Comparison of *in vivo* and *in sacco* methods to estimate mean retention time of fermentable organic matter in the rumen

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Fermentation in the rumen, measured by a steady state *in vivo* method, was compared to *in sacco* estimates obtained by different methods of calculation. These methods included a first-order model calculated over 24 h, a first-order model calculated over 48 h, a first-order model incorporating lag time, a moment-generating function of a gamma distribution and the calculation of mean retention time (MRT) by a discrete approximation to the mean of a continuous random variable. A comparison of *in vivo* and *in sacco* fermentation showed, in general, good agreement between the two methods. Care should, however, be taken when selecting a method to calculate *in sacco* estimates, as some methods induce large differences between the *in vivo* and *in sacco* estimates. The MRT method, based on the discrete approximation of a continuous random variable, is recommended since estimates obtained in this manner are explicable in terms of rumen dynamics.

Fermentasie in die rumen, soos bepaal met 'n *in vivo*-metode, gebaseer op ewewigstoestande, is vergelyk met *in sacco*-beramings wat verkry is met verskillende berekeningsmetodes. Hierdie berekeningsmetodes het die volgende ingesluit: 'n eerste-orde model wat bereken is oor 24 h, 'n eerste-orde model wat bereken is oor 48 h, 'n eerste-orde model met 'n dooie fase, 'n moment-genererende funksie van die gammaverdeling en die berekening van gemiddelde retensietyd (MRT) deur 'n diskrete benadering tot die gemiddeld van 'n kontinue ewekansige veranderlike. 'n Vergelyking van *in vivo*- en *in sacco*-fermentasie het oor die algemeen 'n goeie ooreenkoms tussen die twee metodes getoon. Die keuse van die korrekte berekeningsmetode is egter deurslaggewend aangesien die gebruik van sekere metodes groot verskille tussen die *in vivo*- en *in sacco*-beramings kan veroorsaak. Die MRT-metode gebaseer op die diskrete benaderings tot die gemiddeld van 'n kontinue ewekansige veranderlike word aanbeveel, aangesien dit beramings gee wat verklaarbaar is in terme van rumendinamiek.

Keywords: Fermentable organic matter, *in sacco*, *in vivo*, mean retention time, methods of calculation, rumen, sheep.

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Introduction

Different *in vitro* and *in situ* methods for approximating the *in vivo* fermentation process in the rumen are available. Probably the best known of these methods is the *in sacco* artificial bag technique (Ørskov & McDonald, 1979). This method has some advantages, the most obvious being simplicity and the number of samples which may be handled simultaneously. However, many factors can influence the results obtained with this method (Cronjé, 1983b; Meyer & Mackie, 1986).

All previous evaluations of the *in sacco* technique were either comparisons between different versions of the same (*in sacco*) technique or else a very indirect comparison where many other variables may possibly have influenced the results. An example of this would be the comparison of protein flow at the duodenum to the proportion of bypass protein leaving the rumen, as predicted by the *in sacco* method (Stern & Satter, 1984).

Forage quality and voluntary intake may be expressed in terms of potential digestibility, fermentation, outflow and rumen fill. These parameters have already been measured *in vivo* (Pienaar, Roux, Morgan & Grattarola, 1980). For these measurements to be applied to a practical feeding system, the accuracy of presently available *in sacco* or *in vitro* methods will have to be assessed in terms of the *in vivo* estimates.

The aim of this experiment was to compare the estimates of the disappearance of fermentable organic matter from the rumen, expressed as mean retention times (MRTs) as obtained by the steady state *in vivo* method (Pienaar *et al.*, 1980) and various *in sacco* estimates. Different models were used to describe *in sacco* fermentation. MRT is ideally suited to the comparison of different models, which are otherwise difficult to compare, since they all use different parameters. Since MRT as a technique has other convenient aspects described by Roux & Meissner (1984), it was decided to compare fermentation distributions using this technique.

Methods

Animals and diets

A group of mature South African Mutton Merino wethers (n = 6), fitted with large rumen cannulae (83 mm ID) and weighing 68,2 ± 7,7 kg were used in this study. The animals were accustomed to metabolism crates and automatic feeders which supplied the animals with 1/6 of the daily ration every 4 h. Three of the animals were fed a diet which consisted of ground lucerne hay supplemented with micro- and macrominerals and 3,5% molasses. The other three animals received a diet which consisted of ground maize-cob leaves supplemented with micro- and macrominerals, 7% fish-meal, 0,28% urea and 3,7% molasses. These diets were formulated to provide more than the minimum amounts of minerals and protein required for maintenance according to National Research Council (1975), and were offered at a level of voluntary intake plus 10%, calculated from a moving average of the previous four days' intake.

In vivo fermentation

A method, termed the steady-state method, which describes the disappearance of fermentable OM from the rumen was used to describe *in vivo* fermentation (Pienaar, Roux & van Zyl, 1983).

The MRT of OM *in vivo* was calculated by dividing the intake of potentially fermentable OM, by the potentially fermentable OM content of the rumen. This calculation is theoretically valid only if steady-state conditions in terms of fermentable OM intake and rumen contents exist, or, in statistical terms, if the probability distribution of retention times does not change with time. Although phasic deviations from steady state do normally occur, the magnitude of these may be kept within acceptable limits by frequent feeding and correct spacing of sampling times, so that these effects will cancel out when averages are calculated.

The rumen of each sheep was emptied four times, twice at 08h00 and twice at 15h00, over a five-day period with at least 17 h between evacuations. These were times at which minimum and maximum rumen fill, respectively, were expected for the present feeding regime. Pilot experiments have shown that, for this regime, mean maximum and minimum fill differed by less than 10%. The potentially fermentable fraction of OM was estimated by the method of Tilley & Terry (1963) which was modified to give a 72-h fermentation in the microbial phase of digestion.

The steady-state method of calculation used in this study included the disappearance of the fermentable OM through both fermentation and outflow. Fermentation was expressed in terms of MRT of fermentable OM. Expressing *in vivo* fermentation in terms of MRT is preferable to expressing it in terms of rate constants, since the mathematical relationship between retention time, flow and volume holds true regardless of the form of the flow and fermentation curves (Roux & Meissner, 1984).

In sacco fermentation

The method used was described by Cronjé (1983a), and is similar to the one described by Ørskov & McDonald (1979), except that polyester material with a pore size of 53 μ was used. Bags were removed after 3, 6, 9, 12, 24, 48 and 72 h incubation.

The *in sacco* experiment was conducted with the same sheep, fed the same diets under the same regime as the *in vivo* experiment. Samples placed inside the bags were the same as those fed to the sheep, but were ground in a Wiley Laboratory Mill to pass a 5-mm screen.

Calculations

Expressing fermentation by the MRT method is easily understood if it is recalled that a first-order rate constant is expressed in terms of fraction per hour or sometimes % per hour (Eliman & Ørskov, 1984). Thus a digestion rate of 0,063 h^{-1} or 6,3% h^{-1} implies a retention time of 1/0,063 = 15,9, a value with dimension in hours. When working with simple first-order reactions, values may easily be expressed in either form. However, when more complex reactions such as the moment-generating function of the gamma distribution (Mahlooji, Ellis, Matis & Pond, 1984) or a first-order model with lag phase (McDonald, 1981) are used, the equation is expressed in terms of two or more variables. It is only possible to compare these expressions when they are expressed in terms of a common dimension. MRT is ideally suited for this purpose.

In vivo MRT

The *in vivo* MRT for fermentation was calculated by dividing the intake of potentially fermentable OM by the potentially fermentable OM content of the rumen, as described above and demonstrated in Table 1.

Table 1 Calculation of *in vivo* mean retension time for fermentation of sheep No. 4 on the lucerne diet

Mean OM intake (g/d)	1366	
Mean insoluble fermentable OM intake (g/d)	530	
Mean OM content of rumen (g)	904	
Mean insoluble fermentable OM content of rumen (g)	184	
Mean retention time of fermentable OM in rumen (h) =	184	
	530	× 24
=	8,33 h	

In sacco MRT

The *in sacco* MRT was calculated using five different models.

Model 1: An iterative least-squares procedure was used to fit McDonald's (1981) model to the data. This model may be expressed as:

$$p = a' + b' (1 - e^{-ct}),$$

where p is per cent substrate fermented at time t; a′ is a measure of the rapidly soluble fraction of the substrate but is also influenced by a lag time t_0 (McDonald, 1981); b′ represents a fraction which will ferment in time; and c represents the rate at which the b′ fraction ferments. MRT was calculated as $^1/_c + t_0$ for this model and t_0 was included since it forms part of the probability distribution of fermentation time.

Models 2 and 3: The natural log (1n) of the mass of potentially fermentable OM in the bag was regressed against time from 3 h up to either 24 h (Model 2) or 48 h (Model 3) as an indication of the accuracy of the approximation of fermentation by a first-order process. For the purpose of estimation of rate constants in terms of slopes, MRT was calculated 1/slope for this model.

Model 4: The moment-generating function of a gamma distribution described by Mahlooji *et al.* (1984) was also fitted to the *in sacco* data set. An iterative least-squares procedure was used to fit this model:

$$F(t) = D \text{ for } 0 \leq t < \tau \text{ and}$$
$$f(t) = D [1 + \beta (t - \tau)]^{\alpha} \text{ for } t \geq \tau,$$

where D denotes the digestible fraction, α and β

are shape and spread constants, respectively, τ denotes lag time and t denotes time. MRT was calculated as $1/(\alpha\beta) + \tau$ for this model and τ was included since it forms part of the fermentation probability distribution.

Model 5: In contrast to the above-mentioned descriptions, MRT (t) for fermentation was calculated by a discrete approximation to the mean of a continuous random variable as described by Graham & Williams (1962):

$$t = (1/N)\ \Sigma\ [\tfrac{1}{2}n\ (t' + t)]$$

where n is the mass of insoluble organic matter which disappeared between times t and t', Σ signifies the sum of such quantities for successive intervals (t' to t) until n becomes zero, and N is the total mass of insoluble organic matter that disappeared.

Statistics

Different methods of calculation on the *in sacco* results do not lend themselves to statistical comparison, since they are all based on the same observations. Hence no statistical test was applied to compare these means. The *in vivo* results are, however, statistically comparable to the *in sacco* results since these were obtained independently. A *t* test was carried out on the average differences between pairs with error mean squares obtained by one-way analysis of variance.

Results

A comparison of results for two different diets estimated by the *in vivo* method and five different *in sacco* methods is presented in Table 2.

Similar results were obtained with the *in vivo* and *in sacco* 24 h first-order estimates (Model 2) on both the lucerne and maize-cob leaf diets. Differences between these two methods were not significant ($P > 0,20$) for either diet. On the maize-cob leaf diet, mean values of the models of Mahlooji *et al.* (1984) (Model 4), McDonald (1981) (Model 1), and Graham & Williams (1962) (Model 5), differed significantly from those of the *in vivo* method ($P < 0,025$). The 48 h first-order (Model 3) estimates obtained on the lucerne and maize-cob leaf diets, also differed significantly from the *in vivo* results.

It should be noted that the *in vivo* estimates include both fermentation and the outflow of fermentable OM, thus somewhat shorter retention times for the *in vivo* method may be expected.

An estimate of the outflow of fermentable OM from the rumen, with MRT = 81,66 h (24/0,2939) on diets similar to the ones used in this experiment was made by Pienaar *et al.* (1980). Possible reasons for obtaining such a large value are given by Pienaar & Roux (1984). A combined estimate for the MRT of outflow and fermentation may then be calculated using the *in sacco* MRT in Table 2 (Model 5), and the estimate for outflow obtained by Pienaar *et al.* (1980) on the assumption that both may be approximated by first-order processes. The formula: 1/MRT outflow + 1/MRT fermentation = 1/MRT combined, may be derived from the above considerations. This gives a combined MRT estimate of 8,07 h = (1/81,66 + 1/8,96) for lucerne and 16,16 h = (1/81,66 + 1/20,14) for the maize-cob leaf diets, respectively. These values are very close to the observed value obtained at 8,52 h and 16,12 h using the *in vivo* method on the same two diets. Thus, when an estimate of the outflow of fermentable OM is included, the overestimation of MRT obtained with the *in sacco* technique on Model 5 is corrected closely to the *in vivo* values. This would also hold true for Models 1 and 4, which yielded estimates that were not unlike those obtained with Model 5.

Table 2 Calculation of mean retention times of fermentable OM (MRT) estimated from *in vivo* and *in sacco* fermentation

		MRT (h) of fermentable OM calculated from data obtained						
			In sacco					
Diet	Sheep number	*In vivo*	Model 1 $1/c + t_0$	Model 2 $1/k$	Model 3 $1/k$	Model 4 $1/(\alpha\beta) +$	Model 5 * $\bar{t}$	Model 5 ** $\bar{t}$
Lucerne	4	8,33	7,39	7,25	13,26	8,30	8,39	–
	7	10,17	8,21	11,06	14,72	8,69	10,17	–
	12	7,06	8,43	6,19	22,86	8,33	8,33	–
Means	–	8,52[a]	8,01[a]	8,17[a]	16,95[b]	8,44[a]	8,96[a]	8,07
Maize	1	16,22	21,55	17,78	23,30	20,80	22,43	–
Cob	8	15,69	21,39	18,18	20,51	21,17	19,67	–
Leaves	10	16,44	19,48	15,19	17,27	18,54	18,32	–
Means	–	16,12[a]	20,81[b]	17,05[a]	20,36[b]	20,17[b]	20,14[b]	16,16

* Uncorrected.

** Corrected for outflow.

[a,b] Indicate significance ($P \leq 0,05$) of differences between means of *in vivo* and *in sacco* results.

Discussion

The similarity observed between the *in vivo* and *in sacco* estimates of OM disappearance is reassuring. It shows that these two methods yield answers that are quite comparable, despite all the criticism voiced against the nylon bag technique. This criticism does not seem to be quantitatively important under the present experimental conditions, where only roughage diets were considered and the same diet that was fed to the animal under a constant feeding regime was used in the bag (Meyer & Mackie, 1986).

The different estimates, obtained from the various models that were used to describe the *in sacco* fermentation, showed that the design of the model is an important consideration. These differences originate from the difference between assumptions built into each model.

Since all the models are statistically valid when simple first-order kinetics are assumed, they should all yield approximately the same MRT. This was not the case in our experience. The difference observed between 24 h *in sacco* (Model 2) and 48 h *in sacco* (Model 3) methods highlights an important aspect of this form of modelling. The only real difference in calculation procedure between these two first-order methods is the inclusion or exclusion of a single point near the end of the incubation time. The large difference between these two means (5,95 h) illustrates two important aspects. The first of these is that the data deviate significantly from simple first-order kinetics. The second is the disproportionate effect of a single data point near the end of a series of values when an unweighted least-squares regression analysis is used. The MRT method (Model 5) as suggested here, overcomes one of the said disadvantages because weighted means are calculated which are therefore less sensitive to outliers. In contrast to this, rate constants estimated by regression analysis are sensitive to inaccuracy in the independent variable. This may influence the estimation of the slope (Snedecor & Cochran, 1971), and in the case of the iterative least-squares method (McDonald, 1981), also the estimation of b′, although the effect of the inaccuracy in b′ may be cancelled by the other parameters in the calculation of MRT.

The similarity observed between the *in vivo* and 24 h *in sacco* values (Model 2) is consistent, in general, with the results of Stern & Satter (1984). However, the difference between the 24 h first-order method (Model 2) used here and the 24 h first-order method used by Stern & Satter (1984) lies in the fact that we used potentially degradable OM as the dependant variable while they used the nitrogen residue in the bag. With roughage diets, their procedure may be misleading since more than 40% of the contents of the bag is often potentially indigestible at the onset of fermentation. This fraction may cause a huge bias when included in the calculation of the rate constant, and was consequently not included by us in the calculation of first-order rate constants.

Despite the similarity between values obtained by the 24 h *in sacco* and *in vivo* methods, the former appears to be inappropriate for routine use because the values obtained on lucerne hay diets were smaller than those found *in vivo*. This, in principle, indicates a preclusion of the outflow of fermentable OM since correcting for outflow would make these values even smaller. This is especially important on the maize-cob leaf diet which is highly digestible and slow fermenting and thus has a large potential for outflow of fermentable OM. The result obtained with the method of Graham & Williams (1962) (Model 5) seems best, if the effect of outflow of fermentable OM is taken into account.

The fact that the estimate for MRT for fermentation, corrected for outflow, yielded a value very close to the observed *in vivo* result, emphasizes two aspects. Firstly, that the 24 h first-order model (Model 2) should not necessarily be considered to be correct, since it eliminates any possibility of the outflow of potentially fermentable OM from the rumen. Secondly, it suggests that, in the present situation, the combination of outflow and fermentation may be approximated as implied by first-order kinetics. This is, however, not a general result. In a subsequent paper (Pienaar & Roux, unpublished), it will be shown to be a reasonable approximation for integer gamma processes of a relatively low order, and that general gamma functions provide an adequate fit to fermentation and outflow processes.

In conclusion, the calculation of MRT provides a useful method by which different fermentation models can be compared. Care should be taken to select the correct model when calculating MRTs of fermentation.

In sacco and *in vivo* OM disappearances are in close agreement when *in sacco* MRT of fermentation is calculated according to the method of Graham & Williams (1962) and *in sacco* MRT are corrected for the outflow of fermentable OM.

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Soli Deo Gloria

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