued that any errors from this source would be minimal if the periods of incubation were kept short. He recognized however that while the initial rate of production might be unaltered under these conditions, the increments in VFA concentration during incubation could be influenced by changes in the physical environment within the incubation flask. Thus increments observed in vitro would not be linear with respect to time even if production rates in vivo were constant with time. Since the initial rate of production can be estimated only from finite increments observed in vitro any mathematical approach to the problem must take account of these departures from linearity in the increment–time relationships. Stewart *et al.* (1958) assumed that their increments with time were best described by a quadratic function whereas Sutherland (1963, and unpublished results) assumed that an exponential function was more appropriate to conditions in which a highly fermentable substrate was available to rumen micro-organisms. Both methods of calculation were used in the present work and were found to give closely similar results. If the estimates of VFA production rate obtained by in vitro incubation are in error, as they appear to be from the foregoing discussion, a possible explanation is that neither of these mathematical models adequately describes the process of fermentation in the incubation flask.

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