Onderstepoort J. vet. Res. 43 (1), 11–22 (1976)

VOLATILE FATTY ACID METABOLISM IN SHEEP. 1. AVERAGE DAILY VOLATILE FATTY ACID PRODUCTION IN THE RUMEN OF SHEEP FED LUCERNE HAY

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ABSTRACT

VAN DER WALT, J. G. & BRIEL, B. J., 1976. Volatile fatty acid metabolism in sheep. 1. Average daily volatile fatty acid production in the rumen of sheep fed lucerne hay. *Onderstepoort J. vet. Res.* 43 (1), 11–22 (1976).

Changes in the total concentration of the volatile fatty acid (VFA) pool in the rumen were followed over a 24 hour period in 2 groups of sheep, 1 fed at 08h00 and the other twice daily at 08h00 and 20h00. Although similar maximum (143 and 147 meq/*l*) and average (100,3 and 102,1 meq/*l*) levels were found in the 12 and 24 h groups respectively, the twice daily feeding regimen resulted in a lower variation (S.D.=17,0 meq/*l* and 28,9 meq/*l* respectively). It was concluded from changes in the percentage molar composition of the VFA pool over the same period that the order of VFA absorption from the rumen was propionate > acetate > butyrate for both groups, but that the differences were less marked for the twice daily fed sheep.

Long term infusions of ^{14}C labelled acetic, propionic and butyric acids into the rumen of sheep fed a total of 1 600 g lucerne hay twice daily (08h00 and 20h00), gave an average net total VFA production rate of 4,52±1,01 moles/800 g/12 hours irrespective of the acid infused.

The net individual turnover rates for acetic (2,81 moles/12 h), propionic acid (0,82 moles/12 h) and butyric acid (0,55 moles/12 h), derived by the subtraction of the inter-conversion factors from the gross production rates of the acids, and expressed as the percentage contribution of each acid to the total net VFA turnover (acetic=62%, propionic=18% and butyric=12%) closely resembled the percentage molar composition of the VFA pool in the rumen (acctic=60%, propionic=23% and butyric=12%).

The total net VFA production was found to be directly proportional to the total VFA concentration in the rumen (correlation coefficient=0,83), and the relationship can be described by the equation $y=0,034\,x+0,16$ where y=VFA production in moles/12 hour and x=VFA concentration in meq/*l*.

A specific VFA production rate of 0,85 moles per 100 g digestible organic matter was calculated from the average daily VFA production rate and the composition of the lucerne hay.

Résumé

VAN DER WALT, J. G. & BRIEL, B. J., 1976. *Métabolisme des acides volatiles gras des moutons. 1. Production moyenne journalière des acides gras volatiles dans le rumen des moutons nourris de foins de luzerne. Onderstepoort J. vet. Res.* 43 (1), 11–22 (1976)

Les changements de la concentration totale du fonds des acides volatiles gras (AVG) du rumen ont été déterminés sur une période de 24 heures en 2 groupes de moutons, un groupe nourri à 8 heures du matin et l'autre groupe deux fois par jour soit à 8 heures du matin et à 8 heures du soir.

Bien que les maximums (143 et 147 meq/l) et les niveaux moyens (100,3 et 102,1 meq/l) aient été trouvés les mêmes dans les groupes de 12 et de 24 heures respectivement, le groupe nourri deux fois par jour avait une variation inférieure (S.D. = 17,0 meq/l et 28,9 meq/l respectivement). Du changement du pourcentage de la composition molaire du fonds des AVG pendant la même période on a conclu que l'ordre d'absorption des AVG du rumen était propionate > acétate > butyrate pour les deux groupes, mais que les différences étaient moins marquées pour les moutons nourris deux fois par jour.

Des infusions à long terme des acides acétiques, propioniques et butyriques ^{14}C dans le rumen des moutons nourris d' un total de 1 600 g de luzerne sèche deux fois par jour (8 heures du matin et 8 heures du soir) ont donné un taux de production total net moyen de 4,52±1,01 moles/800 g/12 heures indépendamment de l'acide infusé.

Le taux net de la consommation individuelle de l'acide acétique (2,81 moles/12 h), de l'acide propionique (0,82 moles/12 h), et de l'acide butyrique (0,55 moles/12 h) a été obtenu par la soustraction des facteurs interconversionnels de la production brute des acides et a été donné comme le pourcentage de la contribution de chaque acide au taux total net de AVG (acétique=62%, propionique=18% et butyrique=12%) et ressemblait intimement au pourcentage de la composition molaire du fonds de AVG dans le rumen.

La production totale nette de AVG à été trouvée directement proportionnelle à la concentration totale des AVG dans le rumen (coefficient de corrélation=0,83), et la relation peut être exprimée par l'équation $y=0,034\,x+0,16$ où y=production AVG en moles/12 heures et où x = concentration AVG en meq/l. Un taux spécifique de production AVG de 0,85 moles par 100 g de matière organique digestible a été calculé en employant le taux de production moyenne journalière de AVG et la composition de luzerne sèche.

INTRODUCTION

The main end-products of microbial fermentation in the ovine rumen are the volatile fatty acids (VFA) which form an essential link in the transfer of energy from the diet to the animal. A knowledge of their metabolism, both in the rumen and beyond, is an essential basis for any investigation into the intermediary metabolism of the sheep. The VFA production rate is an important index of ruminal fermentation and is influenced by the quality and quantity of the diet. Factors, such as frequency of feeding, crude protein level, roughage content and digestibility, affect the ecological balance of the ruminal flora and therefore determine the final composition of the individual fatty acid end-products (Weston & Hogan, 1968; Weller, Pilgrim & Gray, 1969; Leng, 1970).

The original work in this field was based chiefly on the measurement of ruminal VFA levels together with *in vitro* studies of their production rates, and has been extensively reviewed (Annison, 1965). The conclusions drawn from this earlier work depended on the concept of the existence of an equilibrium between the processes of production and absorption, resulting in a constant VFA concentration in the rumen (Gray & Pilgrim, 1951).

Introduction of tracer techniques in the measurement of blood glucose turnover in dogs (Steele, Wall, De Bodo & Altszuler, 1956) led to the development of similar methods in the determination of the VFA production rates in ruminants (Annison & Lindsay, 1961; Gray, Jones & Pilgrim, 1960). The most commonly used technique (Leng, 1970) is based on the rate of dilution of a continuously infused, isotopically labelled acid. Single injection methods have also been used (Davis, 1967), although certain difficulties, such as slow mixing of rumen contents coupled with layering of material and liquid, considerably reduce the potential accuracy of these determinations.

In order to minimise the same difficulties in the continuous infusion method, some workers (*inter alia* Bergman, Reid, Murray, Brockway & Whitelaw, 1965; Leng & Leonard, 1965) have mixed the rumen contents artificially and maintained their sheep on high frequency feeding cycles (e.g. $^1/_{24}$ of daily ration offered every hour). Although these measures would improve the accuracy of the technique by encouraging the homogeneous distribution of the labelled infusate, they could, at the same time, alter the normal ecology of the ruminal flora (Black, 1969) and so affect the magnitude of the measured parameters. The impact of different feeding frequencies on the basic ruminal VFA parameters was, however, investigated by Gray, Weller, Pilgrim & Jones (1967) who reported little practical difference between the 1 and 12 hour cycles, indicating no significant change in the rumen ecology.

Further difficulties in the use of this technique could arise from the considerable recycling and interchange of label between the VFA, a result of the versatility of the ruminal microbial metabolism. Consequently the production rate of VFA, measured by the continuous infusion of labelled acid, is a total or gross production rate and comprises the effective or net production (available to the animal), plus the combined rates of interconversion with the other VFA. These interconversion rates may be calculated from the extent of label interchange and may be subtracted from the gross production rates to yield the net production rates of each VFA (Leng & Leonard, 1965). These individual rates may then be added together to obtain a net total production rate of the principal VFA. However, Weston & Hogan (1968) showed that the net total production rate of all of the VFA may be estimated from the results of the infusion of any one of the labelled VFA.

As our interests lie in the control of glucose intermediary metabolism in sheep, and as propionate has been shown to be a major precursor of glucose in this animal, a need arose to establish the basic parameters of ruminal VFA production when the sheep were maintained on lucerne hay. Consequently, the relatively simple single acid infusion technique described, *inter alia*, by Weller, Grey, Pilgrim & Jones (1967) was adopted in principle and these parameters, including net total VFA production rate, individual VFA production rates, extent of label interchange and VFA concentration patterns in the rumen were found to agree closely with the accepted normal values reported by Weston & Hogan (1968) and Leng (1970).

Methods

Pilot study

A group of 6 German Merino wethers (age 5 years) with mass 71,4–80,4 kg were fitted with rumen cannulae. They were maintained on a ration of 1 200 g lucerne hay, supplemented with 15 g of a trace element and salt mixture*, as well as 1 g Vitamin A† per week. Half of this group received its full ration (1 200 g) at 08h00 while the other 3 sheep were fed twice daily: 600 g at 08h00 and the other 600 g at 20h00. Both groups had free access to water.

After a 6 week adaptation period rumen fluid samples were collected at 1 hour intervals over a 24 hour period from each sheep. Each sample was filtered through a double layer of cheesecloth and a 15 ml aliquot was stored at —80 °C until it was analysed as described in the main experiment below.

Main experiment

Animals and feeding. Four wethers were selected from the above group for further study. Their management was similar to that described for the pilot experiment except that the ration was increased from 1 200 to 1 600 g lucerne hay.

Hay samples taken daily were pooled at the end of each week and a representative amount analysed for crude protein and fibre content. During the course of the experiment, the mean values were found to be 13,8% (±1,8) for the crude protein and 68,2% (±3,7) and 45,5% (±2,65) for the holo- and α-cellulose respectively. The same batch of hay was used by Taljaard (1973) and he reported an average dry matter digestibility of 63,6% (±3,9).

Experimental procedure. Two of the sheep were placed in adjustable holding pens in an air-conditioned room (23 °C and 60% relative humidity) on the day prior to the start of the experiment and were fed normally. One end of the spring coil section of an infusion catheter, made from nylon tubing (No. 5, ID=1,88 mm, OD=1,77 mm) was taped to a 30 cm length of curved, stiff, plastic rod and inserted to a dorsal, posterior position in the rumen of each sheep. The stiff plastic rod enabled the point of infusion to be precisely located for the duration of the infusion while the coil was fastened to a pivot point above each pen and was attached to a silicone rubber tube leading via a peristaltic infusion pump to the infusate reservoir (Fig. 1). The infusate, 4 *l* of a labelled VFA‡ (c. 250 μCi, 5 mM) solution sterilized by §Millipore filtration, was infused at *ca* 0,6 ml/min over a 22 h period, which started at 22h00 and ended the next day at the close of the experiment at 20h00.

Rumen fluid was automatically sampled using the apparatus diagrammatically shown in Fig. 1 from 08h00 to 20h00, when the experiment ended. This corresponded to the complete 12 h period of an average feeding cycle.

* Kimtrafos 25, Kynoch Feeds, Kimberley, RSA
† Vitamin A, Peter Hand Panvet, Johannesburg, RSA
‡ Na-1-^{14}C acetate, Na-2-^{14}C propionate or Na-1-^{14}C-butyrate purchased from the Radiochemical Centre, Amersham, England
§ Millipore GSWP 09025, Millipore Filter Corporation, USA

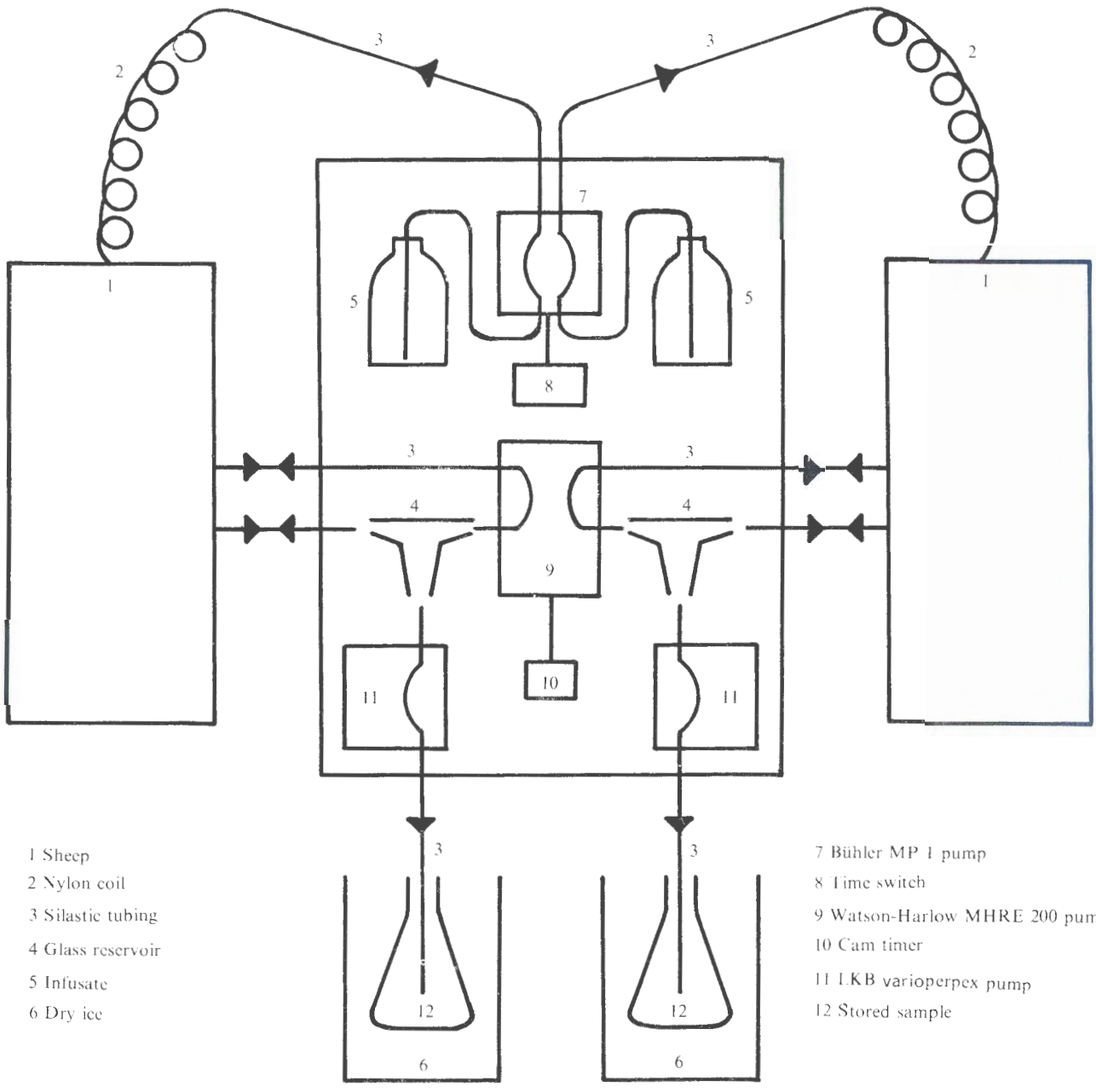

FIG. 1 Diagrammatic representation of the apparatus used to infuse the labelled VFA and automatically collect rumen fluid for the duration of the experiment

In order to prevent blockage of the lumen by particulate matter, filters made from a coarse weave nylon material were attached to the ends of the twin sampling catheters (silicone rubber tubing ID=2 mm) which were taped to a 30 cm stiff plastic rod and inserted in a ventral, anterior position of the rumen of each sheep. The 2 sampling catheters from each sheep were connected to a 2 ml glass reservoir via a peristaltic pump controlled by a cam timer. The direction of the rumen fluid flow through the reservoir was changed every 30 seconds by the switching action of this timer in order to minimise build-up of particulate matter on the sampling filters. The flow rate was adjusted so as to completely flush the glass reservoir with fresh rumen fluid during each cycle. Rumen fluid (0,5 ml/min) was constantly removed from this glass reservoir by another peristaltic pump and stored on dry ice. The 360 ml rumen fluid collected in this fashion over the 12 h period represented an average sample, and 15 ml aliquots were used for further analysis.

Analytical methods

VFA extraction. Total VFA were ether-extracted from 15 ml rumen fluid aliquots, using the method of Neish (1952) as modified by C. Roché (personal communication). Duplicate samples (15 ml) of thawed rumen fluid were acidified and deproteinized by centrifuging at 10 000 *g* for 20 min after the addition of 0,75 ml 5 N hydrochloric acid and 1,5 ml 10% sodium tungstate. A 15 ml aliquot of supernatant was ether-extracted continuously for 3 h in the apparatus described by Neish (1952), and the VFA trapped in sodium hydroxide in the receiving flask. The total ether soluble acids extracted were estimated by back titration of the excess sodium hydroxide with 0,1 N hydrochloric acid.

Sufficient 5 N sodium hydroxide was added to the flask to raise the pH of the solution above 11 prior to drying. This high pH ensured the complete recovery of the VFA as shown below:

pH.....	8,5	9,5	10,0	10,5
% Recovery.....	58,4	81,7	86,0	101,7

The VFA were extracted from the salts of the total ether soluble acids by the method of Gray, Pilgrim & Weller (1951). The slurry, made by adding 3 g potassium hydrogen sulphate plus 0,5 ml water to the dried salts, was extracted 5-fold with 4 ml portions of dry 7,5% n-butanol in benzene solution. After filtration through a *Whatman glass fibre filter disc, the extracts were combined in a 25 ml standard flask which was then made up to the mark. Aliquots from this solution were taken for all further analyses.

Liquid chromatography. The VFA mixture isolated from rumen fluid was separated on a †Celite column, using a technique derived from Gray, Pilgrim & Weller (1951) and Wiseman & Irvin (1957), into 3 major fractions containing acetic, propionic and "butyric" acids respectively. This "butyric" acid fraction contained the C_4 and C_5 acids, iso-valeric, iso-butyric, 2-methyl butyric and valeric and was corrected according to the results of the gas liquid chromatographic analysis prior to the calculation of any specific activity data.

* Whatman GF/A discs, Whatman, England
† Celite Analytical Filter Aid, Johns-Manville Products, Celite Division, USA

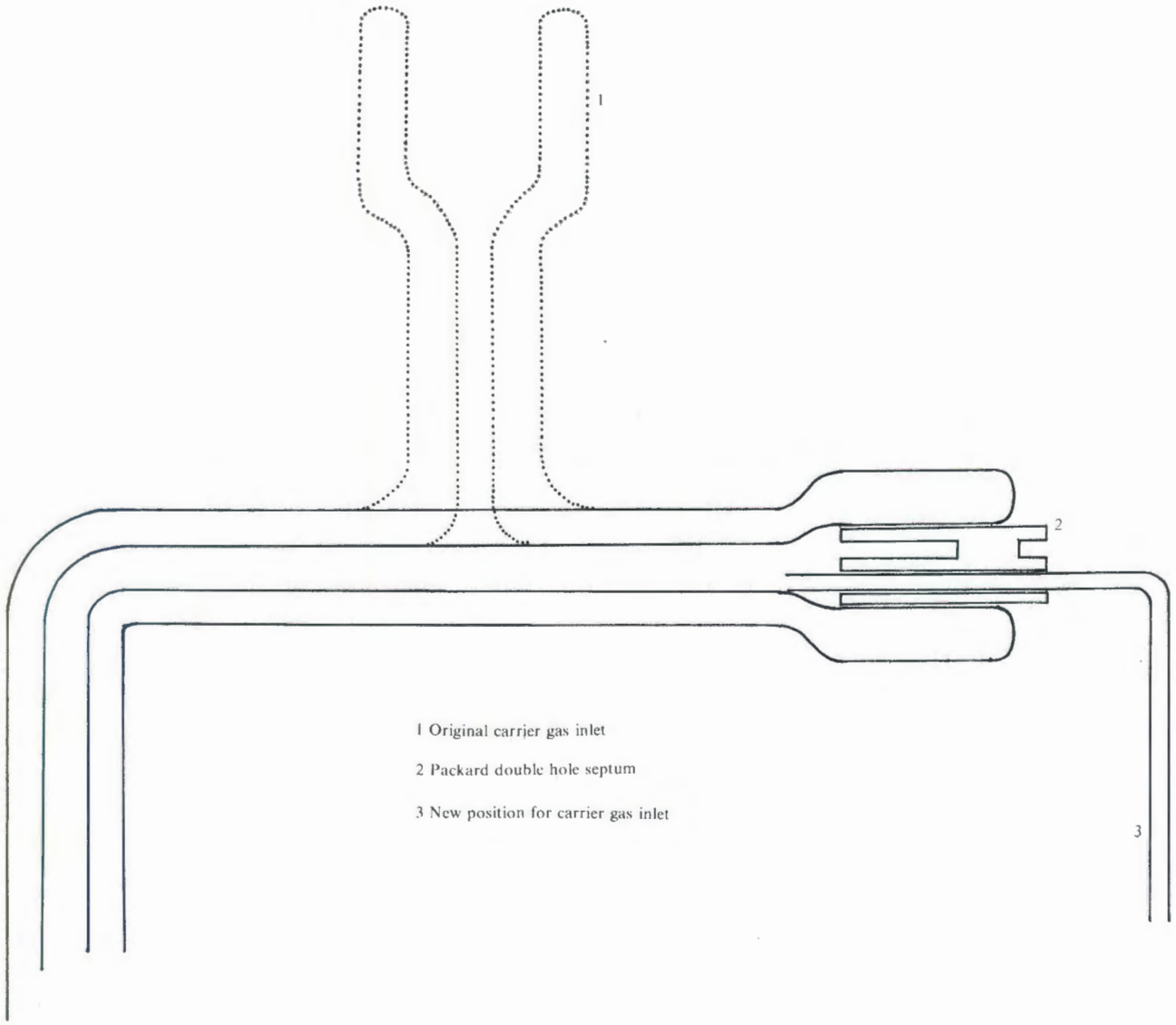

FIG. 2 A modification to the glass column of the Perkin Elmer 880 GLC as described in the text

Glass columns (350×12 mm) were packed to a height of 220–250 mm with a Celite suspension (75 g in 750 ml of a 1:1 hexane: acetone solution plus Alphamine Red-R as indicator). Three solutions were used for eluting the 3 main peaks at a rate of *ca* 1 ml/min; benzene, 3% (v/v) and 7,5% (v/v) n-butanol in benzene, all equilibrated against water before use. After 25 elutions, the Celite was discarded and the columns repacked.

Although the packing included an indicator, which was used to establish a preliminary calibration, precise collection points were determined for each column, using standard mixtures of labelled VFA in order to minimise cross contamination of peaks.

Duplicate 1 ml aliquots of the 25 ml samples were run through the column and pure fractions of each peak were collected, equilibrated against 20 ml water and titrated against 0,005 N sodium hydroxide, using a *Metrohm Combi-titrator set to an end point of pH 9. The salts were taken to dryness after the addition of excess base had raised the final pH to>11.

Gas-liquid chromatography. The complete distribution pattern of the VFA in each 25 ml sample as well as the purity of each peak eluted from the Celite column was determined as follows. Duplicate 1 ml aliquots of each 25 ml sample were titrated and evaporated in order to obtain the dried VFA salts which, together with the sodium salts of the individual peaks, were redissolved in 0,5 ml of dilute formic acid (0,2 N for the eluted "peak" and 2 N for the total acid sample) immediately prior to injection. Triplicate aliquots (0,75 μl) were injected onto a 2 m, 6 mm OD glass column, which was packed with †Chromosorb 101 (60–80 mesh) and fitted to a Perkin Elmer 880 gas liquid chromatograph. Helium was used as carrier gas (40 ml/min at 500 kPa) and all of the effluent passed through the flame ionization detector. The column was maintained at 180 °C while the inlet was kept at 220 °C and the detector at 190 °C.

In order to minimise the "ghosting" problems described by Geddes & Gilmour (1970), the design of the inlet area of the column was altered so as to eliminate the potential dead space behind the septum (Fig. 2), and the column was rinsed with injections of dilute formic acid between injections. A typical separation obtained under the above conditions is shown in Fig. 3 where the last peak, valeric acid, required 10 min for elution. The integral peak values provided by the Perkin Elmer D24 Integrator were normalized into relative molar proportions, using relative response factors.

Radioactivity determination. After the addition of 10 ml of scintillation fluid‡ (INSTA-GEL) to each sample, the radio-activity was determined in a Packard Tricarb Liquid Scintillation counter (Series 3000)‡. The channels ratio method of quench correction was used to calculate absolute activities with the aid of an §Olivetti Programma 101 calculator.

* Metrohm, Herisau, Switzerland

† Chromosorb 101, Johns–Manville Products, Celite Division, USA

‡ Packard Instrument Company, USA

§ Olivetti, Florence, Italy

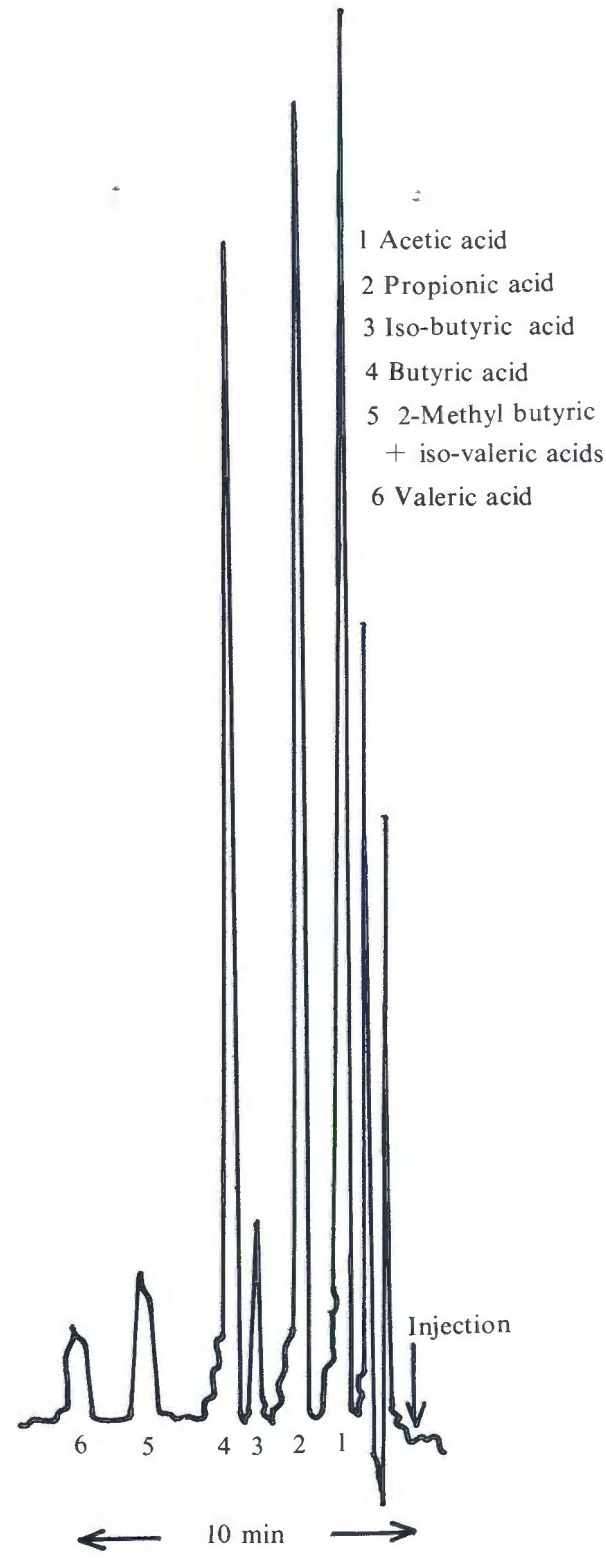

FIG. 3 A typical separation of the VFA present in rumen fluid by the GLC technique described in the text

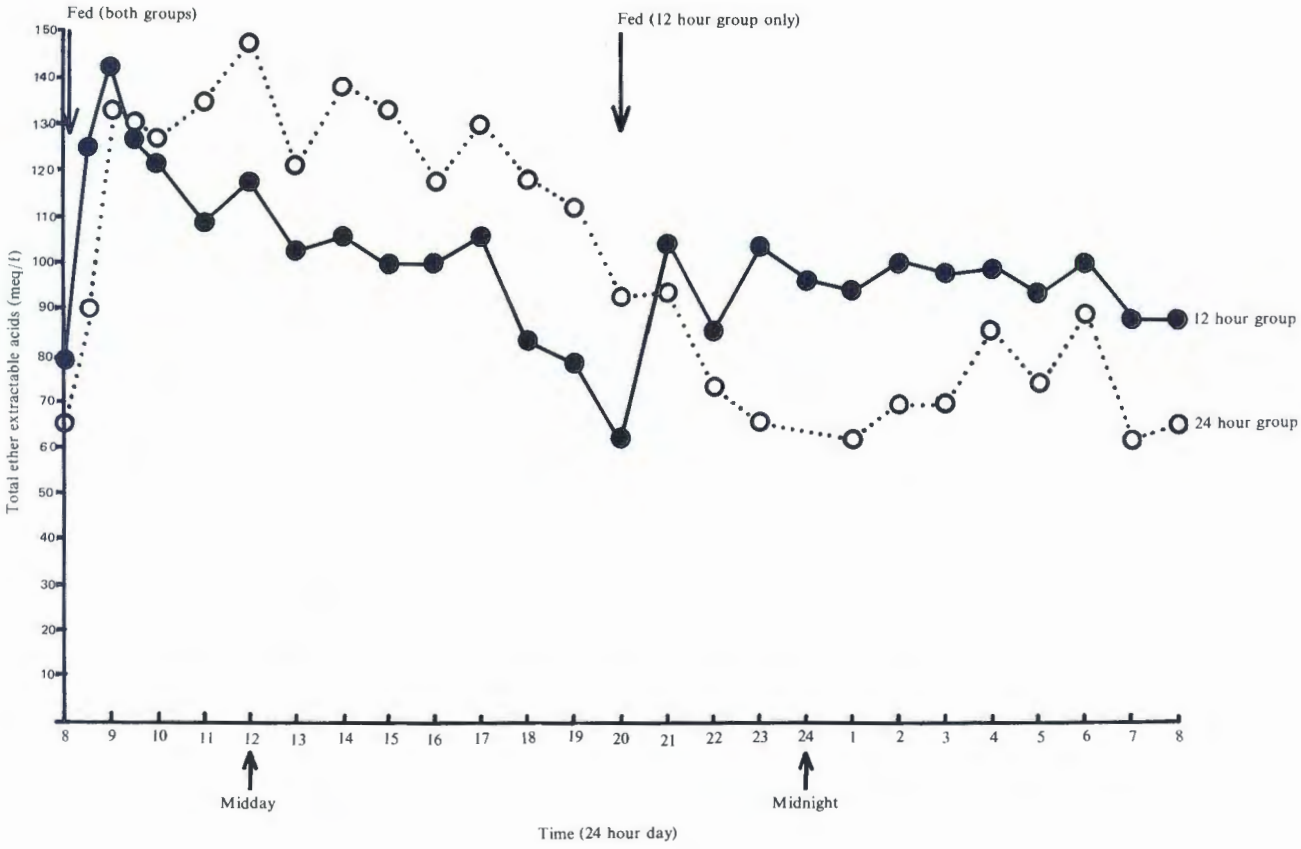

FIG. 4 The effect of a change in the feeding frequency (from once to twice daily) on the levels of the total ether extractable acids in the rumen of sheep fed 1 600 g lucerne hay per 24 hours

Results

Pilot study

The varying levels of the total ether soluble acids in the rumen over a 24 h period are shown in Fig. 4. The 24 h group (fed once daily at 08h00) exhibited maximum levels around midday whereas the 12 h group (fed twice daily at 08h00 and 20h00) showed a peak value at 09h00. Furthermore, the 24 h group required about 5 h to consume the bulk of the 1 200 g ration offered and maintained a relatively high level of acid (120–140 meq/*l*) during this period. This level then declined to basal values of 60–70 meq/*l* soon afterwards. The small peak noted at 04h00–06h00 was due to a renewed interest, at first light, in what remained of the fodder.

Although the maximum level of total acids (143 meq/*l*) attained by the 12 h group was similar to that of the 24 h group (147 meq/*l*), the concentration of the former fell rapidly to a plateau varying in value between 100 to 110 meq/*l*. This was somewhat lower than the 120–140 meq/*l* level maintained by the 24 h group during the same period. Immediately prior to the evening ration (20h00), the 12 h group showed a drop in the total acid level parallel to that of the 24 h group. Soon after feeding, a plateau in the level of the total acids in the rumen was restored albeit at a slightly lower level (90–100 meq/*l*) than earlier. The absence of any major peak in the level of the total acids during the nocturnal 12 h period was due to the more even intake of feed caused by a change in feeding behaviour (Fig. 4 & 5).

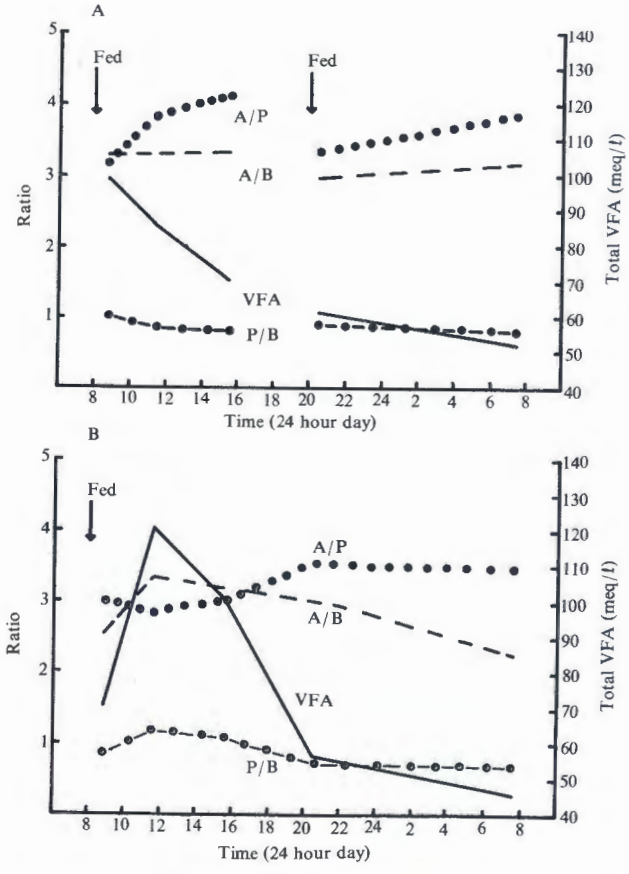

FIG. 5 The relationship between the total VFA level in the rumen and the relative molar composition of the VFA pool is shown for a group of sheep fed twice daily in A and once daily in B

As the amount of VFA in the rumen has been shown to be proportional to ruminal VFA production, an estimate of the relative changes occurring in the production rate over a 24 h period for twice daily fed sheep may be obtained from Fig. 4. An analysis of the areas under the VFA concentration curve showed that the diurnal and nocturnal 12 h periods contributed 56,5% and 43,5% respectively towards the total 24 h production rate.

That variations in the linked processes of production and absorption have also been reduced by the twice daily feeding cycle can be seen in Fig. 5. In the 24 hour group the $^{A}/_{B}$ and $^{P}/_{B}$ ratios rose with increasing production of total acids and fell slowly (by 32% and 44% respectively) as production decreased and absorption predominated. The inverse was true for the $^{A}/_{P}$ ratio, where an initial fall in the value was followed by a 19,5% rise over a period of declining production. The magnitude of these changes was considerably reduced by the introduction of the 12 h feeding cycle, although the basic pattern remained the same. The $^{A}/_{B}$ and $^{P}/_{B}$ ratios now rose by an average of 4% and 15% respectively, while the change in $^{A}/_{P}$ ratio remained essentially the same at 18,0%.

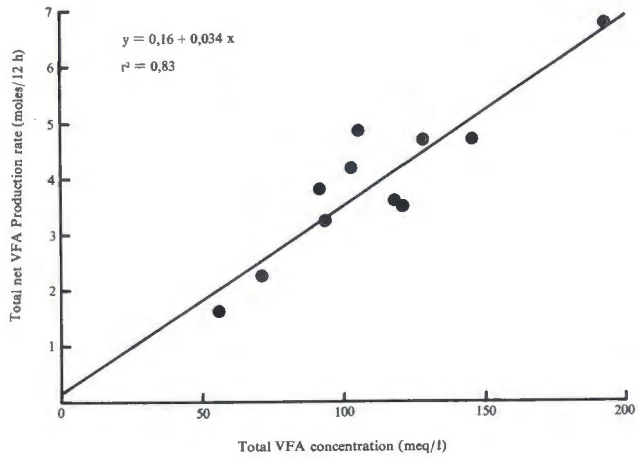

FIG. 6 The correlation between the total VFA concentration and the total net VFA production in the rumen

TABLE 1 The net total and gross individual VFA production rates in the rumen together with the associated levels of total VFA concentrations

Acid infused	Sheep No.	Ration consumed g	Total VFA concentration meq/l	VFA production rate (moles/12 h)		Specific VFA production rate (moles/1 000 g lucerne)	
				Net	Gross	Net	Gross
				Total	Acetic	Total	Acetic
Acetic–1–^{14}C.....	P53	550	93,6	3,27	2,39	5,95	4,35
	P57	800	118,5	3,61	3,03	4,51	3,79
	P60	800	—	—	—	—	—
	P64	800	192,5	6,77	5,45	8,46	6,81
	Average	*800	*155,5±52	*5,19±2,2	*4,24±1,77	†6,31±2,0	†4,98±1,61
				Total	Propionic	Total	Propionic
Propionic–2–^{14}C.....	P53	450	71,4	2,29	0,61	5,09	1,36
	P57	800	128,9	4,68	1,16	5,85	1,45
	P60	800	121,5	3,52	1,05	4,40	1,31
	P64	800	145,4	4,71	1,07	5,89	1,34
	Average	*800	*132,1±12	*4,30±0,68	*1,09±0,06	†5,31±0,71	†1,37±0,06
				Total	Butyric	Total	Butyric
Butyric–1–^{14}C.....	P53	450	55,8	1,64	0,43	3,64	0,96
	P57	800	102,8	4,18	1,15	5,23	1,44
	P60	800	91,9	3,79	1,17	4,74	1,46
	P64	800	106,0	4,88	1,47	6,10	1,84
	Average	*800	*100,0±7	*4,28±0,55	*1,26±0,18	†4,93±1,0	†1,42±0,36
				Total	A+P+B	Total	A+P+B
Overall mean.....	P53	480	73,6±19	2,40±0,82	3,43	4,89±1,17	6,67
	P57	800	116,7±13	4,16±0,54	5,34	5,20±0,67	6,68
	P60	800	106,7±21	3,66±0,19	—	4,57±0,24	—
	P64	800	148,1±43	5,45±1,14	7,99	6,82±1,43	9,99
	Average	*800	*126,0±32	*4,52±1,01	*6,48	†5,44±1,27 *5,65±1,31	†7,77 *8,10

* Average values (±standard deviation) for sheep consuming the full ration
† Average values (±standard deviation) for all sheep

Main experiment

The specific activity data obtained from the infusion of a single labelled acid [either acetate (1–^{14}C), propionate (2–^{14}C) or butyrate (1–^{14}C) as described above] were used to calculate the net production of the total VFA pool in the rumen of each sheep as described by Leng & Leonard (1965). The average values given in this and subsequent tables do not include the data obtained from P53 as this sheep showed an inconsistent eating pattern and failed to consume the entire daily ration (left 450, 450 and 550 g when butyric, propionic and acetic acids respectively were infused). These net production rates, given in Table 1, all fell within the range 3,52–6,77 moles/12 h, irrespective of the acid infused. There were no significant differences between the average values derived from the acetic (5,19±2,2 moles/12 h), propionic (4,30±0,68 moles/12 h) and butyric (4,28±0,55 moles/12 h) acid infusions, and the overall mean for the 3 sheep P57, 60 and 64 of 4,52 moles/12 h corresponded to a specific net production rate of 5,65 moles/1 000 g lucerne hay.

The consistent (but statistically insignificant, $P < 0,9$, Student's t test) differences in the net VFA production rates shown by the individual sheep in Table 1 corresponded well with their observed feeding behaviour. The highest production rates and hence the most efficient fermentation was always associated with P64 which was the fastest feeder and invariably finished its ration within an hour of being fed. Although the lowest absolute production rates were shown by P53, which never finished its ration, the average value, when calculated as the specific VFA production rate was 5,11±2,15 moles/1 000 g, similar to the overall mean of 5,65 moles/1 000 g.

The gross production rates of the individual acids, also derived from these specific activity data, are shown in Table 2. These rates indicated the total amount of each major acid that passed through the VFA pool during the 12 h period under consideration, and included all the various rates of inter-conversion between the VFA in the rumen. An average total production of 4,25±1,77 moles/12 h was obtained for acetic acid, together with the corresponding values of 1,09±0,06 and 1,26±0,18 moles/12 h for propionic and butyric acids, respectively.

The various percentage inter-conversions between the acid components of the VFA pool, derived from the specific activity data presented in Table 2, were used to calculate the individual inter-conversion rates according to Leng & Leonard (1965). The extent of the label interchange between acetic and butyric acid, where 51,9% of the butyric acid in the VFA pool came from acetic acid and, conversely, 31,2% of acetic acid originated from butyric acid, showed this to be the major pathway. On the other hand the propionic acid pool showed little interaction with either acetic or butyric acid, contributing only 1,74% to the former and 8,1% to the latter, while 4,5% and 8,6% were returned to the pool by acetic and butyric acid, respectively.

TABLE 2 Derivation of the inter-conversion factors from the VFA specific activities present in the rumen during the period of infusion

Acid infused	Sheep No.	Specific activity (μ Ci/Atom C)			Percentage inter-conversion between the acids	
		Acetic acid (A)	Propionic acid (P)	Butyric acid (B)		
					P from A	B from A
Acetic (1–^{14}C).....	P53	0,454	0,040	0,403	8,8	88,8
	P57	0,369	0,050	0,153	13,6	41,5
	P60	—	—	—	—	—
	P64	0,221	0,018	0,153	8,1	69,2
	Average*	0,295	0,034	0,153	11,5	51,9
					A from P	B from P
Propionic (2–^{14}C).....	P53	0,056	1,040	0,075	5,4	7,2
	P57	0,018	0,597	0,050	3,0	8,4
	P60	0,011	0,657	0,059	1,7	8,9
	P64	0,005	0,643	0,045	0,8	7,0
	Average*	0,011	0,632	0,051	1,74	8,1
					P from B	A from B
Butyric (1–^{14}C).....	P53	0,403	0,150	1,228	12,2	32,8
	P57	0,106	0,029	0,447	6,5	23,7
	P60	0,127	0,040	0,356	11,2	35,7
	P64	0,105	0,023	0,284	8,1	36,9
	Average*	0,113	0,031	0,362	8,6	31,2

* P53 not included in the average

TABLE 3 Net (or effective) average production rates of the VFA as calculated from the gross production rates listed in Table 1 and the inter-conversion factors appearing in Table 2

VFA	Gross production rate moles/12 h	Inter-conversion rates moles/12 h						Net production rate moles/12 h
		A to P	A to B	P to A	P to B	B to P	B to A	
Acetic	4,24	0,13	1,31	—	—	—	—	2,80
Propionic	1,09	—	—	0,07	0,20	—	—	0,82
Butyric	1,26	—	—	—	—	0,05	0,66	0,55
A+P+B	6,59	0,13	1,31	0,07	0,20	0,05	0,66	4,17

The calculation of the average net or effective production rates of the individual VFA, according to Leng & Leonard (1965), is shown in Table 3. The net production of acetic acid (2,81 moles/12 h) was much lower than the gross production rate of 4,25 moles/12 h (33%). Similarly, the net production of butyric acid (0,55 moles/12 h) was 56% lower than the gross production rate of 1,26 moles/12 h. The difference between the net and gross propionic acid production rate was not as great (28%) owing to its low inter-conversion rate with the other acids as indicated by Table 2. Addition of the net production of the individual acid rates gave a net total production rate of 4,18 moles/12 h, somewhat lower than the total net production of 4,52 moles/12 h calculated from Table 1.

The difference of 0,34 moles/12 h cycle appeared to be due to the combined production rates of the other VFA present in the rumen such as isobutyric, 2-methyl butyric, isovaleric and valeric acids. A comparison between the average molar percentage composition of the VFA pool in the rumen and the net individual production rates is made in Table 4. The similarity between the percentage molar concentration of each acid and its contribution to the total VFA production supports the hypothesis that all of the label infused remained within the total VFA pool and that the absorption of each VFA was concentration dependent. However, the only values which did not agree closely were those for propionic acid (23% concentration compared to 18% of the total VFA production) and the combined "other" VFA (5% concentration compared to 8% of the total production rate). The rates of inter-conversion between propionic and the other 2 acids were higher than those reported by Leng & Brett (1966) and Bergman *et al.* (1965), and contributed to the low net propionic acid production rate listed in Table 4. Investigation revealed the cause to be inefficient column separation resulting in contamination of the butyrate peak with labelled propionate (Van der Walt, unpublished observations). Consequently the net production of propionic acid would appear to correspond closely with the gross value (1,09±0,06 moles/12 h) reported in Tables 1 and 3.

A detailed analysis of the VFA pool was obtained from the gas-liquid chromatography data and is presented in Table 5. The 5% fraction of "other" VFA was found to be comprised of 1,7% iso-butyric acid, 1,8% valeric acid, and 1,6% combined iso-valeric plus 2-methyl butyric acid.

TABLE 4 Comparison between the relative concentration and production rate of the VFA in the rumen

VFA	Concentration		Production rate	
	meq/*l*	%	moles/12 h	%
Acetic	74,4	60	2,80	62
Propionic	28,4	23	0,82	18
Butyric	14,7	12	0,55	12
Other†	6,3	5	0,35	8
Total	123,8	100	*4,52	100

* From Table 1
† For composition of "other" VFA see Table 5

TABLE 5 Composition of VFA in the automatically pooled rumen fluid samples

Sheep* No.	VFA concentration (meq/l)							Molar percentage of total					
	A	P	IB	B	IV+2 MB	V	Total	A	P	IB	B	IV+2 MB	V
P53	41,2	14,0	2,9	9,5	4,7	1,5	74	56,0	19,0	4,0	13,0	6,0	2,0
P57	70,0	25,1	1,8	15,2	2,3	2,3	117	60,0	21,5	1,5	13,0	2,0	2,0
P60	62,9	24,5	1,6	13,3	2,1	2,1	107	59,0	23,0	1,5	12,5	2,0	2,0
P64	90,3	35,6	2,9	15,6	1,5	2,2	148	61,0	24,0	2,0	10,5	1,0	1,5
Overall mean†	74,4	28,4	2,1	14,7	2,0	2,2	124	60,0	22,8	1,7	12,0	1,7	1,8

* Average of 3 experiments
† P53 not included in average

Analysis of the data in Table 1 showed a straight line relationship between the net total VFA production per 12 h cycle and the average VFA level over that period, which was described by the equation.

$$y=0,034\ x\ +0,16$$

with a correlation coefficient of 0,83 and a standard error of y on x of 0,59 where y=production rate in moles/12 h and x=VFA concentration in the rumen in moles/*l*. If the data obtained from P53 were excluded, a similar equation resulted,

$$y=0,027\ x\ +1,08$$

with, however, a far lower correlation coefficient (0,67) and a higher standard error (0,65). This confirmed the observation that, although the absolute VFA production rates found for P53 were low (Table 1) and were thus not included in any group average data, they nevertheless correlated well with the amount of ration consumed on the day of the experiment.

Discussion

A major problem associated with the use of the isotope dilution technique in the estimation of the daily average VFA turnover in the rumen is the stratification of the contents (Balch, 1961) leading to an uneven regional variation in VFA levels (Annison, 1965). Further variations in these levels occur shortly after feeding, owing to an increase in production of the VFA (Gray & Pilgrim, 1951) and, together with the regional differences, lead to considerable difficulties in the use of this technique. In an attempt to minimise these obstacles, Bergman *et al.* (1965) not only fed the sheep continuously, using a moving belt system, but also constantly mixed the rumen contents with a recirculating pump. Leng & Leonard (1965) fed their sheep with equal portions of ration automatically offered at hourly intervals from 08h00 to 19h00.

The dangers inherent in such interference in the ecological balance in the rumen were pointed out by Weller, Gray, Pilgrim & Jones (1967). However, Gray *et al.* (1967) compared daily average VFA production rates obtained from sheep adapted to feeding cycle intervals of 1, 2 and 12 hours on the same diet and found no significant differences, despite increased variations in the VFA levels associated with the 12 h cycle.

It follows from the straight line relationship between the VFA production and concentration in the rumen depicted in Fig. 6 that relative rates of VFA production could be estimated from the magnitude of the areas contained by the concentration curves shown in Fig. 4. Despite the significant reduction in the variation of the VFA levels shown by the 12 h group when compared to the 24 h group, the total areas and hence the total average 24 h production rates would appear to be very similar. An anomalous difference was noted between the diurnal and the nocturnal portions of the VFA concentration curve obtained from the sheep fed twice daily. Despite receiving equal portions (800 g) of the ration at 08h00 and 20h00, the area and hence the apparent production rate associated with the diurnal period was higher than that found with the nocturnal period (56,5% and 43,5%) of the total area respectively.

A possible explanation may lie in the difference between the diurnal and nocturnal feeding patterns observed during the experiment. A portion of the nocturnal ration (offered at 20h00) was left overnight and only consumed the following morning. By comparison the diurnal ration was completely consumed within 3 hours of being offered. Consequently the nocturnal portion consumed immediately prior to the diurnal ration would tend to contribute to the parameters of the fermentation process of that period. The resultant increase in VFA levels found in the first half of the diurnal period raised the apparent average production rate disproportionately for that whole period.

Changes in the composition of the VFA pool in the rumen may be used to deduce the relative rates of production and absorption of the various acid components during the processes of fermentation and digestion (Gray & Pilgrim, 1951). From the information shown graphically in Fig. 5 it was concluded that, although relatively higher proportions of propionate and acetate were formed during peak production periods in both the 12 and the 24 h groups, the change in composition of the VFA pool was less marked in the former. Similarly the relative rates of uptake, inferred from the change in VFA composition taking place after the phase of peak production, were found to be propionate>acetate>butyrate for the 24 h group, and a less marked difference, propionate>acetate>butyrate for the 12 h group. This comparatively constant composition of the VFA in the rumen resulting from the twice daily feeding regimen considerably stabilized the specific activity data presented in Table 2.

The results of the infusion experiment listed in Table 1 agreed with the conclusions of Gray, Weller, Pilgrim & Jones (1966) and Weston & Hogan (1968) that the net total VFA production rate in the rumen could be derived from the specific activity data of the entire VFA pool after the infusion of any 1 of the 3 main VFA, radioactively labelled. This further supports the hypothesis that all of the label infused remains associated with the VFA pool in the rumen, and that the process of VFA absorption from the rumen is gradient controlled.

However, Leng (1970) showed that the absorption of propionate was different from that of acetate or butyrate, although this difference was small and of doubtful biological significance. The slight differences noted in Fig. 5 in the compositional changes taking place in the VFA pool during the 12 h cycle were also found to have little effect on the results, and thus were biologically unimportant.

The considerable extent of inter-conversion between the individual acids in the rumen VFA pool is clearly shown in Table 2. Similar results were obtained by Bergman *et al.* (1965), despite the different diet and experimental procedures used. The values reported by Leng & Leonard (1965) and Leng & Brett (1966) were somewhat lower than those listed in Table 2, in particular, the percentage inter-conversion of butyric and propionic acids. However, Weller *et al.* (1967) found that a lengthy period of infusion was required for complete equilibrium to be established between the incoming tracer and the rumen VFA pool, and the lower values obtained from relatively short infusion periods (4 h compared to the 20 h employed for this study) may be ascribed to this reason. Another possible explanation lies in the percentage molar composition of the VFA pool detailed in Table 5 where acetic acid comprised 60% and butyric acid 12% of the total acid concentration. Relatively higher levels of acetic acid (70%) and lower levels of butyric acid (8%) were reported by Leng & Brett (1966) and Weller *et al.* (1967) for sheep maintained on a lucerne diet.

These differences may well have arisen from the increased contribution of acetic to the butyric acid pool (c. 50%), shown in Table 2, somewhere between the value of 46,8% reported by Leng & Brett (1966) on a similar diet and 60% reported by Bergman *et al.* (1965) for a diet of dried grass. These relative percentage molar changes in the VFA pool are a reflection of the status of the rumen microbial ecology, which is considerably influenced by factors such as the composition of the diet.

The net individual VFA production rates, which were derived by the subtraction of the various interconversion rates from the gross production rates (Table 3), were expressed in terms of their percentage contribution to the total VFA net production and compared to the relative molar VFA composition of the acid pool. The close correspondence between the 2 sets of data supports the hypothesis that the absorption rate of each acid is mainly controlled by the concentration gradient existing between the interior and the exterior of the rumen, and that the differences noted in Fig. 5 between the rates of absorption of the various acids are biologically insignificant.

Despite differences in the composition of the diet, the amount, the frequency of feeding and various experimental techniques, the results obtained by various workers show remarkable similarity when expressed in specific terms. The straight line relationship shown in Fig. 6 between VFA production and concentration in the rumen showed a slope of 0,034 moles per 12 hour per meq per *l*. When similarly expressed, the line derived by Weston & Hogan (1968) from the results of Bergman *et al.* (1965) and Leng & Brett (1966) gave the identical constant (0,034), while the constant reported by Leng (1970) from the analysis of 121 data sets was very similar (0,032). The fact that the VFA pool showed a better correlation with the VFA production than did the VFA concentration (Weston & Hogan, 1968) led to the conclusion that the total amount of VFA present in the rumen was more important than the concentration.

Furthermore, by using a range of roughage diets with a crude protein content varying between 6% and 32%, Weston & Hogan (1968) showed that the amount of VFA produced in the rumen from the microbial fermentation was directly proportional to the digestible organic matter (DOM) content of the diet. They derived a specific production rate of 0,85 moles VFA/100 g DOM, regardless of the ration fed. An equivalent value of 0,86 moles/100 g DOM was calculated from the results of Bergman *et al.* (1965) after the reported total net VFA production rate was corrected by the addition of the assumed branched chain VFA contribution (5%).

The organic matter digestibility (58,6%) of the lucerne hay used in this experiment was calculated by subtracting 5% (for ash content) from the 63,6% average dry matter digestibility reported by Taljaard (1973) for the same batch of hay. The total net VFA production rate of 5,65 moles/1 000 g lucerne hay, calculated from the results in Table 3, was determined during the diurnal 12 h period of the feeding cycle. An estimate of 4,35 moles/1 000 g lucerne hay was made for the nocturnal 12 h period from the ratio of the areas under the twice daily fed curve drawn in Fig. 4 (diurnal area=56,5% and nocturnal area=43,5% of the total). Together, an estimated 5,65+4,35=10 moles/2 000 g lucerne hay was formed over a 24 h period. This is equivalent to 10 moles VFA/1 172 g DOM, which yields a specific production rate of 0,85 moles VFA/100 g DOM, identical to the value calculated by Weston & Hogan (1968), and similar to the value of 0,86 moles/100 g DOM derived from the results of Bergman *et al.* (1965).

ACKNOWLEDGEMENTS

Many thanks are due to Mr G. Vosloo for his excellent technical assistance with the analyses.

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