Short Communications

A comparison of methods used to estimate a rate constant for outflow from the rumen

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A comparison of a 'steady-state' method with a number of marker-based methods, together with autoregression analysis showed that estimates of rate constants for outflow from the rumen should include a time dependent phase together with a first order estimate. A two compartment flow analysis where the time dependent phase is included with the second compartment and calculated as being outside the rumen, gives results which underestimate rumen residence time.

'n Vergelyking van die 'ewewigsmetode' met 'n aantal beramings wat op merkers gebasseer is, saam met outogressie analise het getoon dat beramings van uitvloei uit die rumen 'n tydsafhanklike fase saam met 'n eerste orde uitvloeifase moet insluit. Indien die vloeianalise vir 'n tweekompartement model gedoen word, waar die tydsafhanklike fase by die beraming van die tweede kompartement ingesluit word, en as buite die rumen gereken word, word verblyftyd in die rumen onderberaam.

Keywords: Sheep, rumen, outflow, rate constant, methods

A popular method for describing the outflow of particulate matter from the rumen (Ørskov & McDonald, 1979) involves the use of a marker as described by Grovum & Williams (1973). A single dose of marked material is placed in the rumen and its pattern of disappearance from the rumen or its appearance in the faeces is studied. Data obtained in this manner may also be used to calculate the retention time of marked material in the digestive tract as described by Graham & Williams (1962). Pienaar, Roux, Morgan & Grattarola (1980) described an alternative method for calculating a rate constant for outflow from the rumen. Their approach is based on a steady-state principle and uses the mass of unfermentable organic matter (OM) in the rumen and the intake of unfermentable OM to calculate the rate constant for outflow of unfermentable OM from the rumen.

To compare these methods simultaneously in the same sheep, chromium mordanted lucerne hay (Udén, Colucci & van Soest, 1980) was used as the marked material for the single dose method. The mordanted material was a representative sample from the basal diet.

Four mature (42 kg) S.A. mutton merino wethers, fitted with large rumen cannulae (81 mm internal diameter) were used. The animals were adapted to metabolism cages and fed for a period of at least six months on a ground (12 mm sieve) lucerne diet. This experiment comprised the last two months of the six-month period. Feed was supplied with automatic feeders every four hours and feed intakes were monitored daily for the whole period. The masses of OM in the rumens were determined by emptying their rumens manually, weighing the contents, and determining the organic matter content thereof. The *in vitro* digestibility technique of Tilley & Terry (1963), altered to give a 72-hour incubation in the microbial phase, was used to estimate the digestible (fermentable) fraction of the freeze-dried rumen contents. The total digestibility (fermentability) of the diet was estimated by the *in vivo* digestibility of the feed at maintenance intake. During digestibility determinations, faeces collections were made daily, but during the five days that chromium excretion was monitored, collections were made at two-hour intervals.

Faeces collections for digestibility determinations were first done at maintenance intake for ten days before intake was changed to *ad libitum*. The next faeces collection was started after the animals were fed for about one month on *ad libitum*.

The rumens were emptied while they were fed on *ad libitum* intake. The times at which the rumens were emptied were planned to give an indication of minimum and maximum rumen fill. Previous unpublished work showed that with the feeding regime followed, a minimum rumen load could be expected at about 08h00 and a maximum load at 15h00 with a difference of about 10% in mass of organic matter between minimum and maximum load. Each sheep's rumen was emptied twice before and twice after faeces collection for chromium excretion. The second time the rumen was emptied (08h00) about 10 g of ^{51}Cr-mordanted lucerne hay was mixed well into the digesta and the digesta returned to the rumen.

Faeces collections at two-hour intervals commenced eight hours after the chromium was administered. The faeces collected during chromium excretion were weighed and dried each time and ground through a Wiley mill with an 0,5 mm sieve size.

Tubes for gamma counting were weighed, filled to a constant volume with the dried, ground faeces and weighed again. The tubes were then counted for ^{51}Cr activity and the counts thus obtained were divided by the mass of the sample in the tube to give a corrected value. The corrected values were then plotted against time. The pattern obtained (Figure 1) is well known and similar to the one obtained by Grovum & Williams (1973). It consists of a fast inclining part, a peak and a slow declining part.

The results obtained with the steady-state method of Pienaar *et al.* (1980) and the marker excretion method were analysed to obtain several estimates of rate constants for outflow from the rumen and through the whole digestive tract. Rate constants for outflow of unfermentable OM from the rumen were obtained as described by Pienaar *et al.* (1980). Rate constants for outflow of marked material from the rumen were obtained as described by Grovum & Williams (1973) and comprise the analysis of the declining slope of the line.

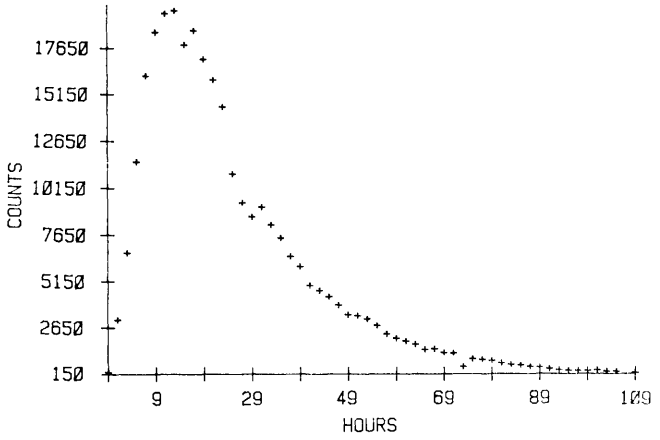

Figure 1 Excretion curve of ^{51}Cr-mordanted feed

Table 1 Different estimates of the rate constants for outflow from the rumen (fraction per day)

Method used	Sheep Number 1	3	5	7	Mean ± SD
Steady state	0,821	0,880	0,939	0,810	0,863 ± 0,060
Single dose marker declining slope	1,176	1,420	1,320	1,152	1,267 ± 0,126
Single dose marker autoregression	1,198	1,132	1,432	1,014	1,194 ± 0,176
Single dose marker Total tract mean retention time	0,679	0,715	0,640	0,652	0,672 ± 0,033
Single dose marker Faecal appearance mean retention time	0,878	0,939	0,813	0,834	0,866 ± 0,056

A rate constant for the outflow of marked material from the rumen was also obtained by autoregression analysis, using the model;

$$y(n) = \alpha + \rho\, y(n-1) + at + bt^2 + \epsilon(n) \quad \text{for } t \leqq T \text{ and } n \leqq N$$
$$= \alpha + \rho y(n-1) + \epsilon(n) \quad \text{for } t \geqq T \text{ and } n \geqq N$$

y(n) = number of counts at the nth time interval, corresponding to time t, rate constant $\gamma = -\ln \rho$ where ρ is the autoregression coefficient and T is time at maximum counts in faeces.

Autoregression analysis (Fuller, 1976, Chapter 8 & 9) showed the inclining part of the curve to be a time related process and both inclining and declining slopes to be consonant with a single first order kinetic-process.

The method described by Graham & Williams (1962) was also used to calculate the rate of passage of marked particles through the total digestive tract. The mean retention time value obtained by this method was expressed as a rate constant by dividing the mean retention time by 24 to obtain the time in days and by taking the inverse of the result to obtain the rate constant. The method described by Graham & Williams (1962) was modified by taking the time at which the marked particles first appeared in the faeces as the value from which the mean retention time was calculated instead of the time at which the material was dosed. This was done with the idea that if no or limited mixing takes place in the gastro-intestinal tract post ruminally, as suggested by the results of Ellis, Lascano & Matis (1979), the pattern of marker excretion in the faeces should be similar to the pattern observed when sampling would have taken place immediately post ruminally.

The results obtained with these methods are summarized in Table 1. It is clear that the fastest rate constants were obtained using the single dose marker, using the declining slope only. The single dose results calculated by autoregression with a time dependent phase in the rising slope gave very similar results.

The steady-state method gave a slower rate constant than the declining slope method. The results obtained with the steady-state method were very similar to the results obtained with the marker mean retention time in the total tract when calculated from the time of first appearance in the faeces. The results with the marker mean retention time in the total tract calculated from the time the marker was dosed, are included to show the effect of subtracting the time to first appearance by this method of calculation. It shows that a faster rate constant is obtained by subtracting the time to first appearance.

There is a striking similarity between the means of the steady-state method and the faecal appearance mean retention time as well as between the single dose declining slope and autoregression methods. It seems that four of these estimates of the rate constants are grouped in pairs of two each, and that each pair has something in common. The declining slope and autoregression methods have in common the fact that both have eliminated the influence of the rising slope of the marker excretion curve. With the declining slope method the rising slope of the curve was ignored. The autoregression method eliminated its influence by the inclusion of a time dependent phase as described by a second order polynomial.

When using the faecal appearance mean retention time method and calculating from the time of first appearance in faeces, the time of increase in counts was also included in the estimate. The steady-state method which uses the total mass of unfermentable OM in the rumen would also include the effect of a time lag in the outflow of digesta if the time lag were present in the rumen. The fact that both methods which eliminated the influence of the time of increase in counts gave similar results, and that both methods which did not eliminate this part also gave similar results, confirms the finding of Ellis *et al.* (1979) that the rising slope of the curve originates from the rumen itself.

It is suggested that members of both pairs of corresponding methods be used in the description of the outflow of a single dose of marked particles. Both the time of increase in counts and the declining slope should be included, using the methods of Grovum & Williams (1973) and of Graham & Williams (1962) and modified by taking the time of first appearance of marker in the faeces as the starting point.

By calculating both the rate constant with the declining slope method and the total tract method from first appearance in faeces, estimates of the effects of both the time dependent phase and the part of the outflow which follows first order kinetics may be obtained.

The practical implications of these two methods of description may be shown by calculating the pool size

relating to the fast rate constant (active mass) and comparing it with the total pool size (mass of unfermentable OM in rumen) which relates to the slower rate constant (Table 2). The method of calculating pool size follows from first order kinetics as described, among others, by Pienaar *et al.* (1980).

If $\frac{dz_2}{dt} = \gamma_2 Z_2$

then $Z_2 = \frac{dz_2}{dt} / \gamma_2$,

where $\frac{dz_2}{dt}$ = rate of outflow of unfermentable OM from the rumen,

z_2 = mass of unfermentable OM in the rumen,

and γ_2 = a rate constant for outflow from the rumen.

Table 2 Total and active masses of unfermentable organic matter in rumen

	Sheep Number				
	1	3	5	7	Mean ± SD
Mass of unfermentable OM in rumen	421,6	406,7	327,0	494,4	412,4 ± 68,6
Mass of unfermentable OM in rumen calculated from fast rate constant	294,2	325,4	263,8	347,6	307,8 ± 36,6

These results show that only 75% of the unfermentable OM present in the rumen is fully active in leaving the rumen and relates to the first order rate constant for outflow which is calculated from the declining slope. The alternative rate constant is related to the total mass of unfermentable OM in the rumen owing to a time lag during which feed particles become part of the active particle pool.

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