

A model for the estimation of volatile fatty acid production in the rumen in non-steady-state conditions

BY S. V. MORANT, J. L. RIDLEY AND J. D. SUTTON

National Institute for Research in Dairying, Shinfield, Reading RG2 9AT

(Received 16 May 1977 – Accepted 15 August 1977)

A method of estimating volatile fatty acid (VFA) production in the rumen in non-steady-state conditions was proposed. Laboratory models indicated that good estimates of total production over a complete feeding cycle could be obtained even when a wide range of production rates and volume changes occurred during the experiment. But estimates of instantaneous production rates might have contained large errors unless some attempt was made to match the infusion rate of labelled VFA to the rate at which VFA was produced in the rumen.

Volatile fatty acids (VFA) in the rumen provide approximately half the energy absorbed from the digestive tract. Many attempts have therefore been made to measure the rate of VFA production, and these have mainly been based on the use of radioactive isotopes. Tracer methods, apart from the 'matched infusion' technique proposed by Gray, Weller, Pilgrim & Jones (1966), require steady-state conditions, and to establish a constant production rate and a constant rumen volume, animals have to be fed more frequently than they would be under normal husbandry routines. But changes in the frequency of feeding probably influence the type of fermentation in the rumen, so it is important that a method of estimating VFA production accurately in animals fed once or twice daily should be developed.

This paper provides a theoretical basis for the use of radioactive tracers to estimate VFA production in non-steady-state conditions.

AN ALGEBRAIC MODEL

Notation

The following definitions are needed:

V_R (l/h)	flow of fluid into the rumen by eating, drinking, or passage of water through the rumen wall;
α (l/h)	flow of fluid out of the rumen into the omasum;
V (l)	volume of rumen fluid;
M_R (mol/h)	rate of production of VFA;
β (mol/h)	rate of absorption of VFA through the rumen wall;
M (mol/l)	concentration of VFA in the rumen fluid;
I_R (mCi/h)	rate of infusion of isotope;
I (mCi/l)	concentration of isotope in the rumen fluid;
P_R (g/h)	rate of infusion of inert marker;
P (g/l)	concentration of inert marker in the rumen fluid;
Q (g/l)	concentration of second inert marker in the rumen fluid.

Steady-state estimates of total VFA production

In this notation the steady-state equation is:

$$\frac{M_R}{I_R} = \frac{M}{I}. \quad (1)$$

Using the subscript 'i' to indicate concentrations measured in the *i*th sample of a series of *n* samples, this simple equation leads to three alternative estimates of total production:

$$M_R = nI_R \left(\sum_{i=1}^n \frac{I_i}{M_i} \right)^{-1} \quad (2)$$

$$= \frac{\text{infusion rate}}{\text{mean specific activity}},$$

$$M_R = I_R \frac{\sum_{i=1}^n M_i}{\sum_{i=1}^n I_i} \quad (3)$$

$$= \frac{\text{infusion rate}}{\text{specific activity of pooled rumen sample}},$$

$$M_R = \frac{I_R}{n} \sum_{i=1}^n \frac{I_i}{M_i} \quad (4)$$

$$= \text{infusion rate} \times \text{mean reciprocal specific activity}.$$

These three estimates will differ only by small random amounts when the system is in steady-state. But there will be systematic differences between them which may be large when, as a result of infrequent feeding, for example, the range in specific activity is wide.

Estimation of variable production rate when the rumen volume is variable but assumed to be known

The rate at which the amount of VFA in the rumen changes per unit time is:

$$\frac{d}{dt}(MV) = M_R - \alpha M - \beta, \quad (5)$$

and the rate at which the amount of labelled VFA changes is:

$$\frac{d}{dt}(IV) = I_R - \alpha I - \frac{\beta I}{M}. \quad (6)$$

It is assumed that the amount of labelled VFA infused is negligible compared with the total amount in the rumen. Eliminating $\alpha M + \beta$ gives:

$$M_R = \frac{M}{I} \left(I_R - \frac{d}{dt}(IV) \right) + \frac{d}{dt}(MV). \quad (7)$$

The derivatives of the products *IV* and *MV* can be written as:

$$\frac{d}{dt}(IV) = \frac{I dV}{dt} + \frac{V dI}{dt}, \quad (8)$$

and

$$\frac{d}{dt}(MV) = \frac{M dV}{dt} + \frac{V dM}{dt}, \quad (9)$$

Estimation of VFA production in the rumen 453

and substituting these expressions in equation no. 7 gives:

$$M_R = I_R \frac{M}{I} + V \left(\frac{dM}{dt} - \frac{M}{I} \frac{dI}{dt} \right). \quad (10)$$

By noting that the derivative of M/I can be expanded to give:

$$\frac{d}{dt} \left(\frac{M}{I} \right) = \frac{1}{I} \left(\frac{dM}{dt} - \frac{M}{I} \frac{dI}{dt} \right), \quad (11)$$

equation no. 10 can be further simplified to give:

$$M_R = I_R \frac{M}{I} + IV \frac{d}{dt} \left(\frac{M}{I} \right), \quad (12)$$

in which the volume is the only quantity on the right-hand side that can not be measured directly.

This equation reduces to the steady-state equation if, as is also the instance in matched infusion experiments, M/I is constant so that $d/dt(M/I) = 0$ throughout the experiment. But if the specific activity is not constant the estimates of production rate also depend on the pattern of volume changes in the rumen.

Estimation of variable rumen volumes

A technique that is commonly used to estimate constant flow-rates (i.e. V_R and α , assuming they are constant and equal) is to infuse an inert marker into the rumen until an equilibrium concentration is reached. The ratio, infusion rate:concentration is then an estimate of the constant flow-rate. A second technique is to inject a single dose of a marker, and under the same steady-state conditions its concentration decreases exponentially. The slope of the straight-line plot of log concentration v. time gives the flow-rate, and extrapolation to zero time gives an estimate of the volume. These methods have been reviewed by Warner & Stacy (1968).

If these two techniques are used simultaneously with different inert markers it is not necessary to make any steady-state assumptions in order to estimate the rumen volume at any time.

The rate of change of the amount of an infused marker per unit time in the rumen is:

$$\frac{d}{dt}(PV) = P_R - \alpha P, \quad (13)$$

which is identical in form to equation no. 5 except that the absorption rate β is zero for an inert marker. A similar equation holds for the second marker which has a concentration Q (g/l) at time t after a single injection, but which has a zero infusion rate:

$$\frac{d}{dt}(QV) = -\alpha Q. \quad (14)$$

Eliminating α gives:

$$\frac{d}{dt}(PV) = P_R + \frac{P}{Q} \frac{d}{dt}(QV). \quad (15)$$

Making substitutions similar to those in equations nos. 8 and 9 leads to the algebraic equation for the volume:

$$V = \frac{P_R}{Q} \left[\frac{d}{dt} \left(\frac{P}{Q} \right) \right]^{-1}. \quad (16)$$

It is more convenient for numerical work to rewrite this equation as:

$$V = \frac{P_R}{P} \left[\frac{d}{dt} \left(\log_e \frac{P}{Q} \right) \right]^{-1}, \quad (17)$$

because it does not involve division by Q , a concentration which decreases approximately exponentially, and because the change in $\log_e (P/Q)$ with time is likely to be easier to smooth than the trend in P/Q itself.

Estimation of variable production rate when the rumen volume is variable and unknown

In principle it would be possible to obtain numerical estimates of the volume from equation no. 17 and to substitute them in equation no. 12. But to reduce the number of arithmetic steps required to estimate the production rate we can make the substitution algebraically and simplify the equations.

Eliminating V between equations nos. 12 and 17 leads to the equation:

$$M_R = I_R \frac{M}{I} + \frac{IP_R \frac{d}{dt} \left(\frac{M}{I} \right)}{P \frac{d}{dt} \left(\log_e \frac{P}{Q} \right)}, \quad (18)$$

which, substituting Y for M/I and X for $\log_e (P/Q)$, becomes:

$$M_R = I_R \frac{M}{I} + \frac{IP_R}{P} \frac{dY}{dX}. \quad (19)$$

Estimates of variable production rates can therefore be derived from four concentration curves (P , Q , M and I) and two infusion rates (I_R and P_R), and require only one rate of change to be estimated.

LABORATORY MODELS

Estimation of variable production rate when the rumen volume is known

Experimental procedure. A small-scale laboratory model was set up to test the validity of the algebraic model, and to indicate the magnitude of the effect that volume changes in the rumen fluid might have on the estimates of total production.

In all the experiments the flow-rates were such that a 3 h period in the model corresponded to 12 h in the rumen, which is approximately a complete feeding cycle in cows fed twice daily. But in order to make direct comparisons between results from the models and from animal experiments, all quantities that apply to the models are quoted in their corresponding units expressed per h in the rumen.

In four experiments the volume of water in a 1 l beaker, representing fluid in the rumen, was held constant by pumping water into the beaker and out again at a constant rate. In two instances the turnover rate was 25 % of the volume expressed per h, in the other two the value was 8.3 %.

A dye, methylene blue, was infused at a constant rate to simulate the infusion of a labelled VFA into the rumen. A second dye, saffranine, was infused at a variable rate to simulate the production of VFA; the infusion rate was increased to 5 or 10 times its basal rate for 2 h in each experiment. The contents of the beaker were stirred continuously to ensure thorough mixing of the dyes.

Outflow of liquid from the beaker corresponded to passage of the rumen fluid into the omasum. Absorption of the VFA through the rumen wall was simulated by further

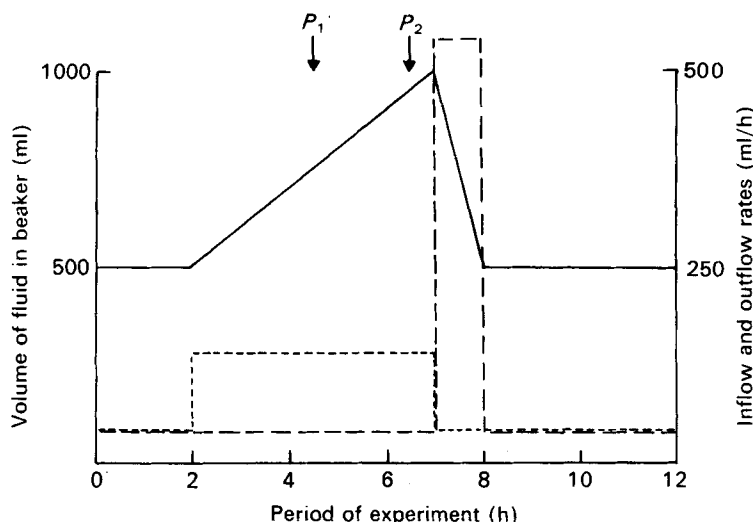


Fig. 1. Expt 6. The volumes and flow-rates in an experimental model simulating volatile fatty acid production in the rumen. —, volume (ml); - - -, inflow (ml/h); - · - ·, outflow (ml/h); P_1 , 10-fold increase in production rate; P_2 , 10-fold reduction in production rate.

Table 1. Summary of production rates, absorption rates, turnover rates, and volume changes in six laboratory experiments to compare methods of estimating volatile fatty acid production

Expt no.	<i>n</i> -Fold increase in production	Approximate <i>n</i> -fold increase in absorption rate	Average turnover rate (%/h)	Volume changes
1	10	12	8.3	None
2	10	15	25.0	None
3	5	4	25.0	None
4	5	4	8.3	None
5	10	5	13.3	Doubled in 1 h, halved in 5 h
6	10	Proportional to concentration	13.3	Doubled in 5 h, halved in 1 h

extraction of the liquid which was replaced by clean water at the same rate so that there was no change in the volume. The 'absorption' rate could not be controlled directly, but it could be altered by changing the rate at which the contents of the beakers were replaced by clean water.

At hourly, and sometimes half-hourly intervals, a small sample of the solution in the beaker was taken and the concentrations of the two dyes determined simultaneously using two spectrophotometers. At the same time the beaker and its contents were weighed, so that the volume of water could be calculated.

In two further experiments (Expts 5 and 6) the volume of water in the beaker was varied by altering the inflow and outflow rates. The flow-rates and resulting volume changes in Expt 6 are shown in Fig. 1. In both experiments there was a 10-fold increase in the production rate, and in Expt 5 the change in the rate at which the solution in the beaker was replaced by clean water corresponded approximately to a 5-fold increase in the absorption rate. The time intervals at which changes in flow-rate occurred relative to the volume changes in Expt 6 are also shown in Fig. 1. In this experiment there was no attempt to

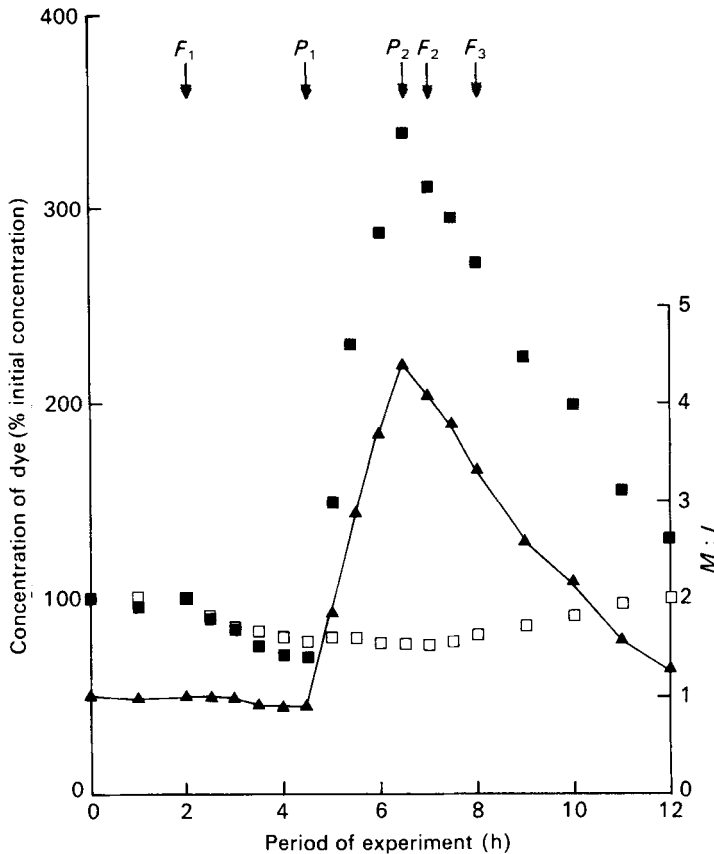


Fig. 2. Expt 6. The concentrations of two dyes, methylene blue and saffranine, representing volatile fatty acids (VFA) and labelled VFA in an experimental model simulating VFA production. ■, concentration of 'VFA' (M); □, concentration of 'labelled VFA' (I); ▲, M/I ; F_1 , increase in inflow rate; F_2 , reduction in inflow rate and increase in outflow rate; F_3 , reduction in outflow rate; P_1 , 10-fold increase in production rate; P_2 , 10-fold reduction in production rate.

manipulate the absorption rate, which was therefore proportional to the concentration of the dye throughout the experiment. The production, absorption and turnover rates in all six experiments are summarized in Table 1.

Estimation of total production. The concentration curves obtained from the laboratory experiments were broadly similar to those obtained from cows. The concentrations of the two markers and their relative concentrations in Expt 6 are shown in Fig. 2.

Three estimates of the total production in each experiment were obtained from equations nos. 2, 3 and 4. A fourth was derived from equation no. 12 using the known volume of water at each sampling time; the observed concentrations of the two markers in each sample taken from the beaker were substituted in turn into the equation, and the rate of change of M/I at a given time was estimated from the difference between M/I values at the two adjacent points.

The four estimates of total production, expressed as a percentage of the true production in each experiment, ranked in the same order in all six experiments (Table 2). The commonly-used estimate based on the mean specific activity underestimated the total production by 22–46% in these experiments. The third estimate, based on the mean reciprocal specific activity, underestimated it by a maximum of 16%. The fourth estimate, derived from

Table 2. Estimates of total production as a percentage of the known total in six laboratory experiments to compare methods of estimating volatile fatty acid production

Method of estimation*	Expt no.					
	1	2	3	4	5	6
Equation no. 2	57	54	78	78	58	66
Equation no. 3	75	77	87	84	78	83
Equation no. 4	84	86	92	88	85	85
Equation no. 12 (known volumes)	96	96	98	95	107	99
n-Fold range in specific activity	7.0	7.5	3.5	3.0	7.2	4.9

* For details, see p. 452.

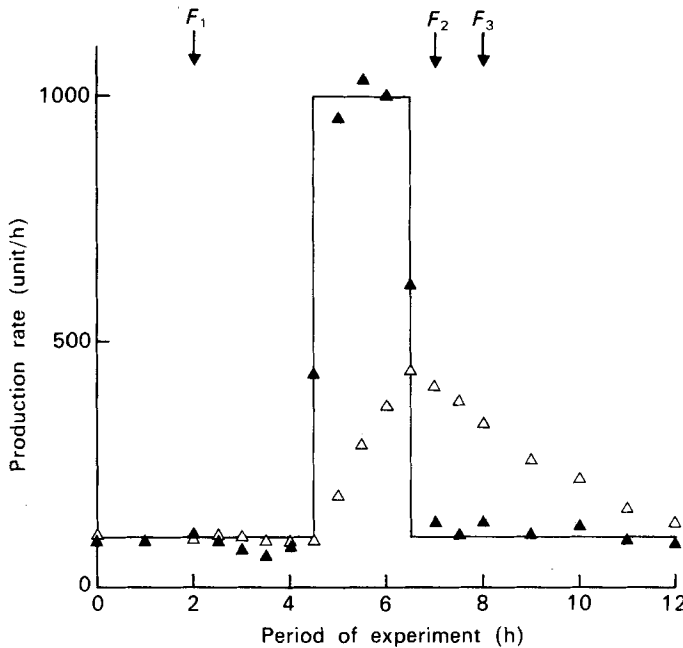


Fig. 3. Expt 6. Estimates of instantaneous 'production rates' in an experimental model simulating volatile fatty acid production. ▲, estimates derived from equation no. 12

$$\left(M_R = I_R \frac{M}{I} + IV \frac{d}{dt} \left(\frac{M}{I} \right) \right);$$

△, estimates based on the mean reciprocal specific activity from equation no. 4

$$\left(M_R = \frac{I_R}{n} \sum_{i=1}^n \frac{I_i}{M_i} \right);$$

—, known production rate; F_1 , increase in inflow rate; F_2 , reduction in inflow rate and increase in outflow rate; F_3 , reduction in outflow rate. For details of notation for equations, see p. 451.

equation no. 8 and using the known volumes, differed from the true total production by a maximum of 7%.

The differences between estimates obtained with equations nos. 2-4 arose because M/I was not constant. The pattern of changes in M/I , the reciprocal specific activity, also determined the contribution of the second term in equation no. 12 to the estimate of total

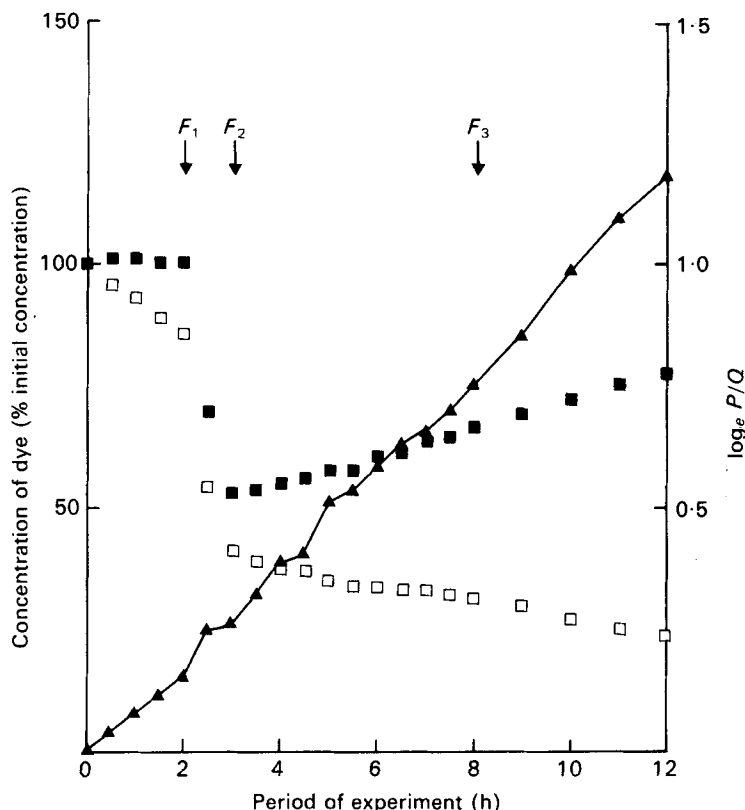


Fig. 4. The concentrations of two dyes, methylene blue and saffranine, representing inert markers in the rumen (volume changes as in Expt 5) in an experimental model simulating volume changes in the rumen. ■, infused marker (P); □, injected marker (Q); ▲, $\log_e(P/Q)$; F_1 , increase in inflow rate; F_2 , reduction in inflow rate and increase in outflow rate; F_3 , reduction in outflow rate.

production; and it was this term that accounted for the difference between the estimate from equation no. 4 and that derived from equation no. 12 in each example. In the first four examples this difference was also directly proportional to the volume of liquid in the beaker, which was constant. Errors in the estimation of the volume in these examples would have had a relatively small effect on the total production. In Expt 1, for example, an error of 10% in the estimation of the volume would alter the estimate of total production by only $(96.5 - 84.1) \times 0.1 = 1.24\%$ of the true total. In the other three examples the effect was even smaller.

Estimation of instantaneous production rates. When the volume was known the instantaneous production rate could be estimated from equation no. 12. These values were calculated in the estimation of total production and they are shown in Fig. 3, which illustrates the effect of ignoring the term that depends on the volume. In all the experiments it had a more serious effect on the individual estimates of production rates than on the estimates of total production.

Estimation of variable rumen volumes

Experimental procedure. The experiments set up in the laboratory to test the method of estimating volume changes were similar to those used to simulate VFA production, but the two dyes, methylene blue and saffranine, represented the inert markers in the rumen. The

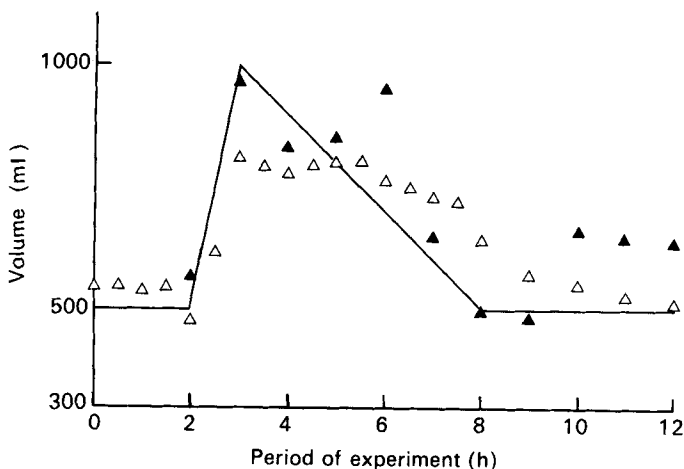


Fig. 5. Estimates of volume changes in two laboratory experiments simulating volatile fatty acid production. \blacktriangle , high turnover (mean 26.7%/h); \triangle , low turnover (mean 13.3%/h); —, known volume.

volume of solution in the beakers was again controlled by manipulating the inflow and outflow rates, but there was no exchange of the solution for clean water because there is no absorption of inert markers across the rumen wall.

In two of the experiments the inflow and outflow rates were the same as those used in Expts 5 and 6, and in the other two the same patterns of volume changes were created, but with a faster average turnover rate (26.7%/h).

Estimation of volume changes. The concentrations of the two markers, P and Q , and $\log_e(P/Q)$ are shown in Fig. 4. There were large errors in the individual estimates of volume (Fig. 5), even though the curve of $\log_e(P/Q)$ was smoothed by least-squares regression. The reason was that the procedure involved division by an estimated rate of change, an arithmetic process which is unstable even in the presence of the relatively small random errors in the laboratory values.

Estimation of variable production rate when the rumen volume is variable and unknown

The results of Expts 5 and 6 were each combined with those from the corresponding experiment in which the dyes represented inert markers in the rumen, and production rates were estimated from equation no. 19. The slopes of the relationships between Y and X at a given point were calculated from the differences between the two adjacent points, which is equivalent to fitting a parabola through the three points when they are equidistant in time.

There were clearly large discrepancies between the individual estimates of production rate and the known rates (Fig. 6); indeed, some of the estimates were negative. But if an estimate of Y or X led to an over-estimate of the slope at one point, it was also likely to produce an underestimate at another, and the errors tended to cancel. The net effect in Expts 5 and 6 was that the estimates of total production were, respectively, 99% and 97% of the true totals.

DISCUSSION

Current methods of estimating VFA production in the rumen require either that the system be in steady-state, or that the rate at which the labelled VFA is infused can be varied so as to keep its concentration constant relative to the natural VFA. Steady-state conditions are

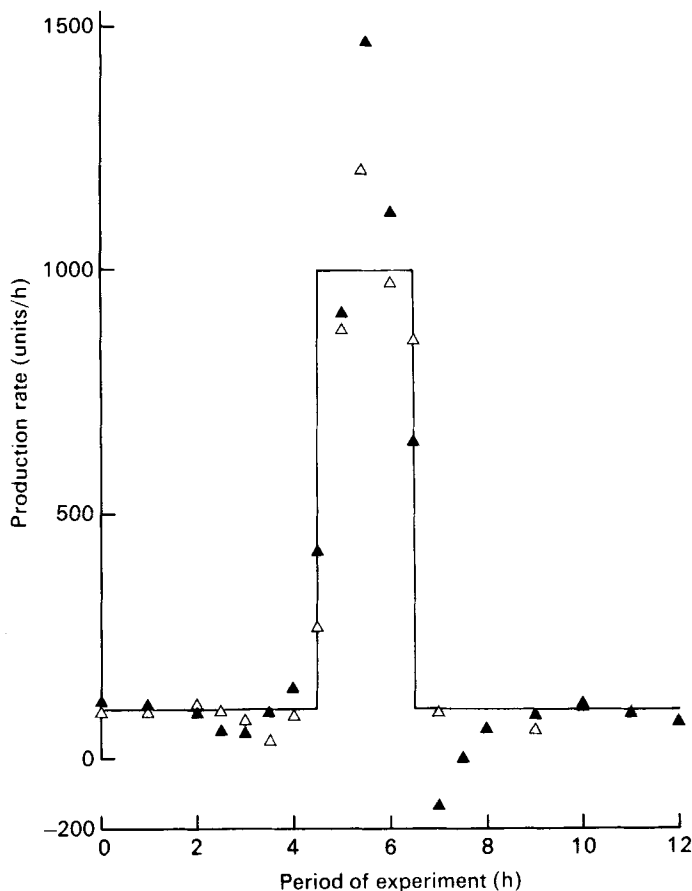


Fig. 6. Estimates of instantaneous production rates in two laboratory experiments simulating volatile fatty acid production when the volume of rumen fluid is not constant. ▲, Expt 5; △, Expt 6; —, known production rate.

unlikely to occur in practice, particularly in dairy cows, for example, which are often fed twice/day on high-concentrate rations. Estimates of the total production obtained from steady-state experiments may not, therefore, be a reliable guide to the production that would have been obtained with more practical feeding frequencies, and if the feeding frequency does influence the digestive processes and the production of VFA its effects may themselves be of practical interest.

The method of 'matched infusion' proposed by Gray *et al.* (1966) does, as the authors claim, provide an unbiased estimate of total production and of the instantaneous production rate. But in cattle, where the production rate may vary 10-fold or more, it may be difficult to keep the specific activity of the VFA constant.

The methods proposed in this paper overcome the objections to both these techniques; there is, in principle, no need to establish a steady-state system, or to keep the specific activity constant.

The volume changes that were produced in the laboratory models were probably more extreme than those that normally occur in the rumen. Direct measurement of the volume of rumen contents could be obtained by emptying the rumen of fistulated animals periodically. But no estimates of the diurnal variation in the volume of rumen fluid in milking cows fed

twice daily and watered normally appears to have been published. Using once daily feeding, Reid (1965) found that the maximum volume was approximately 70% greater than the minimum in a single milking cow, and Campling, Freer & Balch (1961) reported increases up to 65% in dry cows.

The laboratory models suggested that equation no. 12 may yield reasonable estimates of instantaneous production rate and total production even if the volume of the rumen fluid is assumed constant. Even in the two experiments where the volume was doubled, estimates of average volume obtained from the concentration curve of the injected marker were used to obtain estimates of total production that were 88% and 90% of the true totals.

In the laboratory models there was an instantaneous 10-fold increase in the production rate, and an instantaneous return to the basal rate. While the range of the production rate in cows may be even greater it cannot change instantaneously, and it probably decreases relatively slowly after reaching its peak. The contribution of the last term in equation no. 19 or in equation no. 12 if the volume is assumed constant, which depends on the rate at which M/I changes, would then be considerably smaller in relation to the first term than it was in the laboratory experiments. The importance of this term could also be reduced by making some attempt to match the infusion of labelled VFA to the production rate. This would reduce the large random errors in individual estimates of the production rate, and may also reduce the tendency for the total production to be underestimated.

In theory, equation no. 19 can be used to calculate VFA production in any non-steady-state conditions; in practice it appeared to give good estimates of total production, but poor estimates of instantaneous production rates when the range in specific activity was large. We suggest, therefore, that some attempt should be made to match the infusion of labelled VFA to the production of natural VFA, so that the range in specific activity is reduced. But we also recommend that the two inert markers be used, so that their concentrations may be used in the estimation of the last term in equation no. 19 when it is not negligible.

When this term is negligible, estimates of total production from equation no. 19 reduce to the estimate from equation no. 4, which should always be used in preference to those from equations nos. 2 and 3. If the specific activity can be held constant equation no. 19 gives unbiased estimates of total production and instantaneous production rates in any non-steady-state conditions.

If the specific activity is not constant, but the volume of rumen fluid is approximately so, equation no. 12 can be used instead of equation no. 19. Errors in the estimate of the constant volume have relatively small effects on estimates of total production, depending on the pattern of changes in the specific activity; and even estimates of mean volume when it is variable are likely to lead to better estimates of total production than using equation no. 4.

Laboratory models were used to validate the algebraic solutions that have been developed because we knew of no other method of estimating variable production rates in animals.

Results from animal experiments will clearly be subject to much greater random variation than results from laboratory models, particularly as a result of local variations in the production rate of VFA in the rumen, incomplete mixing of the rumen fluid, and the difficulties of obtaining representative samples of the fluid (Sutton, Macleod, Sissons & Johnson, 1972). Results from steady-state or 'matched infusion' experiments are also subject to this type of error, but when equations nos. 12 or 19 are used to estimate instantaneous production rates it may be necessary to use a more powerful technique than simple differencing to estimate the rates of change. The particular method used will depend on the extent of the random variation in biological values and the pattern of systematic variation in each instance.

In this paper, no account was taken of the interconversions that are known to occur among the VFA, nor of the likely differences in the absorption rates of different VFA when

high-concentrate diets are fed. The effect of these complications on the theoretical solutions developed in this paper are now being studied.

REFERENCES

- Campling, R. C., Freer, M. & Balch, C. C. (1961). *Br. J. Nutr.* **15**, 531.
Gray, F. V., Weller, R. A., Pilgrim, A. F. & Jones, G. B. (1966). *Aust. J. agric. Res.* **17**, 69.
Reid, C. S. W. (1965). *Proc. N.Z. Soc. Anim. Prod.* **25**, 65.
Sutton, J. D., Macleod, G. K., Sissons, J. W. & Johnson, V. W. (1972). *Proc. Nutr. Soc.* **31**, 67A.
Warner, A. C. I. & Stacy, B. D. (1968). *Br. J. Nutr.* **22**, 369.