Rates of Production of Acetate, Propionate, and Butyrate in the Rumen of Lactating Dairy Cows Given Normal and Low-Roughage Diets

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ABSTRACT

Five lactating dairy cows with a permanent cannula in the rumen were given (kg DM/d) a normal diet (7.8 concentrates, 5.1 hay) or a low-roughage (LR) diet (11.5 concentrates, 1.2 hay) in two meals daily in a two-period crossover design. Milk fat (g/kg) was severely reduced on diet LR. To measure rates of production of individual volatile fatty acids (VFA) in the rumen, 0.5 mCi 1-¹⁴C-acetic acid, 2-¹⁴C-propionic acid, or 1-¹⁴C-*n*-butyric acid were infused into the rumen for 22 h at intervals of 2 to 6 d; rumen samples were taken over the last 12 h. To measure rumen volume, we infused Cr-EDTA into the rumen continuously, and polyethylene glycol was injected 2 h before the morning feed. Results were very variable, so volumes measured by rumen emptying were used instead. Net production of propionic acid more than doubled on LR, but acetate and butyrate production was only numerically lower. Net production rates pooled across both diets were significantly related to concentrations for each VFA. Molar proportions of net production were only slightly higher than molar proportions of concentrations for acetate and propionate but were lower for butyrate. The net energy value (MJ/d) of production of the three VFA increased from 89.5 on normal to 109.1 on LR, equivalent to 55 and 64% of digestible energy, respectively. Fully interchanging, three-pool models of VFA C fluxes are presented. It is concluded that net production rates of VFA can be measured in non-steady states without the need to measure rumen volumes.

(**Key words:** dairy cow, volatile fatty acid, rumen fermentation, milk fat depression)

Abbreviation key: ADE_R = energy apparently digested in the rumen, DDM = digestible DM, DE =

digestible energy, LR = low roughage, PEG = polyethylene glycol.

INTRODUCTION

It has been known for many years that marked changes in the molar proportions of the concentrations of VFA in the rumen can be induced in response to a wide variety of dietary manipulations. The consequences of these changes for metabolism in the body and ultimately for meat and milk production have been explored in numerous papers.

One particular topic of VFA utilization that has received much attention over the last 50 yr is the close association of VFA proportions with the depression in milk fat content when dairy cows change from a conventional diet containing approximately equal proportions of forage and concentrates to a low-roughage (LR) diet. The suggestion that milk fat depression is related to rumen VFA proportions and specifically to a reduction in the ratio of the concentrations of acetic acid to propionic acid appears to have originated in abstracts published by Wisconsin workers (Stoddard et al., 1949; Tyznick and Allen, 1951). The first full report appears to be that published from the National Institute for Research in Dairying, UK, by Balch et al. (1955). Measurements of actual rates of production of individual VFA would clearly be of greater value than simple rumen concentrations, but technical difficulties have proven to be a serious problem.

The most common method for measuring rates of VFA production in the rumen in vivo has relied on the use of VFA labeled with ¹⁴C. The majority of published estimates have been with sheep or steers, and there have been very few with lactating dairy cows. In a review, Sutton (1985) was unable to find any reports in which the rates of production of all three of the major VFA had been measured successfully in lactating cows, and there appear to have been no further triple ¹⁴C-VFA infusions since that time. Acetic and

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propionic acid production rates were measured in dairy cows by Annison et al. (1974) and Lebzién et al. (1981), acetic acid and butyric acid by Wiltrout and Satter (1972), acetic acid only by Davis (1967), and propionic acid only by Bauman et al. (1971). Unless all three of the major VFA production rates are measured, it is not possible to calculate net production rates that take interconversions into account and are considered to be the nearest estimate of the rates at which the VFA become available for absorption (Leng, 1970).

In the present paper, rates of production of the three major VFA in the rumen were measured by isotope dilution techniques in cows given normal and LR diets in two feeds daily. The accuracy of the measurement of production (or entry) rates of metabolites is greater when undertaken under steady-state conditions, but Sutton et al. (1985) demonstrated that milk fat depression caused by feeding LR diets is greatly reduced when cows are fed their daily ration in frequent feeds rather than in two feeds daily. Thus, frequent feeding of such diets to produce steady-state conditions generates an inappropriate model for the study of VFA production and its relation to milk fat depression in cows fed twice daily.

Mathematical equations to allow calculation of rates of production of a single VFA in non-steady states in which liquid volume and turnover rate and metabolite entry rates were varied were explored in laboratory studies by Morant et al. (1978). These studies were taken further in a fully interchanging multi-pool modeling analysis of production rates of individual VFA by France et al. (1987, 1991a) and in an analysis of variable rumen volume by France et al. (1991b). These models form the basis of the approach used in the present experiment.

MATERIALS AND METHODS

Cows, Diets, and Management

Five multiparous Friesian cows in midlactation and weighing approximately 650 kg were used. Each cow had a large rumen cannula (i.d. 10 cm).

Two contrasting diets designed to provide similar intakes of digestible energy (DE) at 160 to 170 MJ/d were fed. They were two of the four diets used in a related experiment and designated 60B and 90B (Sutton et al., 1980). The concentrate:hay ratio on an air-dry basis was 60:40 for the normal diet (60B) and 90:10 for the low-roughage (LR) diet (90B). The cows were offered the same amounts of feed DM as those offered in the related experiment. The daily rations were normal 7.8 kg DM (9.0 kg air dry) 60B concentrates, and 5.1 kg DM (6.0 kg air dry) hay (total 12.9 kg DM); and

LR 11.5 kg DM (12.9 kg air dry) 90B concentrates, and 1.2 kg DM (1.4 kg air dry) hay (total 12.7 kg DM). The concentrates were offered as a loose mix. The hay was harvested without chopping from a perennial ryegrass sward at a mature stage. Concentrate 60B contained (per tonne) 820 kg of rolled barley, 160 kg of extracted soybean meal (504 g of CP/kg DM), and 20 kg of vitamins and minerals. Concentrate 90B contained 870 kg of rolled barley, 110 kg of extracted soybean meal, and 20 kg of vitamins and minerals. The vitamin/mineral mix was a standard dairy supplement.

The cows were housed in individual tie stalls with rubber mats and individual feeding facilities. They were milked in their tie stalls at 0600 and 1500 h, and the daily ration was given in two equal portions at each milking, except for 1 wk before and during measurements of VFA production, when it was given at 12-h intervals (0900 and 2100 h). Orts were removed and weighed at 1400 h, except during measurements of VFA production, when they were not removed until the end of the 12-h measurement period. Drinking water was available throughout.

Design

Five cows were used in a two-period crossover design. Three of the cows were given the diet sequence LR followed by normal; the other two were given the diets in the reverse sequence. At least 4 wk were allowed for diet adaptation before measurements were made. For each cow, the three ^{14}C -VFA infusions followed a random order, but the same order was used for both diets. Intervals of 2 to 6 d were allowed between each infusion.

Rumen Infusions and Sampling

The methods of infusing and sampling used in this experiment were similar to those described by Annison et al. (1974). Infusion points and samplers were kept in their positions in the rumen by combination of a frame (50 cm long) attached to the rumen cannula by rubber pressure tubing and weights attached to the samplers. The ^{14}C -VFA and Cr-EDTA solutions were infused in separate lines until the last 5 cm, when the ^{14}C -VFA infusate was combined with the Cr-EDTA infusate to maximize the total infusate volume in an attempt to improve dispersion of the infusates through the rumen fluid. Both of these infusates were infused into two sites about 5 to 10 cm below the surface of the contents and 30 cm apart.

Rumen samples were taken through filters (pot scrubbers) anchored by weights in the bottom of the reticulum and the ventral sac of the rumen 1 and 0.1

h before the morning feed, 0.5 h after the feed, and then every hour from 1 to 12 h after the feed. Equal volumes from the two sites were mixed. Samples for measurement of VFA were preserved by the addition of 5 ml of saturated mercuric chloride solution (50 g/L) to 100 ml of rumen fluid and stored at -20°C . Samples for polyethylene glycol (PEG) and Cr analysis were stored similarly but without the addition of preservative.

Volatile fatty acid production was measured by continuous infusion into the rumen for 22 h of an aqueous solution containing 0.5 mCi of either $1\text{-}^{14}\text{C}$ -acetic acid, $2\text{-}^{14}\text{C}$ -propionic acid, or $1\text{-}^{14}\text{C}$ -*n*-butyric acid at 150 ml/h using a peristaltic pump. To reduce the possibility of breakdown of the radioactive VFA in the infusate by microbial attack, a carrier of the nonradioactive sodium salt of the appropriate VFA at a concentration of $12.5\text{ }\mu\text{mol/ml}$ was included in the infusate. Also, the infusate was made up in deionized water, which was boiled immediately before use; glassware was placed in an oven at 100°C or rinsed with absolute alcohol, and infusion lines were flushed with absolute alcohol for 15 min before an infusion commenced.

To provide estimates of rumen volume and changes in volume with time, two liquid-phase markers were used. A solution (500 ml) containing 50 g of PEG (MW 4000) was injected into several points in the rumen contents 2 h before the morning feed on the measurement day. Also, 270 ml of Cr-EDTA solution (2250 mg of Cr/L) was injected into the rumen as a primer at 1000 h on the day preceding measurement day followed by a continuous infusion into the rumen of Cr-EDTA solution (450 mg of Cr/L) at 200 ml/h for the next 36 h.

Following the first two infusions of isotope and marker in each set of three, water continued to be infused into the rumen at 350 ml/h to prevent the infusion lines from becoming blocked.

Rumen digesta contents were also measured by complete removal, weighing, sampling, and return of the rumen digesta 1 h before and 1, 2, 4, 7, and 11 h after the morning feed, as described by Gasa et al. (1991). The rumen emptyings were begun 3 to 8 d after the last of the three ^{14}C -VFA infusions in each period. They were carried out over 3 to 4 d, with a maximum of two emptyings at intervals of not less than 3 h on any one day. There was no evidence that this routine affected feed intake in any of the cows. Samples were analyzed for oven DM content (100°C for 48 h) to allow calculation of total liquid weight as the difference between total digesta weight and total digesta DM weight.

The procedure for each series of infusions was as follows:

Day 1	09.00 h	Feed given (half daily ration)
	1000 h	Cr-EDTA primer followed by infusion at 200 ml/h
	21.00 h	Feed given (half daily ration)
	2200 h	Start ^{14}C -VFA infusion at 150 ml/h
Day 2	0700 h	PEG injection
	0800 h	Rumen sample 1
	0845 h	Rumen sample 2
	0900 h	Feed given (half daily ration)
	0930 h	Rumen sample 3
	1000 h	Rumen samples hourly to 2100 h
	2100 h	Feed given (half daily ration)
Days 3, 4*		Continuous water infusion at 350 ml/h
Days 5 to 12*		Repeat Days 1-4 for the other two ^{14}C -VFA
Days 14, 15, 16		Empty rumen twice daily in a random sequence of 0800, 1000, 1100, 1300, 1600, and 2000 h over the 3 d.

*The interval between infusion days varied from 2 to 6 d.

Methods of Analysis

Rumen samples were thawed and centrifuged for 30 min at $2500 \times g$ before analysis for concentrations of VFA by gas chromatography, Cr, and PEG. To determine the specific activity of individual VFA, some of the samples were bulked as follows: 1 and 0.1 h before the feed, 3 and 4 h; 5 and 6 h; 7, 8, and 9 h; and 10, 11, and 12 h after the feed. Samples taken 0.5, 1, and 2 h after the feed were analyzed as singles. The total VFA in 6-ml rumen sample were separated by steam distillation (Annison, 1954). The distillate was made alkaline with 2 N NaOH and taken to dryness over a hot plate at 80°C . It was then reacidified with 0.5 ml of 2 N H_2SO_4 and 100 to 200 μl was injected onto a $2\text{-m} \times 0.95\text{-cm}$ glass column packed with 75g/kg of PEG 400 monostearate and 7.5 g/kg of orthophosphoric acid on 60/80 mesh Chromosorb W/AW in a Pye 106 preparative gas chromatograph (Pye Unicam, Cambridge, UK). Acetic acid, propionic acid, and *n*-butyric acid were collected separately from the main part of their respective peaks, taking care to avoid both the initial and final parts of the peaks. The concentration and purity of the collected peaks were determined on an analytical gas-liquid chromatograph, and their radio-

Figure 1. Net production (mol/d) vs. concentration (mM) (on normal and low roughage diets respectively: ●, ○ = acetate; ■, □ = propionate; ▲, △ = butyrate). The regression lines for each VFA are shown.

Figure 2. Molar proportions of net production vs. molar proportions of concentration (on normal and low roughage diets respectively: ●, ○ = acetate; ■, □ = propionate; ▲, △ = butyrate). The regression lines for each VFA and the line of equality are also shown.

activity was measured in a Packard Tricarb model B2450 liquid scintillation spectrometer.

The concentrations of Cr and PEG in centrifuged rumen fluid were determined by atomic absorption spectrometry and the turbidimetric method of Smith (1958), respectively.

Milk samples were taken at each milking during measurement periods and analyzed for fat, protein, and lactose using an infrared milk analyzer (AOAC, 1984; IRMA, Grubb Parsons, Newcastle-upon-Tyne, UK).

Calculations of VFA Production and Rumen Volume

The purpose of the experiment was to measure net rates of production of the three major VFA in non-steady states. Calculation of non-steady-state production rates was based on the equations of Morant et al. (1978) as presented in France et al. (1991b). However, these were developed for measurement of production rates of one VFA only (i.e., gross production rates). To allow calculation of net production rates of all three major VFA in non-steady-states, the equations were therefore taken further in a fully interconverting three-pool model as described by France et al. (1991a) (see Figures 3 and 4 below). The isotopic form of each VFA in turn is continuously infused into the rumen at a constant rate, and for each infusion the specific activities ($\mu\text{Ci g-atom}^{-1} \text{C}$) of acetate (s_a), propionate

(s_p), and butyrate (s_b) are monitored. The rate:state equations for this non-steady-state system are as follows. The movement of tracee acetate, Q_a (g-atom C), is described by:

$$\frac{dQ_a}{dt} = F_{ao} + F_{ap} + F_{ab} - F_{oa} - F_{pa} - F_{ba},$$

where the F (g-atom C h^{-1}) denote the flows between the three pools and into and out of the system, and t (h) is the time variable. For instance, F_{ao} is de novo synthesis of acetate and F_{ap} is the flow to acetate from propionate. Following the infusion of labeled acetate, I_a ($\mu\text{Ci h}^{-1}$), the movement of label through the acetate pool, q_a (μCi), is described by:

$$\frac{dq_a}{dt} = I_a + s_p F_{ap} + s_b F_{ab} - s_a (F_{oa} + F_{pa} + F_{ba}),$$

through the propionate pool, q_p , by:

$$\frac{dq_p}{dt} = s_a F_{pa} + s_b F_{pb} - s_p (F_{op} + F_{bp} + F_{bp}),$$

and through the butyrate pool, q_b , by:

$$\frac{dq_b}{dt} = s_a F_{ba} + s_p F_{bp} - s_b (F_{ob} + F_{ab} + F_{pb}).$$

Similar equations may be derived to describe the movement of tracee propionate and butyrate and the movement of label when labeled propionate and butyrate are infused into the rumen, giving a total of 12 dynamic differential equations.

These rate:state equations can be solved as follows. Instantaneous values of the derivatives are determined by monitoring the variable liquid volume of the rumen and its tracee (in addition to isotopic) concentration of acetate, propionate, and butyrate. An algebraic expression for each derivative term in the equation set is obtained by fitting a polynomial of the form:

$$\phi(t) = \sum_{i=0}^n a_i t^i,$$

where the a_i denote constant coefficients and n is the number of sampling times at which monitoring takes place, to serial data on isotope/tracee pool size and differentiating analytically to find $d\phi/dt$. Having determined the instantaneous values of the 12 derivatives at a sampling time from their respective algebraic expressions (i.e., from their $d\phi/dt$), the corresponding instantaneous values of the flows are then found by solving the resulting 12 simultaneous equations in 12 unknowns. This was done using a computational procedure similar to that described by France et al. (1987) for the steady-state situation. The process of solving a unique set of 12 simultaneous equations in 12 unknowns is repeated for each sampling time.

There is some confusion of terms in the published literature. For instance, entry of a VFA into a pool directly from fermentation of a substrate is termed direct secretory rate by Bergman et al. (1965) and de novo synthesis by Sharp et al. (1982), whereas Leng and Brett (1966) do not report this value at all, using the term production rate, which refers to the sum of all inflows into a pool and is the sum of Bergman's direct secretory rate plus flux from other VFA. Outflow of a VFA from its pool to leave the system as measured in a fully interconverting three-pool model is generally considered to be the closest estimate of the rate at which VFA become available for absorption from the rumen and is termed net production rate, irreversible outflow, and effective production rate, respectively, by the same three groups of authors. A further term, gross production rate, is normally confined to the calculated production rate based on the infusion of only one labeled VFA (e.g., Bergman et al., 1965; Davis, 1967; Bauman et al., 1971) and necessarily ignores all interconversions. For the purposes of the present paper, de novo synthesis and net production rate, respectively, will be used to refer to VFA entering the pool from

Table 1. Mean composition of the hay and concentrates (g/kg DM).

	Hay	60B	90B
Ash	59	41	33
NDF	703	178	179
ADF	414	86	86
Starch	—	508	528
CP	82	166	148

substrate fermentation and VFA leaving the pool and not returning.

Changes in rumen digesta water based on the two-marker system were calculated according to the equations of Morant et al. (1978). Rumen digesta weights were also measured by emptying at six unequally spaced times over 12 h. For interpolation purposes, to obtain rumen digesta weights at the sampling times, rumen-emptying profiles were modeled using smoothing spline function SSPLINE in GenStat (Payne, 2000). SSPLINE function specifies a cubic smoothing spline for the effect of a variate (in this study the time emptying since feeding), i.e., they are constructed from segments of cubic polynomials between the distinct values of the variate and constrained to be smooth at the junctions. The degree of smoothness is controlled by specifying effective degrees of freedom (Hastie and Tibshirani, 1990). Two sets of interpolated rumen digesta weights to match sampling times were obtained for 3 and 4 degrees of freedom. The average of these two sets at each time was used in further calculations.

Statistical Analyses

Diets were compared in an analysis of variance as a two-period crossover design, with means being adjusted for incomplete balance in the design.

RESULTS

Feed Intake and Milk Production

The composition of the feeds is shown in Table 1. The hay was mature with a high fiber concentration and low CP concentration. Both concentrates, consisting mainly of rolled barley and extracted soybean meal, had a high starch concentration. The calculated

Table 2. Calculated composition of the diets (g/kg DM).

	Normal	Low roughage
NDF	385	229
ADF	216	117
Starch	307	478
CP	133	142

Table 3. Milk yield and composition measured on the sampling day and next 2 d for each infusion.

	Normal	Low roughage	SED	P
Yield, kg/d	17.8	19.6	0.99	0.148
Fat, g/kg	34.5	22.0	0.85	<0.001
Protein, g/kg	30.0	30.4	0.85	0.691
Lactose, g/kg	44.4	45.7	0.42	0.042

composition of the diets (Table 2) shows the particularly low fiber and high starch contents of diet LR.

For each diet, all the cows were given the same fresh weight of feed. There were no measurableorts. Thus on normal and LR diets, respectively, total DMI were 12.9 and 12.7 kg/d. Based on the digestibility of the same diets measured with other cows (Sutton et al., 1980; unpublished), digestible DM (**DDM**) intakes were calculated to be 9.2 and 9.4 kg/d, digestible OM intakes 9.0 and 9.1 kg/d, and DE intakes 161 and 171 MJ/d. The cows ate the normal ration within 50 to 90 min (mean 61 min), and the LR ration within 30 to 60 min (mean 41 min).

There was no diet effect on milk yield or milk protein content (Table 3). However, milk fat content was severely reduced on LR, whereas lactose content was increased. Mean milk yield varied from 13.5 to 22.7 kg/d among individual cows, reflecting the relatively low DMI and different stages of lactation. Milk fat content ranged from 28.9 to 38.4 g/kg on normal and 18.0 to 26.3 g/kg on LR.

Rumen Digesta

Weights of both total digesta and digesta water as measured by emptying were higher on normal than LR at each emptying time (Table 4). Weights of digesta water increased rapidly after feeding, with increases of 17% in the first hour on both diets. The maximum increase in digesta water was similar for both diets at 21% on normal and 19% on LR. By 11 h after feeding,

the mean weights of digesta water were within 4% of the value 1 h before the morning feed 12 h earlier.

Attempts were also made to measure rumen water volumes by the use of two liquid-phase markers as described already. It was hoped that the combination of the two markers would allow changes in volume over the day to be measured. In general the results showed a similar pattern of volume change to that measured by emptying, but the results were very variable, and in some instances the mean volumes were up to three times greater than the volume measured by emptying.

To determine the consequences of these different estimates of rumen volume for calculation of VFA production rates, net production rates were calculated using three estimates of rumen water volume based on emptying and three based on the two-marker method. For the rumen emptying methods, estimates were based on use of all six emptyings (splined) or the 2- or 11-h emptyings only. The use of one emptying only was examined to reduce the possible stress on the cow. The 2-h emptying was chosen as representing the maximum rumen volume when diet effects were greatest. The 11-h emptying was selected as being close to the minimum volume and at a time when the infusions and associated rumen sampling were completed. For the two-marker method, estimates were based on the two-marker results alone or on those results scaled to the 2- or 11-h emptyings only. The scaling was done to try to overcome the consequences of the occasional high estimates of rumen volume ob-

Table 4. Weights (kg) of total digesta and digesta water in the rumen at different times relative to the morning feed as measured by emptying.

Time, h	Total digesta				Digesta water			
	Normal	Low roughage	SED	P	Normal	Low roughage	SED	P
-1	67.8	63.3	0.70	<0.001	59.6	47.1	0.67	<0.001
1	82.3	66.3	1.50	0.002	69.7	55.3	1.74	0.004
2	83.7	66.0	4.33	0.026	71.8	55.8	3.66	0.022
4	79.0	64.4	3.33	0.022	68.8	55.9	3.19	0.028
7	74.2	59.1	1.07	<0.001	65.0	52.2	0.98	<0.001
11	69.3	55.3	0.60	<0.001	61.8	49.0	0.61	<0.001
Mean ¹	77.0	60.9	1.45	0.002	66.9	52.8	1.20	<0.001

¹Area under curve.

Table 5. Calculated net production rates of VFA (mol/d) using different methods of calculating rumen digesta water volume.

Volume estimate	Normal			Low roughage		
	Acetic	Propionic	Butyric	Acetic	Propionic	Butyric
Rumen emptying						
All points (splined)	56.5	16.8	6.5	49.0	36.2	4.8
2 h only	56.4	16.8	6.4	48.7	36.0	4.8
11 h only	56.4	16.8	6.5	48.8	36.1	4.8
2-Marker system						
Unscaled	57.2	16.9	5.6	48.1	34.9	4.7
Scaled to 2 h emptying only	56.6	16.8	6.3	48.5	35.8	4.8
Scaled to 11 h emptying only	57.2	16.9	5.6	48.1	34.9	4.7

tained by the marker method. The differences among the resulting estimates of net production (Table 5) are relatively small, implying that the calculations were not sensitive to the differences in rumen volume estimates obtained in the present experiments. It was decided to use the results of the rumen emptying technique, appropriately splined, as providing the most reliable estimate of the weight of water in the rumen in the present experiment.

Rumen pH

Although rumen pH just before the morning feed and just before the evening feed 12 h later were similar on the two diets at between 6.9 and 7.1, both the mean and minimum pH were significantly lower on LR than on normal, while the range in pH from maximum to minimum was much greater on LR (Table 6).

VFA Proportions and Production

The pattern of rumen fermentation changed from high acetate on normal to high propionate on LR with

large and significant reductions in the concentrations and proportions of acetic and *n*-butyric acids, which were balanced by large and significant increases in the concentrations and proportions of propionic and *n*-valeric acids (Table 6).

In terms of de novo synthesis rates, propionic acid production was increased by 18.7 mol/d (122%) on LR compared with normal ($P < 0.001$), whereas butyric acid production was reduced by 2.5 mol/d ($P < 0.05$; 43%) (Table 7). Acetic acid production was 4.7 mol/d lower (8%) on LR, but the difference was not significant. When calculated in terms of net production, taking interconversions into account, the pattern was similar, but differed in some details. Net production rate of propionic acid increased by 19.4 mol/d (115%; $P < 0.001$) on LR, but the accompanying reductions in acetic acid (7.5 mol/d or 13%) and butyric acid (1.7 mol/d or 26%) were not significant. When net production rates are expressed in terms of molar proportions (mol individual VFA as percentage of sum of the three major VFA), the change on LR compared with normal was a reduction of 16.5% units in acetic acid ($P < 0.001$) and an increase of 18.9% units in propionic acid ($P < 0.001$)

Table 6. Rumen pH and VFA concentrations and molar proportions.

	Normal	Low roughage	SED	<i>P</i>
pH, mean	6.77	6.49	0.022	0.001
pH, minimum	6.53	5.78	0.112	0.007
pH, range ¹	0.45	1.29	0.132	0.008
Total VFA, mM	86.6	92.8	2.65	0.099
Acetic, mM	57.9	46.5	2.71	0.025
Propionic, mM	16.4	34.3	1.32	0.001
<i>N</i> -Butyric, mM	10.7	8.4	0.31	0.005
<i>iso</i> -Valeric, mM	0.7	0.3	0.28	0.198
<i>N</i> -Valeric, mM	0.8	3.3	0.66	0.032
Molar proportions, mol/100 mol				
Acetic	67.0	50.1	1.87	0.003
Propionic	18.9	37.0	1.56	0.001
<i>N</i> -Butyric	12.4	9.1	0.65	0.015
<i>iso</i> -Valeric	0.9	0.3	0.31	0.169
<i>N</i> -Valeric	0.9	3.5	0.64	0.026

¹pH range = maximum minus minimum.

Table 7. Rates of production and concentrations of acetic, propionic, and n-butyric acids in the rumen.

	Normal	Low roughage	SED	P
De novo synthesis, mol/d				
Acetic	60.1	55.4	4.62	0.382
Propionic	15.3	34.0	1.11	<0.001
Butyric	5.8	3.3	0.70	0.037
Net production, mol/d				
Acetic	56.5	49.0	3.48	0.121
Propionic	16.8	36.2	1.35	<0.001
Butyric	6.5	4.8	1.26	0.280
Molar proportions of net production, mol/100 mol total VFA ¹				
Acetic	71.0	54.5	1.15	<0.001
Propionic	21.2	40.1	1.48	<0.001
Butyric	7.9	5.4	1.14	0.123
Concentration, mM				
Acetic	58.7	48.6	2.67	0.032
Propionic	16.9	36.6	1.48	<0.001
Butyric	11.0	8.8	0.32	0.007
Molar proportions of concentrations, mol/100 mol total VFA				
Acetic	68.0	51.6	1.94	0.003
Propionic	19.4	39.0	1.59	0.001
Butyric	12.6	9.4	0.63	0.014

¹Total VFA calculated as sum of acetic, propionic and butyric acids.

with a nonsignificant reduction in butyric acid of 2.5% units.

The diet differences in the mean concentrations of acetic, propionic, and *n*-butyric acids broadly reflect the differences in net production rates (Table 7). The relation of net production rate of each VFA to its concentration for each cow and both diets is illustrated in Figure 1. Diet effects were not significant for any of the VFA, so the data from both diets were pooled. The calculated regression of net production rate (Y, mol/d) on concentration (X, mM) for each VFA was:

$$\text{Acetic: } Y = 15.64 (\pm 14.695) + 0.691 (\pm 0.2718)X, \\ (r^2 = 0.378)$$

$$\text{Propionic: } Y = -0.39 (\pm 3.133) + 1.005 (\pm 0.1086)X, \\ (r^2 = 0.904)$$

$$\text{Butyric: } Y = -9.68 (\pm 3.982) + 1.546 (\pm 0.3958)X, \\ (r^2 = 0.613).$$

Both the intercepts and the regression coefficients were widely different for the three VFA. Only for propionic acid was the intercept close to zero.

Net production rates and concentrations are compared in terms of their molar proportions in Figure 2. Diet effects were not significant, so data from both diets were pooled. The calculated regression of the molar proportions of net production rates (Y, mol/100 mol total VFA) on the molar proportions of concentrations (X, mol/100 mol total VFA) for each VFA was:

$$\text{Acetic acid: } Y = 0.05 (\pm 6.383) + 1.048$$

$$(\pm 0.1056)X, (r^2 = 0.916)$$

$$\text{Propionic acid: } Y = 1.00 (\pm 3.094) + 1.016$$

$$(\pm 0.0999)X, (r^2 = 0.919)$$

$$\text{Butyric acid: } Y = -8.65 (\pm 5.164) + \\ 1.386 (\pm 0.4608)X, (r^2 = 0.472).$$

For acetic and propionic acids, the relationship was very close. In contrast, the proportion of butyric acid produced was markedly lower than the proportional concentration at lower concentrations.

The de novo synthesis rates and net production rates of all three VFA and full interchanges among the three pools are described for both diets in Figures 3 and 4. Units are in g-atoms C/d. Values are simple mathematical means, so the means for de novo synthesis and net production differ slightly from the statistically fitted means in Table 7. De novo synthesis of acetate at 121.2 and 109.6 g-atom C/d represented 83.2 and 85.7% of C entering the pool on normal and LR diets, respectively. The equivalent values for propionate were 44.8 and 103.4 g-atom C/d, equivalent to 82.2 and 87.2% of C entering the pool. However, for butyrate, the values of 22.6 and 13.7 g-atom C/d were equivalent to only 42.3 and 31.9% of C entering the pool, respectively. Butyrate C exchanged most widely with the other VFA. On normal and LR diets, respectively, butyrate C supplied 19.9 and 11.4 g-atom C to acetate (13.7 and 8.9% of acetate C) and 7.3 and 12.3 g-atom C to propionate (13.4 and 10.4% of propionate C), whereas 30.0 and 27.4 g-atom butyrate C (56.1 and 64.1%) came

Figure 3. Fully interchanging, three-pool model of C fluxes in the rumen on normal diet (g-atom C/d). A = acetic acid, P = propionic acid, B = butyric acid.

Figure 4. Fully interchanging, three-pool model of C fluxes in the rumen on low roughage diet (g-atom C/d). A = acetic acid, P = propionic acid, B = butyric acid.

from acetate, but only 0.8 and 1.7 g-atom butyrate C (1.6 and 4.0%) came from propionate.

Net production rates are expressed in terms of net energy contributed by each VFA in Table 8. There were no significant diet effects on the net energy production from acetic acid or butyric acids, although both were numerically higher on normal. However, the net energy from propionic acid on normal was less than half that on LR ($P < 0.001$). On normal, acetic acid contributed over half the VFA energy and twice as much as propionic acid, but on LR propionic acid was the major supplier of energy. The total net energy from the three VFA was 22% higher on LR than on normal ($P = 0.036$).

DISCUSSION

Rumen Techniques

The system for infusing and sampling used in this experiment was similar to that described by Annison et al. (1974) but modified on the basis of preliminary and largely unpublished studies (Sutton et al., 1972; unpublished). In these studies, concentrations of infusates obtained by manual mixing and sampling of the rumen contents were compared with concentrations obtained from samplers kept at four sites within the rumen. The results showed that infusate concentrations in digesta water obtained from samplers in the bulk of the rumen water in the ventral sac and the

reticulum were very similar to those obtained by the "manual" method without the need for repeated disturbance of the cow and the rumen contents necessary with the latter. Additional samplers kept in the dorsal and posterior sacs did not improve the agreement of concentrations from sites samples with those obtained from manual samples, so these positions were discarded for the current experiments.

In lactating cows fed concentrate-based diets twice daily, there are inevitably wide fluctuations in the concentrations of metabolites and infusates in the rumen, and it is difficult to establish when the system comes to equilibrium. In the present experiments, 10 h was allowed between the start of the ^{14}C -VFA infusion one evening and the first rumen sample the following morning. This was based on preliminary studies and published work with lactating cows fed frequently. Thus, Wiltrout and Satter (1972) infused for 9 or 10

Table 8. Net energy value of acetic, propionic, and *n*-butyric acid production in the rumen (MJ/d).

	Normal	Low roughage	SED	<i>P</i>
Acetic	49.5	42.9	3.05	0.121
Propionic	25.8	55.6	2.08	<0.001
Butyric	14.2	10.6	2.76	0.280
Total	89.5	109.1	5.39	0.036

h following a primer dose and Annison et al. (1974) infused for 10 h.

Steady State

The use of markers to measure kinetics of dynamic systems yields the most precise and accurate results when these systems are in steady state. Deviations from steady state introduce difficulties into the measurement and subsequent interpretation of the changes in marker concentration with time and inevitably reduce the accuracy of the resulting estimates of the various outputs calculated from the raw data. However, in many production systems, dairy cows are offered their feed in two or three discrete feedings daily, which introduces wide fluctuations in both fermentation rates and digesta volume in the rumen. To address the problems of attempting to measure VFA production rates in non-steady-state conditions, proposals for mathematical solutions have been published (Morant et al., 1978; France et al., 1991a), but they have not been tested *in vivo*.

The importance of working in non-steady states in studies of milk fat depression was clearly identified by a series of experiments investigating the effects of feeding frequency on milk composition in cows given LR diets (Sutton et al., 1985, 1986, 1988). The depression in milk fat content on LR (long hay) diets was much less when cows were fed frequently (six times daily) than when they were fed the same ration only twice daily (Sutton et al., 1985). More frequent feeding induced a small though nonsignificant reduction in the proportion of propionic acid in the rumen, but a large change in the diurnal pattern of acetic and propionic acid in the peripheral vein, presumably reflecting similar changes in the rates of production in the rumen (Sutton et al., 1986, 1988). It was concluded from the studies that the use of frequently fed cows to improve the accuracy of measurements of VFA production would not provide an appropriate model to study the quantitative contribution of individual VFA to rumen fermentation in cows with milk fat depression.

Rumen Volume

Rumen emptying (or baling) is the most direct method of measuring the amount and composition of the rumen digesta, but it suffers from a number of obvious practical drawbacks, including the uncertainty of its effect on the cow and hence on the measurements themselves. Nevertheless, it has been widely used and, with appropriate concern for the cow such as restricting the frequency of emptying, should provide the most reliable measure against which to compare other, indirect techniques based on markers.

The mean increases in rumen digesta water after feeding of 21 and 19% on normal and LR, respectively, were rather less than the increases of 27 to 33% measured in similar cows fed four diets based on grass silage and concentrates twice daily (Gasa et al., 1991; unpublished). They were, however, much less than the increases of 100% in the laboratory modeling study of Morant et al. (1978), who noted the sensitivity of these equations to even the minor errors in measuring marker concentrations that occur in carefully controlled laboratory conditions. Errors are inevitably much greater in measurements of markers in the rumen of a lactating dairy cow, particularly those arising from uneven distribution of infusates through the digesta. Such errors probably account for the sometimes large differences between the volumes measured by the markers and those measured by emptying.

However, the differences in estimates of rumen volume found in the current study were found to result in only minor differences in estimates of the net production rates of VFA production (Table 5), suggesting that, in practice, attempts to make accurate measurement of the diurnal changes in rumen volume, or even a mean daily value, may not be necessary in such studies.

Measurements of VFA Production

Net rates of VFA production, which take into account interconversions among the individual VFA, provide the closest estimate of the amounts of VFA available for absorption. Their calculation requires measurement of the production rates of all three major VFA by tracer techniques within a very short time span of a few days and the application of a fully interconverting three-pool model to the data, but there appear to be no reports of such results with lactating cows.

Bergman et al. (1965), using sheep, were the first authors to describe their results in terms of a three-pool model but omitted propionate:butyrate fluxes as being relatively small. Leng and Brett (1966), also using sheep, appear to have used such a model, though they do not refer to their calculations in those terms. Later authors using cattle have relied on variations of the three-pool model (Esdale et al., 1968; Armentano and Young, 1983) or even a four-pool model (Wiltrout and Satter, 1972; Sharp et al., 1982), but in all cases some interconversions have been omitted, usually on the grounds that they were small. Other authors using lactating cows (Annison et al., 1974; Lebzien et al., 1981) have obtained results for only two labeled VFA for a variety of reasons and have, therefore, been unable to apply their data to a fully interchanging three-

pool model. Davis (1967) and Bauman et al. (1971) used only one labeled VFA (acetate and propionate, respectively) in studies of milk fat depression. All of these authors assumed steady-state conditions in their calculations, although the animals were not always given frequent meals to try to attain such a state.

The high proportion of acetate and propionate C derived from de novo synthesis in the present study is in agreement with other reports (Bergman et al., 1965; Leng and Brett, 1966; Wiltrout and Satter, 1972; Sharp et al., 1982) as is the large amount of C exchange between acetate and butyrate. Several authors have reported very little C exchange between propionate and butyrate and have therefore omitted the propionic:butyric C exchanges from their models (Bergman et al., 1965; Annison et al., 1974; Sharp et al., 1982). However, although only 2 and 4% of butyrate C was derived from propionate on normal and LR diets, respectively, 13 and 10% of propionate C, respectively, was derived from butyrate, which agrees well with the value of 10% of Wiltrout and Satter (1972) and argues against excluding the propionate:butyrate C exchange from three-pool models.

Significant regressions of net production rates on concentrations were established for each VFA in the present experiment, the r^2 values being much higher for acetic and propionic acids than for butyric acid. No significant diet effects on the regressions were established, so the data were pooled. However, this conclusion must be viewed with some reservations in view of the limited number of data and the fact that the data tended to form two blocks for each VFA based on the two contrasting diet types may have influenced the final regression coefficients obtained. Both the slopes and the intercepts from the resulting regressions were widely different for the three VFA. For both acetate (positive intercept) and butyrate (negative intercept) it would clearly be dangerous to extrapolate the relationship beyond the limits of the current data. One implication of the regression for butyrate is that the net production rate is lower than would be expected from its concentration relative to the other VFA, as was also found by Wiltrout and Satter (1972), and is effectively zero at concentrations of less than 6.3 mM in the rumen. In contrast to the present regressions, Leng (1970), using data then available for sheep fed a variety of diets, found no differences between the VFA in the slopes, although the intercepts differed between acetate and butyrate.

One approach to evaluating the present data is to relate the production rates to diet intake. In a previous review, Sutton (1985) related net production rates to DDM intake from the small number of published data then available for lactating cows. Values for acetic acid

ranged widely from 2.2 to 6.5 mol/kg DDM on normal diets and from 2.0 to 4.2 mol/kg DDM for LR diets. The values for propionic acid ranged between 1.1 and 1.5 mol/kg DDM on normal diets and 1.2 and 2.9 (only two values) mol/kg DDM on LR diets. The mean values obtained on normal and LR diets, respectively, in the present experiment of 6.1 and 5.2 mol/kg DDM for acetic acid and 1.8 and 3.9 mol/kg DDM for propionic acid tend to be slightly higher than the previously published results, particularly on the LR diet.

Total VFA production in the present experiment was 8.7 and 9.6 mol/kg DDM for normal and LR, respectively. These values are well within the wide range of 4.4 to 15.1 mol/kg DDM that can be calculated from the values of 3.0 to 10.3 mol/kg DM by assuming a DM digestibility of 68%, reported by Martin et al. (2001) in a review of isotope dilution estimates of VFA production in sheep and nonlactating cattle. Making a number of assumptions, Sutton (1985) calculated that published estimates of VFA production by isotope techniques on a variety of diets suggested that VFA energy represented between 21 and 64% of DE (40 to 64% if one set of data is omitted) in lactating cattle. These values are to be compared with the estimates in the present study of 55% of DE on the normal diet and 64% on the LR diet. In a related study in which similar cows with rumen and reentrant duodenal cannulas were given the same two diets as those used in the present study (Sutton et al., 1980), energy apparently digested in the rumen (ADE_R) contributed 74.1 and 71.6% of DE. Using stoichiometry to partition the ADE_R into VFA, methane and heat gives values of 51 and 54% for the energy from acetate, propionate and butyrate as a proportion of DE on normal and LR diets, respectively. These values are 93 and 86%, respectively, of the values based on measured VFA production in the current study.

There are various possible reasons for the differences between these two estimates of the contribution of the energy of acetate, propionate, and butyrate to DE. The measurements were made on different animals with different forms of gut cannulation. Also Sutton (1985), in his calculations of energy partition in the rumen, assumed that all the VFA, including valerate, contributed to ADE_R in proportion to their concentrations in the rumen and that, on this basis, the contribution of the higher VFA was about 10% of ADE_R . The present studies showed that the proportional net production of butyric acid was only about 60% of its proportional concentration. If a similar factor applies to the higher VFA, then the calculations by Sutton (1985) would have underestimated the contribution of acetate and propionate to DE.

Prediction of VFA Production

An important practical question relates to the value of VFA concentrations in predicting VFA production rates and the generality of calculated relationships between concentrations and production rates. An obvious problem relates to the simple matter of the consequences of differences in rumen volume, and so in the available pool of VFA, between different sizes of ruminant. The range of VFA concentrations in the data of Leng (1970) with sheep was similar to the range in the present experiments with dairy cows yet, at the same concentration, net production rates were obviously far lower in the sheep. For instance, at a concentration of 60 mM, the net production rate of acetic acid calculated from the regression equations was 2.9 mol/d in the sheep, whereas in the cows it was 57 mol/d. It is not surprising that such differences exist between sheep and dairy cows, but a more important question in the context of milk production is how widely the present regressions based on low-yielding Friesian cows given hay and concentrates apply to other cattle, particularly the modern high-producing Holstein cow, which may be consuming in excess of 25 kg DM/d of a TMR. There appears to be little information about the effect of feed intake on factors such as VFA concentrations and liquid volumes and turnover rates in dairy cows, which might contribute to a resolution of this question.

Although the results suggest that the use of VFA concentrations to predict net production rates must be treated with some caution, the close relationship of the molar proportions of net production to molar proportions of concentrations, particularly for acetic and propionic acid, was encouraging. This implies that the readily measured and widely published values for VFA proportions in the rumen provide a close estimate of the molar proportions of acetic and propionic acid available for absorption. However, the proportional production of butyric acid, and possibly the higher VFA, may be overestimated by this approach. How closely the proportions produced reflect the proportions ultimately reaching the portal system is a matter of some controversy at present. Earlier studies suggested that a high proportion of the VFA were metabolized during absorption from the rumen with recoveries in the portal vein of only 70, 50, and 10% for acetic, propionic, and butyric acid, respectively, in sheep (Bergman and Wolff, 1971). However, these low recoveries have been contested recently by Kristensen et al. (2000) based on results obtained with the washed rumen of sheep. These workers found recoveries of 109, 95, and 23% for the same three VFA, respectively, suggesting that effectively all the acetic and propionic

acids produced in the rumen reach the portal system though they did confirm the extensive metabolism of butyric acid during absorption.

In view of the present uncertainty regarding the extent of metabolism of VFA in the rumen wall during absorption, estimates of the net supply to the liver of VFA produced in the gut are best obtained by measurement of net absorption across the portal-drained viscera using appropriate cannulation techniques (Reynolds, 2002) rather than attempting to apply correction factors to production rates measured within the rumen. These techniques have been developed to the point at which they have been successfully applied to high-producing dairy cows (Benson et al., 2002).

Milk Fat Depression

The purpose of these studies was to attempt to compare rumen VFA production rates on diets causing the production of milk with normal milk fat content with that on diets causing milk fat depression. The results showed that the main difference between the two diets was that the net production rate of propionic acid more than doubled. The net production rates of acetic and butyric acids were numerically lower (13 and 27%, respectively) but not significantly so. These changes were within the context of a 22% increase in the contribution of VFA production to DE. Further, the molar proportions of concentrations were a reasonably close estimate of the molar proportions of net production, although they slightly underestimated acetic and propionic acid production and, more importantly, overestimated the net production rates of butyric acid. These conclusions are broadly similar to those of Davis (1967) and Bauman et al. (1971), who found that on an LR diet, the gross production rate of acetic acid was not significantly reduced compared with that on a conventional diet, whereas the gross production rate of propionic acid increased by over 130% both in terms of mol/d and as a proportion of DE. They did not measure the production rates of butyric acid. However, in terms of the contribution of VFA to DE, the values obtained by these workers, calculated to be 21 and 40% on normal and LR diets, respectively (Sutton, 1985), were much lower than the values of 55 and 64%, respectively, found in the present studies. One possible reason for this difference is that the Illinois workers used corn-based concentrates that are less completely digested in the rumen than the barley used in the present experiment (Sutton et al., 1980). Annison et al. (1974) also attempted to compare net production rates of VFA in cows given normal and LR diets. They also found no consistent differences in acetic acid production, but

their estimates of propionic acid production were too variable to lead to any clear conclusion.

Until recently, debates over the metabolic causes of milk fat depression have generally been dominated by consideration of the role of rumen VFA either as substrates for milk synthesis or as secretagogues for insulin production. However, in a review of the metabolic causes of milk fat depression, Davis and Brown (1970) suggested that an increase supply of *trans* fatty acids might also be a contributory factor, and this theory has recently gained considerable experimental support (see Bauman and Grinari, 2001). The present paper does not attempt to enter that debate. Its main purpose is to develop reliable estimates of the rates of production of the principal VFA on contrasting diets fed in discrete meals, the importance of which is not confined to the specific situation of milk fat depression but relates to broader questions of metabolism and energy utilization in cattle fed diets containing widely different proportions of forage.

CONCLUSIONS

The results suggest that accurate estimates of the net production rates of the three major VFA in the rumen using all three labeled acids can be obtained in non-steady states without the need to measure changes in rumen volume. The present results show that the principal effect on rumen VFA production of changing from a conventional diet to a LR diet at similar intakes is that propionic acid production is more than doubled and the net energy from total VFA production also increases. Reductions in the production rates of acetic and *n*-butyric acids were not significant but were probably real. The molar proportions of acetic and propionic acids in the rumen only slightly underestimated the proportions of their net production rates, but the molar proportion of butyric acid considerably overestimated its net production rate, particularly at low concentrations.

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