

Some observations on the digestibility of food by sheep, and on related problems

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When, in nutritional studies, it is necessary to determine the losses of a particular nutrient in the faeces, two techniques are employed to ensure that the faeces collected are representative of the intake of food. The first consists of feeding a marker substance, such as carmine, immediately before and at the end of the experiment. This allows a separation of the faeces produced from the food given in the interval, and is the technique employed with non-ruminant species.

With ruminants, however, the use of markers does not result in a clear separation of the faeces marking the beginning and end of the period. Consequently, the method employed to obtain accurate digestion coefficients with ruminants is to give the experimental ration in exact quantities for long periods, in order to ensure that a 'steady state' of faecal excretion is reached, and then to collect the faeces excreted during a measured interval of time. If refusal of food occurs on a particular day, the method is invalid, and so the amount of food given is usually kept small to avoid refusal. The low planes of nutrition which are thus employed are not desirable if results are to be applied in practical feeding (Schneider, 1954). Nor can the method be used where intake is voluntary or uncontrolled, because the faeces produced during a particular 24 h period are not simply referable to the food intake in some previous 24 h period.

In the course of experiments designed to study the effect of methods of fodder preparation and nutritional plane on the energy metabolism of sheep, information was obtained on the passage of food through the digestive tract, its digestibility, and the diurnal variation in the amounts of dry matter and water excreted. This information is presented here, and the results have been examined in some detail in order to elucidate the problems involved in the determination of digestibility coefficients in ruminants. It has been found that the time relationships involved in the passage of food through the gut give rise to equations which allow the prediction of the errors of digestibility coefficients, the digestibility coefficients themselves and the diurnal pattern of faeces excretion. They also permit the estimation of the gut contents or 'fill' of the animal under a variety of feeding systems and show what precautions must be taken in the conduct of digestibility trials.

EXPERIMENTAL

Animals and technique for studying digestibility

The six wether sheep used as experimental animals were harnessed for the separate collection of faeces and were housed in individual cages. Each sheep was given 600, 1200 and 1500 g of artificially dried grass, in successive periods of 21–27 days' duration.

The rations were given twice daily at 12 h intervals. Two sheep were given the dried grass in the long form, two the same material made into cubes after grinding in a hammer mill with a $\frac{1}{4}$ in. diameter sieve, and two were given the same grass made into cubes after it had been passed twice through a hammer mill with a $\frac{1}{16}$ in. diameter sieve. The original sample of dried grass was well mixed, but in the composition of the long material, the 'medium' and the 'fine' cubes given in Table 1, small differences were apparent. Similar differences in composition after processing were observed in experiments in Pennsylvania (Forbes, Fries & Braman, 1925).

Table 1. *Composition on a dry-matter basis of the dried grass used*

| Component | Form in which grass was given | | |
|-------------------|-------------------------------|-------------------------|-------------------------|
| | Long | Medium-ground and cubed | Finely ground and cubed |
| Crude fibre (%) | 22.1 | 21.3 | 21.1 |
| Nitrogen (%) | 2.21 | 2.28 | 2.21 |
| Carbon (%) | 45.4 | 45.2 | 45.8 |
| Calories (Cal./g) | 4.38 | 4.40 | 4.50 |
| Ether extract (%) | 4.1 | 4.7 | 4.4 |
| Ash (%) | 7.4 | 8.2 | 7.7 |

The measurement of the passage of food through the digestive tract was based on the method of Balch (1950) which was developed from that of Lenkeit (1930) and Usuelli (1933). It involved counting the number of previously stained food particles appearing in the faeces in successive intervals of time. Samples of the cubes and long material were dyed in a hot 0.05% aqueous solution of Safranin (Standard Stain, British Drug Houses Ltd) for 6–8 h. After washing, the excess of water was removed by a filter press, and the remainder by drying at 105°. The cubes formed a hard cake, which was broken into pieces, similar in size to the original cubes. The dyed food was substituted for 5% of the daily allowance in one meal. The dyed long material mixed easily with the normal material and was readily eaten. With the pellets, the dyed material was offered first and ordinary pellets added at intervals as it was being eaten. Some animals were reluctant to eat the dyed foods on occasions, but complete consumption was always obtained. Faeces were collected at 6 h intervals during the first 84 h after feeding and subsequently at 12 h intervals. They were weighed to the nearest 0.5 g, and the dry-matter content of a sample was determined in duplicate. These duplicates agreed to $\pm 1\%$ of the amount determined. The counting of particles in the faeces was facilitated by low-power magnification, a standard suspension of faeces being dispersed, over an illuminated area measuring 3 × 3 in., with 10% Teepol L (Shell Chemicals Ltd) as a wetting agent.

Statistical and mathematical method

In dealing with the errors attached to digestibility coefficients, it has, in the past, been customary to use the digestion coefficients themselves as variates (see Raymond, Harris & Harker, 1953). However, when the accepted technique of providing a constant intake is used, variation in the amount of the dietary component excreted per unit time becomes the sole determinant of the error of a digestibility coefficient.

For constant food intake in an individual the variability of the quantity of a food ingredient excreted during a given interval of time may be regarded as arising in two different ways. Firstly, it arises from independent errors which reflect variations in the efficiency of the digestive process from time to time, and also reflect any analytical errors arising from the weighing of the faeces or the chemical estimation of the particular food ingredient concerned. Secondly, errors arise from the fact that the expulsion of faeces is irregular, so that a single defaecation may occur in a particular period or outside it. On some occasions the animal may defaecate just before the end of a 24 h period, increasing the weight of material collected in that period, and reducing the weight collected in the subsequent 24 h. This error is here called the 'end-period' error, and accounts for the negative correlation observed between faecal outputs measured on one day and on the day following.

When the length of a collection period is increased, and if all analyses are made daily, independent errors attached to the mean output daily will fall with \sqrt{n} , where n is the length of the collection period expressed in standard intervals of time, in this instance, days. End-period errors, however, will fall more rapidly as the length of period is increased.

RESULTS

The accuracy of estimates of the output of faecal dry matter

The 12 h collections of faeces, expressed in terms of dry matter, were analysed to find to what extent independent and end-period errors contributed to the errors attached to faecal outputs. For this purpose, the sums of squares of the deviations of faecal

Table 2. *Estimates of variation of faecal dry-matter excretion in g, in relation to the time interval of collection. Pooled values for six sheep given daily 1200 g dried grass*

| Time interval during which collection was made (h) | Pooled no. of observations ($\Sigma n'$) | Pooled no. of degrees of freedom ($\Sigma(n' - 1)$) | Estimated variances | |
|--|--|---|-----------------------------|--------------------------|
| | | | Divisor $\Sigma(n' - 1/n')$ | Divisor $\Sigma(n' - 1)$ |
| 12 | 124 | 118 | 595.4 | 624.2 |
| 24 | 62 | 56 | 502.5 | 550.9 |
| 48 | 31 | 25 | 600.6 | 715.9 |
| 72 | 19 | 13 | 624.4 | 821.3 |
| 96 | 13 | 7 | 864.9 | 1260.4 |

weights from the mean faecal weight were computed over periods of 12, 24, 48, 72 and 96 h. These sums of squares refer to total weights of material, not to mean weights per 12 h interval. This calculation was done for each of eighteen digestibility experiments. The pooled results for six experiments when 1200 g food were given daily are shown in Table 2.

Pooling the individual sums of squares and dividing by the number of degrees of freedom $\Sigma(n' - 1)$ showed that there was a tendency for the estimates of variance of the total quantity of faeces collected in a time interval to increase somewhat with the width of that interval. Few degrees of freedom were available, however, for the longer intervals. If the whole of the error were end-period error the divisor in the analysis of variance would be $\Sigma(n' - 1/n')$. Where n' is large this is equivalent to division by

the number of variates. In Table 2 a column is included which shows that when the divisor is $\Sigma(n' - 1/n')$ there is little if any increase in the estimates of variance with increasing length of period, the final value for collections made at 96 h intervals not being significantly greater than the value for the 72 h interval. The error associated with a collection of faeces is thus virtually independent of the length of time during which the collection is made.

Table 3. *Estimates of variation in the 24 and 72 h faecal excretion of dry matter of dried grass in g, as affected by the amount of food given and hence faeces produced. Pooled values for six sheep*

| | 24 h collection period | | 72 h collection period | |
|---|------------------------|---------------|------------------------|---------------|
| | 600 g ration | 1200 g ration | 600 g ration | 1200 g ration |
| Mean quantity of faeces | 126.1 | 295.6 | 378.3 | 886.8 |
| Pooled sum of squares of deviations from individual means | 23,299.2 | 30,851.2 | 14,124.0 | 10,677.2 |
| No. of variates | 72 | 62 | 24 | 19 |
| Divisor $\Sigma(n' - 1/n')$ | 71.5 | 61.4 | 22.5 | 17.1 |
| Estimated variance (divisor $\Sigma(n' - 1/n')$) | 325.9 | 502.5 | 627.7 | 624.4 |

Such independence suggests that the error is not a function of the amount of faecal material collected, and that it would also be independent of the amount excreted per unit time. To test this supposition the estimated variances obtained with the same six sheep on increasing the ration from 600 to 1200 g daily were compared. The results, given in Table 3, show that doubling the food intake increased the estimated variance slightly in one instance. Further calculations involving 12 and 48 h collections confirmed that the effects were small, although there was a tendency for the errors attached to short periods of collection to be lower when the smaller ration was given. The magnitude of this effect when translated into a standard error was small relative to the mean, and largely arose from one sheep which gave extremely low variability ($\sigma_0^2 = 61$) over short periods of time, and normal variability ($\sigma_0^2 = 380$) for periods of over 24 h. The sums of squares for sixteen experiments, irrespective of feeding level, were therefore pooled as shown in Table 4 and the standard deviations calculated using $\Sigma(n' - 1/n')$ as divisor. These standard deviations of the weight excreted in the periods were then expressed as the standard deviations attached to the weight of faeces excreted/24 h. Similar standard deviations were then calculated with the standard deviation for 12 h collections as a base. The alternative assumptions— independent errors, or end-period errors—were used for this purpose. The observed standard deviations were considerably lower than those calculated on the assumption of independent errors. Where analytical techniques of low accuracy are used, errors will obviously tend more towards the curve for independence. With the common analytical procedures this tendency will not be marked.

Generalization of the end-period error hypothesis

The above analysis shows that the error attached to a collection of faeces is about ± 23 g dry matter irrespective of the length of the collection period or the rate of excretion of faecal dry matter. Since this error is a combination of two end-period

errors, the single end-period error will be ± 16.2 g. This is approximately the weight of a single defaecation. The excretion of faeces by an animal can be regarded, therefore, in the following way. Suppose that faecal material enters the lower part of the gut at a rate of $F(t)$, which need not be a constant rate, the functional notation indicating that it is a continuous function of time. The amount of faeces expelled at the i th defaecation, Z_i , is equal to

$$\int_{t_{i-1}}^{t_i} F(t) dt \dots, \quad (1)$$

where t_i is the time of the i th defaecation and t_{i-1} the time at which the previous defaecation occurred. If $F(t)$ varies considerably over the 24 h, and the amount excreted at any particular time, Z_j , tends to be constant, then the frequency of defaecation will vary with $F(t)$. In deciding on the length of collection periods, the variation in $F(t)$ must be considered. If, for instance, the rate at which faeces enter the lower part of the gut is not constant over the 24 h, and yet the rate pattern repeats

Table 4. *Estimates of the standard deviations attached to mean daily collections of faecal dry matter in g of sheep given dried grass, together with the observed standard deviations*

| Time interval during which collections were made (days) | No. of observations ($\Sigma n'$) | Divisor $\Sigma(n' - 1/n')$ | Pooled sum of squares $\Sigma(s.s.)$ | Standard deviation of mean quantity excreted/24 h | | |
|---|-------------------------------------|-----------------------------|--------------------------------------|---|----------|-------|
| | | | | Observed (divisor $\Sigma(n' - 1/n')$) | Computed | |
| | | | | | (a)* | (b)† |
| 0.5 | 360 | 352.0 | 188,622.8 | 46.30 | 46.30 | 46.30 |
| 1.0 | 180 | 172.0 | 91,528.8 | 23.06 | 23.15 | 32.74 |
| 2.0 | 90 | 87.0 | 45,617.1 | 11.44 | 11.57 | 23.15 |
| 3.0 | 58 | 56.0 | 39,514.2 | 8.86 | 7.72 | 18.90 |
| 4.0 | 42 | 35.7 | 25,984.0 | 6.75 | 5.79 | 16.37 |
| 6.0 | 16 | 12.0 | 12,184.6 | 5.30 | 3.86 | 13.37 |

* On the assumption that the errors are entirely end-period errors (i.e. the error falls in direct proportion to the length of the period).

† On the assumption that the errors are entirely random (i.e. the error falls in proportion to the square root of the length of the period, that is, quadrupling the length of the period reduces the error by half).

every 24 h, collection periods other than exact multiples of 24 h could give rise to considerable over- or under-estimation of the amount excreted in a 24 h interval. Even when collections are made at 24 h intervals, it is desirable that they should be made under conditions where the rate of faeces production, $F(t)$, is fairly constant. Undoubtedly $F(t)$ will be subject to variation arising from unknown causes, and if collections are made when the mean value of $F(t)$ is changing rapidly, then such variation will result in a greater variation in the weights of materials collected in successive intervals than if the mean value of $F(t)$ were constant.

Apart from these considerations, which suggest that information is needed on the rate of faeces production if faecal collections are to be made with maximal precision, two further corollaries follow the fact that the emptying time of the colon and rectum is largely the determinant of the errors attached to faecal weights. Firstly, the error attached to digestion coefficients will fall with increasing food intake. Secondly, for a given intake of food, errors are independent of the magnitude of the digestibility coefficient.

The passage of food through the digestive tract as affected by method of preparation

The results obtained with the dried grass prepared in three different ways are shown graphically in Fig. 1, each graph being the mean of values for two sheep. Variation between sheep was not large, with the exception that one of the sheep given fine cubes passed the food more rapidly than the other.

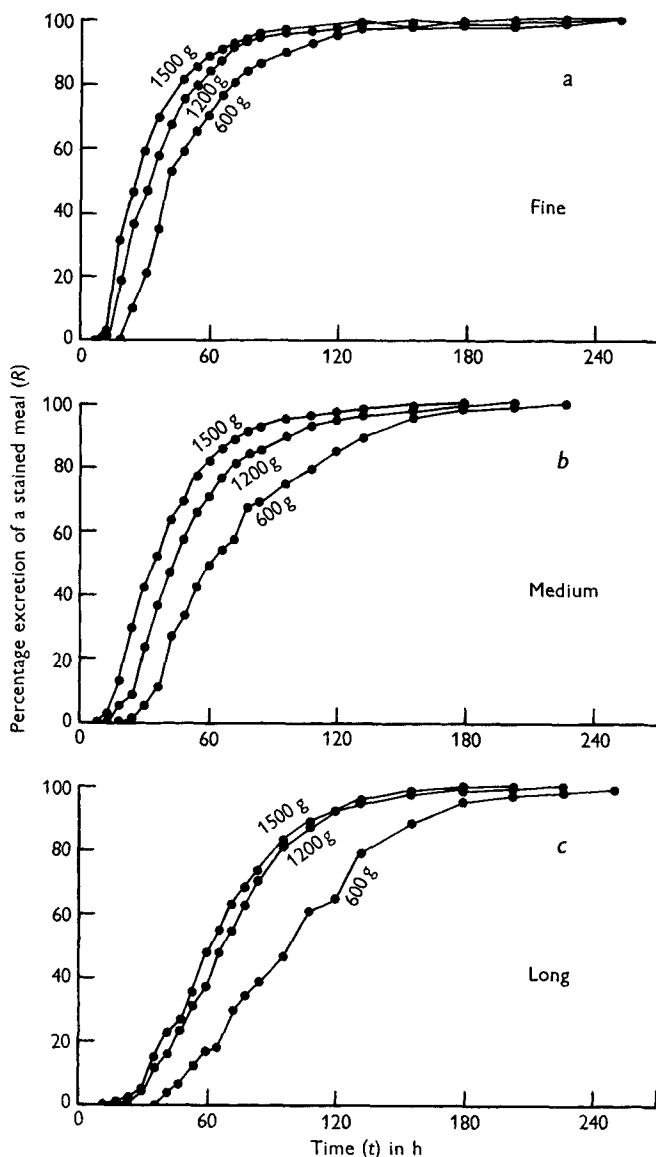


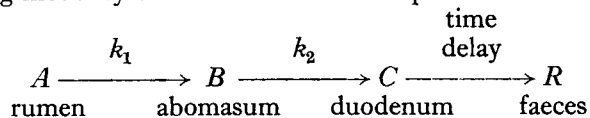
Fig. 1. Passage of dried grass through the digestive tract of sheep as affected by the method of preparation and the amount given. The curves represent the cumulative total of the stained particles excreted, expressed as a percentage, plotted against time. *a*, finely ground and cubed dried grass; *b*, medium-ground and cubed dried grass; *c*, long dried grass. The figures on the curves indicate the daily intake.

The results show that the passage of the grass through the digestive tract increased both with the amount given and with increased comminution. The fact that with sheep, finely ground, cubed grass passed through the digestive tract more quickly than long material is contrary to results obtained by Balch, Balch, Bartlett, Johnson, Rowland & Turner (1954), who used ground hay in experiments with cows. Whether this difference reflects a species difference or a difference between cubes and meal is not known. It is of interest, however, that all Balch's (1950) cows given ground hay excreted it much faster initially than long hay, and in one instance (Balch's cow Y) this initial rapid excretion persisted up to the time when 90% of the meal had been excreted. In the studies of Balch *et al.* (1954) the slow passage of a ration containing little hay may, in part, be explained by the reduction in the total quantity given. In view of the marked effect of level of intake on passage, it is possible that any differences discernible between the present results and those of Balch may be more apparent than real, since the sheep were given exact quantities of food whereas Balch's cows were given part of their ration in proportion to their milk yield.

Although graphical analysis shows clearly the differences in passage of food which occur when different rations are given, a more rigorous treatment of the data is necessary to disentangle interrelationships. The mathematical description of the faecal excretion curves which follows may be regarded as a convenient method of summarizing and then manipulating the unwieldy mass of data involved. Balch (1950) has shown that the time taken to excrete 5% of the stained residues of a meal is approximately equivalent to the time the food takes to pass through the omasum, abomasum and intestines. In addition, the spread of the excretion curve, measured by the interval of time between excretion of 5 and 80% of the particles, is indicative of the time the food spent in the reticulo-rumen. Here a different approach has been used.

The work of Phillipson (1952) has shown that the flow of digesta from rumen to abomasum and from abomasum to duodenum is continuous throughout the 24 h, unlike the emptying of the colon and rectum. Balch's (1950) studies, in which stained material was introduced directly into the abomasum, and Piana's (1952) studies with rumenectomized sheep suggest that events occurring in the rumen are the predominant factor accounting for variations in passage.

These results suggested that the overall process could be regarded as a kinetic one and the following model system was therefore set up:



Here A , B , C and R represent amounts of a unit of food in the compartments concerned. Initially ($t=0$), $A=1$, and all food is present in the first phase; when $t \rightarrow \infty$, $A \rightarrow 0$ and $R \rightarrow 1$. k_1 and k_2 are rate constants. The time delay from C to R is here denoted by τ , food present in the duodenum at time t appearing in the faeces at time $t+\tau$.

We may express this model in three differential equations:

$$\frac{dA}{dt} = -k_1 A, \quad \frac{dB}{dt} = +k_1 A - k_2 B, \quad \frac{dR}{dt} = k_2 B_{t-\tau}. \quad (2)$$

The solution of equations (2) gives

$$\left. \begin{aligned} R(t) &= 1 - \frac{1}{k_1 - k_2} (k_1 e^{-k_2(t-\tau)} - k_2 e^{-k_1(t-\tau)}) & \text{for } t \geq \tau, \\ R(t) &= 0 & \text{for } t \leq \tau. \end{aligned} \right\} \quad (3)$$

The interpretation of the constants is not rigorous, since there is no proof that k_1 and k_2 apply to events occurring in the specific parts of the tract mentioned; their physiological significance has not been tested by direct experimentation, but, as appears later, they are certainly extremely suggestive. Attempts were made by the use of different systems, including diffusion systems, to obtain more suitable models, but all soon led to intractable equations. The present equation, initially arrived at on the basis of kinetic considerations, has been retained because it fits the data so well and is easily manipulated. The method of fitting the equation consisted of minimizing

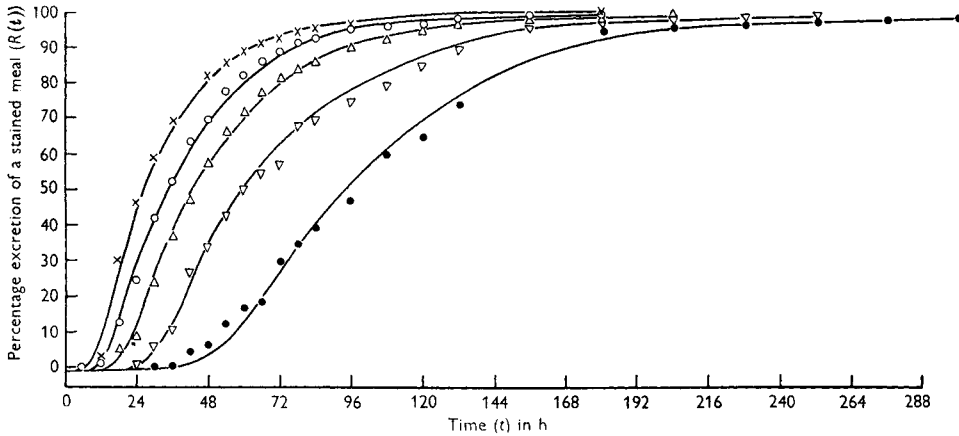


Fig. 2. Selected values from Fig. 1 representing the fastest and slowest rate of passage of food, together with passage curves predicted from equation (3) (see above). ●, sheep given daily 600 g long dried grass; ∇, sheep given daily 600 g medium-ground and cubed dried grass; Δ, sheep given daily 1200 g medium-ground and cubed dried grass; ○, sheep given daily 1500 g medium-ground and cubed dried grass; ×, sheep given daily 1500 g finely ground and cubed dried grass.

the sum of squares of deviations of observed from calculated values. This was done by successive approximation and, in effect, involved finding a minimum in a three-dimensional space. The fit is shown in Fig. 2, which includes the fastest and slowest passages observed, and also in Table 5, which, besides presenting the constants, gives the residual standard errors. These are derived from the sums of squares of the differences between observed and calculated values divided by the number of degrees of freedom ($n - 3$). These residual errors were all well below ± 0.03 on the scale 0-1. It will be noted that, when $k_1 = k_2$, equation (3) is indeterminate. The solution when $k_1 = k_2$ is

$$\left. \begin{aligned} R(t) &= 1 - e^{-k(t-\tau)} (k(t-\tau) + 1) & \text{for } t \geq \tau, \\ R(t) &= 0 & \text{for } t \leq \tau. \end{aligned} \right\}$$

In Table 5 both k_1 and τ show considerable variation but k_2 remains relatively stable. It is evident that high values of k_1 are associated with low values of τ . This

suggests that k_1 and τ both represent events taking place in the rumen or that one is a determinant of the other. It follows that either τ is not a time delay from duodenum to colon or that rapid flow from the rumen (a high value of k_1) accelerates intestinal flow. Without direct experimentation it is impossible to decide which alternative applies. The important point, however, is not so much a rigorous physiological interpretation of the constants but that the excretion of stained material can be accurately described by a simple equation with three constants. Furthermore, the equation gives sensible results on extrapolation, and, unlike empirical relationships, it is based on all the experimental data. It is not contingent upon determining two points accurately as in the 80–5% excretion times which have been used before.

Table 5. *The constants of equation (3) for the nine experiments, each experiment being made with two sheep, t being expressed in h*

| Form in which dried grass was given | Amount of dried grass given daily (g) | k_1 | k_2 | τ | 100 × residual standard error |
|-------------------------------------|---------------------------------------|-------|-------|--------|-------------------------------|
| Long | 600 | 0.031 | 0.031 | 36 | ± 2.31 |
| | 1200 | 0.057 | 0.038 | 26 | ± 1.85 |
| | 1500 | 0.064 | 0.037 | 25 | ± 1.60 |
| Medium-ground and cubed | 600 | 0.102 | 0.026 | 23 | ± 2.65 |
| | 1200 | 0.187 | 0.031 | 15 | ± 1.67 |
| | 1500 | 0.279 | 0.035 | 10 | ± 1.44 |
| Finely ground and cubed | 600 | 0.235 | 0.034 | 17 | ± 1.74 |
| | 1200 | 0.318 | 0.039 | 10 | ± 1.26 |
| | 1500 | 0.503 | 0.045 | 9 | ± 1.67 |

The pattern of excretion of ingesta into the lower gut

From equation (3), which describes the passage of a single meal through the intestinal tract, it is possible to compute the rate at which faecal material enters the lower gut in an animal given a normal sequence of meals. The rate at which a single meal passes through may be obtained by differentiating equation (3) to give

$$\left. \begin{aligned} \frac{dR(t)}{dt} &= \frac{k_1 k_2}{k_1 - k_2} (e^{-k_2(t-\tau)} - e^{-k_1(t-\tau)}) \quad \text{for } t \geq \tau \\ &= 0 \quad \text{for } t < \tau. \end{aligned} \right\} \quad (4)$$

The time spent in eating a single meal may be regarded as very short relative to the time taken for the meal to pass through the digestive tract, and so, in order to obtain the rate at which faeces are produced when the animal is being given a particular pattern of meals, it is only necessary to add up a series of rate-of-passage curves for individual meals, each having a different intercept on the time axis corresponding to the time at which it was given. This can easily be done graphically or, more formally, by mathematical manipulation.

In Fig. 3 the method of graphical summation can be seen. The animal in this instance was given one meal a day at time θ on the 1st day, days being numbered 1, 2, ..., n . Each meal passes through the intestinal tract at the rate $dR(t)/dt$ given by

the solid black lines. At time t on the n th day, the rate at which faeces are being produced consists of the sum of the ordinates at t :

| | |
|--|-------------|
| For each meal given at time $\theta+n-1$ (since none reaches the faeces until time $(\theta+n-1+\tau)$ and τ is > 1) | Rate |
| For the meal given at time $\theta+n-2$ | 0.80 |
| For the meal given at time $\theta+n-3$ | 0.36 |
| For the meal given at time $\theta+n-4$ | 0.18 |
| For the meal given at time $\theta+n-5$ | 0.09 |
| For the meal given at time $\theta+n-6$ | 0.04 |
| For the meal given at time $\theta+n-7$ | 0.00 |
| Total | 1.47 |

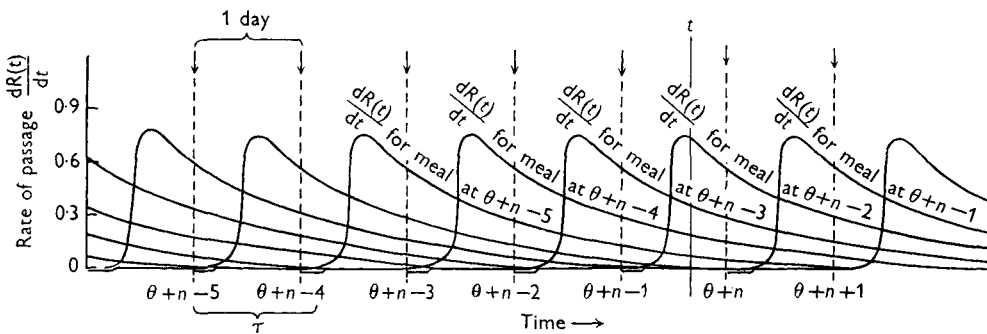


Fig. 3. Diagram showing the nature of the superposition of the rates of passage $\left(\frac{dR(t)}{dt}\right)$ used to obtain the rate of faeces production at a particular time. For explanation see text, p. 77.

This is the total rate expressed in terms of a unit weight excreted/24 h. If the faecal weight excreted/24 h was 400 g, then the rate of excretion at time t would be 588 g/24 h. At some other time the rate would, of course, be less than 400. By the use of the superposition theorem of Heaviside, and Dirac δ -functions, it can be shown that if meals are given at regular intervals, the rate of faeces production between meals can be predicted from equation (5), which is a steady-state equation implying that the animal had received the ration under consideration for such a long period that effects of any previous ration were completely nullified:

$$F_s(t-\tau) = D e^{-k_2 \delta} - G e^{-k_1 \delta}, \tag{5}$$

where
$$D = \frac{k_1 k_2}{k_1 - k_2} \frac{e^{k_2}}{e^{k_2} - 1} \quad \text{and} \quad G = \frac{k_1 k_2}{k_1 - k_2} \frac{e^{k_1}}{e^{k_1} - 1}.$$

The subscript s indicates that the rate of faeces production is the steady-state rate, and δ is the fraction of the time interval between meals, having any value between 0 and 1.

The use of this equation avoids much of the tedious arithmetic involved in summations by the graphical method.

In Fig. 4, graphs computed using equation (5) are given. They show that with animals given their rations in two equal meals at exactly 12 h intervals the rate of

faeces production is not constant over the 24 h. The greatest variation in rate occurred with the food showing the fastest rate of passage; in the instance illustrated, the finely ground and cubed material given at the 1500 g level. With the ration that passed more slowly, the variation in rate was considerably less but, nevertheless, still apparent. These results suggested that diurnal variation in faecal excretion would arise from the diurnal pattern of food consumption, and in these sheep weights of faeces produced in successive 6 h periods did not, in fact, reveal any diurnal variation other than that which can be predicted from equation (5).

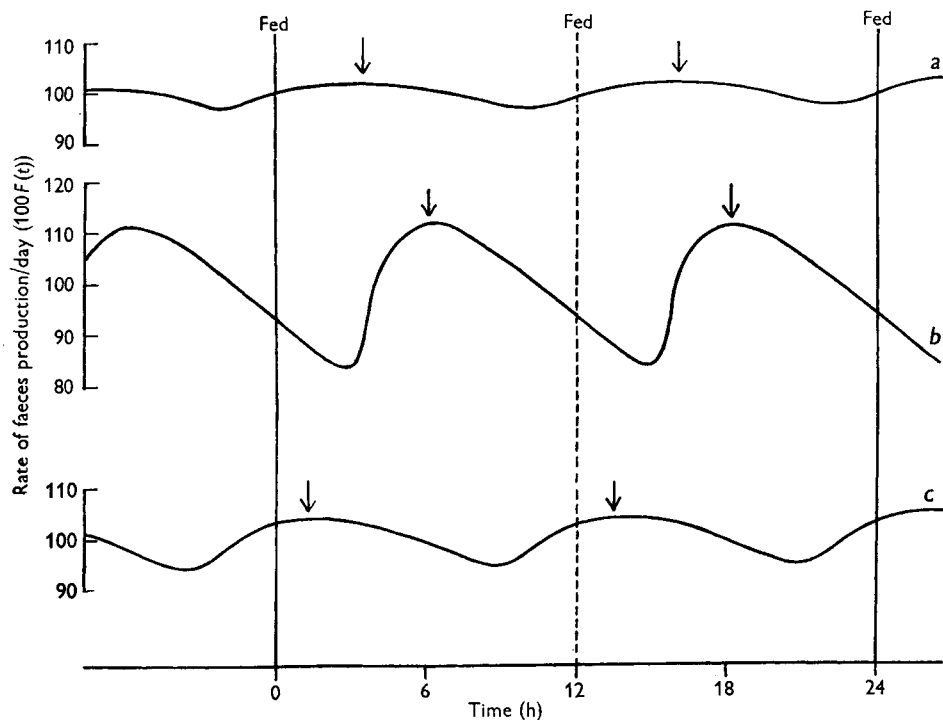


Fig. 4. Diurnal variation in the rate of faeces production $F(t)$ of sheep given two meals daily at 12 h intervals, calculated from the steady-state equation (5) (see p. 78). The mean rate of faeces production over the 24 h has been placed at 100, so that the amplitude of the curves represents the percentage range of diurnal variation in rate. The time when $\frac{dF(t)}{dt}$ is a minimum is indicated by the arrows. *a*, sheep given daily 1200 g long dried grass; *b*, sheep given daily 1500 g finely ground and cubed dried grass; *c*, sheep given daily 1200 g medium-ground and cubed dried grass.

As previously stated, the maximal frequency of defaecation coincides approximately with the maximal rate at which faecal material enters the lower gut. From Fig. 4 it is clear that these maxima occur at different times of the day for the different rations. To obtain comparable accuracy in two experiments it would be desirable to make collections at those times when the frequencies of defaecation are similar. They will clearly vary from ration to ration. There is, in other words, no ideal time of day at which to make faecal collections in digestibility trials.

If the daily ration is given in two feeds which are not equally spaced in the 24 h, equation (5) does not apply. Recent studies (Blaxter, Graham & Wainman, 1956)

have shown that the passage of a single meal/24 h does not differ from that of two meals of half the size given at 12 h intervals. This suggests that it is legitimate to consider unequal spacing of meals in the manner outlined above, and that the main determinant of passage is the total amount given in the day rather than the size of individual meals. Analysis of irregular feeding patterns shows that they give rise to considerably greater variations in rate of production of faeces over the 24 h than do regimens involving equal intervals between meals. It follows that the assumption so often made in conducting digestibility trials, namely, that provided all the ration is given between one morning and the next intervals between meals matter little, cannot be correct. Such methods would lead to considerable errors in the estimates of the quantities excreted/24 h. To minimize errors it is advisable to adhere to a strict routine, and preferably to provide several meals each day at defined times.

Further derivatives may be obtained from the summation of the individual rate curves and used to predict the length of time which must be allowed after a ration has been given to be certain that the faeces produced are characteristic of the food. Heaviside superposition gives equation (6) which is a transient. It gives the change with time of the rate of faeces production from a ration when it is first given, or, alternatively, the rate of faeces production from the ration when it has been changed to another:

$$F_T(t-\tau) = Ge^{-k_1 n} - De^{-k_2 n}, \quad (6)$$

where subscript T represents the transient, G and D have the same values as before and n is the number of days following the commencement or the cessation of feeding the ration. This function is illustrated in Fig. 5 for three foods. When cubes made from the finely ground material were given at the rate of 1500 g daily, 5 days were sufficient to reduce the value of $F(t)$ to below 1%, which means that after 5 days had elapsed 99% of the faeces would be representative of the new ration. With long material 8 days would be necessary. The usual practice of allowing 10 days to elapse before commencing collections of faeces in a digestibility trial thus appears sufficient to annul any effects of a previous ration. Whether such periods of time are long enough to allow readjustment of the flora of the alimentary tract to the change in ration cannot be stated. Measurements of the rate of change of methane production, indicative of changes in the rumen flora, suggest, however, that a similar period may, in fact, be sufficient (Blaxter & Graham, 1955*a*).

By summing the passage curves in other ways, involving meals of different sizes, different feeding frequencies and variation in intervals between meals from day to day, a number of other problems concerned with finding optimal procedure for conducting digestibility trials can be investigated. The approach, for instance, provides explanations for the so-called diurnal variation in the excretion of inert materials used as marker substances in experiments in which digestibility determinations are made by the ratio technique. The variation in the concentration of marker substance in the faeces appears to reflect the pattern of food intake and excretion relative to the pattern of marker intake and excretion. These aspects have been examined and are quoted to illustrate the utility of determinations of the passage of food in studies of ruminant digestion.

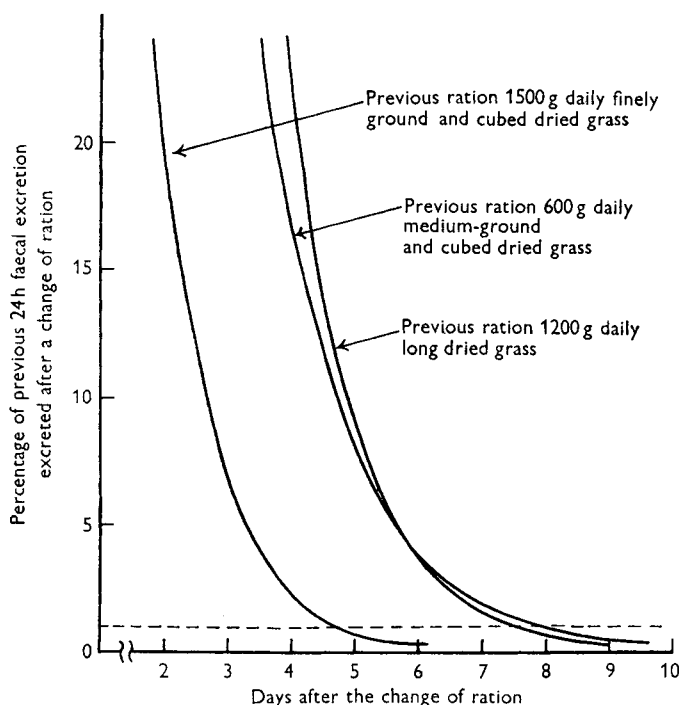


Fig. 5. Residual effect of a previous ration, in terms of the faecal output referable to a previous ration, in relation to the number of days of constant feeding after the change of ration, calculated from the transient equation (6) (see p. 80) for three typical rations. An equilibrium can be accepted as existing when the transient has reached a value below 1%. Transient below dotted line has a value below 1%.

Digestibility and rate of passage of food

In the eighteen experiments, the digestibility of the dry matter of the ration was determined, and the mean coefficients of apparent digestibility are given in Table 6.

It will be noted that, though the samples of grass were all prepared from the one batch, the digestibility varied from 80 to 65%. The increase in the feeding level resulted in a very slight fall in the digestibility of long material, a fall in the digestibility of 'medium' cubes and a considerable fall in the digestibility of the 'fine' cubes.

Table 6. *Mean values for two sheep on each ration for the coefficients of apparent digestibility of the dry matter of dried grass in relation to the mean length of time the food spent in the alimentary tract*

| Form in which dried grass was given | Amount given daily (g) | Apparent digestibility (%) | Mean time spent by the food in the digestive tract (h) |
|-------------------------------------|------------------------|----------------------------|--|
| Long | 600 | 80.3 | 103 |
| | 1200 | 79.1 | 72 |
| | 1500 | 79.4 | 68 |
| Medium-ground and cubed | 600 | 76.9 | 74 |
| | 1200 | 71.5 | 53 |
| | 1500 | 69.9 | 42 |
| Finely ground and cubed | 600 | 75.9 | 53 |
| | 1200 | 68.8 | 39 |
| | 1500 | 65.4 | 34 |

These results parallel in a striking way the passage studies, since those rations that spent the greatest time in the tract were digested to the greatest extent. A limit of about 81% in the potential digestibility is apparent. In Fig. 6 the digestibility coefficients are plotted against the mean length of time the food stayed in the digestive tract. This time was obtained by dividing the sum of the times which individual stained particles spent in the tract by the total number of particles. The figure shows that the relationship was curvilinear, the rate of increase of digestibility with mean time being greater when food passed quickly than when it remained in the tract for long

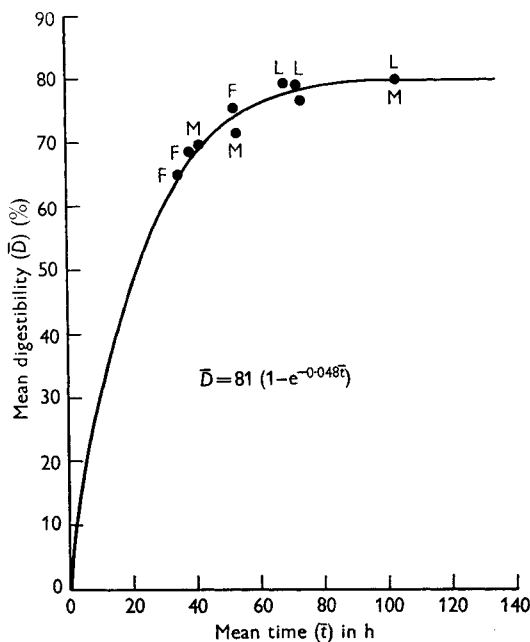


Fig. 6. Relation between the apparent digestibility of the nine rations (groups of two sheep) and the mean time they took to pass through the digestive tract of the sheep. For definition of mean time see text, this page. *L*, long dried grass; *M*, medium-ground and cubed dried grass; *F*, finely ground and cubed dried grass.

periods. What applies to the food considered as a whole should apply to each fraction of each meal. Since it has been shown that, with a single meal, part passes through the gut in a matter of hours whereas part takes a matter of days, the 'mean time' cannot be the ideal criterion to use to establish a relationship between the passage of food and its digestibility. Each particle needs separate consideration.

If we state that the length of time a particle stays in the tract determines its digestibility, and that for any one food of a particular composition there is a maximum potential digestibility dictated by the presence of inert material completely resistant to digestion, such as lignin, silica and cellulosic materials protected by lignin, we can write

$$D = L(1 - e^{-ht}), \quad (7)$$

where D is the digestibility of the particle, L is the potential digestibility, and h is a constant.

Referring once more to the passage curves, the amount of a unit of food which has been excreted up to a particular time, t , is $R(t)$, and so the amount which is excreted in the interval $(t, t + dt)$ is $\frac{dR(t)dt}{dt}$. This amount of non-digested material must obviously have stayed inside the digestive tract for a time t .

If each particle is digested to the extent shown by equation (7) the mean digestibility as determined by experiment (\bar{D}) will be

$$\bar{D} = \int_0^\infty L(1 - e^{-ht}) \frac{dR(t)}{dt} dt, \tag{8}$$

and since

$$\int_0^\infty \frac{dR(t)}{dt} dt = 1, \tag{9}$$

$$\bar{D} = L \left(1 - \int_0^\infty e^{-ht} \frac{dR(t)}{dt} dt \right).$$

Inserting in equation (9) the rate of faeces production given in equation (4), we have

$$\bar{D} = L \left(1 - \int_0^\infty e^{-ht} \frac{k_1 k_2}{k_1 - k_2} (e^{-k_2(t-\tau)} - e^{-k_1(t-\tau)}) dt \right),$$

which gives on simplification

$$L - \bar{D} = L \frac{k_1 k_2}{k_1 - k_2} \left(\frac{e^{-k_2 \tau}}{h + k_2} - \frac{e^{-k_1 \tau}}{h + k_1} \right). \tag{10}$$

In the above equation, k_1 , k_2 and τ are known, the limiting value L can be chosen and the value of h can then be obtained by successive approximation in equation (11):

$$\frac{(L - \bar{D})(k_1 - k_2)}{L k_1 k_2} = \frac{e^{-k_2 \tau}}{h + k_2} - \frac{e^{-k_1 \tau}}{h + k_1}. \tag{11}$$

The left-hand term in equation (11) is a constant for each ration. In the present experiments the value of L was taken to be 0.81, and values of h were computed from the constants of Table 5. The results are given in Table 7.

Table 7. Values of h , the rate constant in the exponential equation relating the digestibility for sheep of the dry matter of dried grass to the time it takes to pass through the digestive tract (estimated from equation (11), see above)

| Amount of dried grass given daily (g) | Form in which dried grass was given | | |
|---------------------------------------|-------------------------------------|-------------------------|------|
| | Finely ground and cubed | Medium-ground and cubed | Long |
| 600 | 0.29 | 0.30 | 0.30 |
| 1200 | 0.15 | 0.16 | 0.35 |
| 1500 | 0.13 | 0.16 | 0.18 |

The values of h show some variation, being larger for the smallest ration. This may reflect the fact that digestibilities of the smallest rations were close to the asymptotic value of 0.81, thus leading to inaccuracies in prediction. A similar argument applies to the experiments with the long dried grass. As an approximation, it would appear that h normally has a value between 0.13 and 0.18, indicating that in 10 h some 60-80% of the potentially digestible components of the dried grass would be digested.

In view of the slow nature of cellulose digestion when incubated with rumen contents *in vitro*, this estimate appears reasonable. Undoubtedly, to regard digestion as described by a single exponential relationship is an oversimplification; it is probably more complex, involving different rates of digestion for different constituents. As a first approximation, however, a single exponential appears to describe the results reasonably well.

Previous experimenters have sought a relationship between rate of passage of food and digestibility. Ewing & Smith (1917), assuming that a higher moisture content of the faeces was indicative of a rapid rate of passage, concluded that rapid passage was associated with high digestibility. No actual determinations of the time taken by food to pass through the gut were, however, made. In Balch's (1950) work the variation in digestibility coefficients from period to period was not large and could not be explained by differences in passage.

The variation in digestibility coefficients met in the present study was certainly much larger than those previously observed in studies with the same food prepared in different ways (Heller, Wall & Briggs, 1911; Bohstedt, Roche, Rupel & Fuller, 1930; Mead & Goss, 1935; Forbes *et al.* 1925; Kick, Gerlaugh, Schalk & Silver, 1937; Cole & Mead, 1943; Swanson & Herman, 1952). Most of these studies have compared finely ground hay with long hay, and differences in digestibility have been of the order of 2-3% in favour of long material. This suggests that the process of cubing may have been of greater importance than that of grinding in determining the rate of passage and hence digestibility. The absence of a marked correlation between digestibility and rate of passage in previous experiments undoubtedly arises from the limited spread of rates of passage of the foods compared.

Gut contents

Estimation of gut contents in terms of indigestible material or 'ballast'

It has long been known that the appetite of ruminants varies with the quality of the food. Foods of high nutritive value are habitually consumed in larger amounts than are those of low value (Blaxter, 1944, 1950-1). Certainly, the dry-matter content of a ration is a poor guide to its ability to satisfy the appetite of an animal. For this reason, other attributes of the ration, thought to be indicative of its 'space-filling' properties, have been used as measures. These include the volume of the ration, the volume after soaking, and the 'ballast' or indigestible material it contains (Lehmann, 1941). The measurement of appetite must take into consideration the passage of food since the gut is not infinitely distensible. From the passage of the food in the present experiments, the weight of gut contents can be deduced.

The amount of indigestible material present in the gut arising from a single meal, which has a passage described by the function $R(t)$, will be $1 - R(t)$. Thus when $t = 0$, the amount of 'ballast' it contributes will be unity and when t becomes very large and the whole of the meal has passed through, $1 - R(t)$, the 'ballast' will be zero.

Methods analogous to those used to arrive at the steady state of faeces production may be used to compute the amount of indigestible material which stays inside the

sheep, and forms the so-called 'ballast'. These methods consist of adding up the ordinates in a graph in which a series of curves of the function $(1 - R(t))$ is plotted, the intercepts of each corresponding to the times of the meals.

Fig. 7 illustrates this. Here, as in Fig. 3, one meal is provided daily at time θ on the 1st day, and days are numbered 1, 2, ..., n . The solid lines are the function $1 - R(t)$, depicting the proportion of each meal that stays inside the sheep. The summation at time t now includes the following terms:

| | |
|---|------|
| For the meal given at time $\theta + n - 1$ | 1.0 |
| For the meal given at time $\theta + n - 2$ | 1.0 |
| For the meal given at time $\theta + n - 3$ | 0.7 |
| For the meal given at time $\theta + n - 4$ | 0.3 |
| For the meal given at time $\theta + n - 5$ | 0.1 |
| For the meal given at time $\theta + n - 6$ | 0.05 |
| For the meal given at time $\theta + n - 7$ | 0.00 |
| Total ballast | 3.15 |

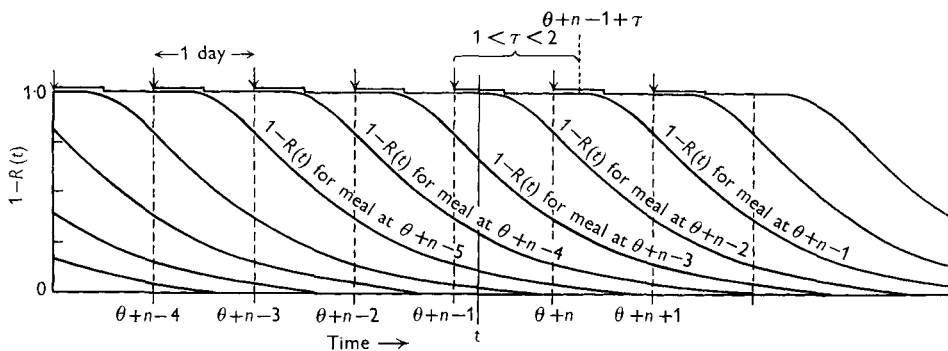


Fig. 7. Diagram showing the nature of the superposition of the function $1 - R(t)$ which describes how much of the indigestible residues of a single meal remains inside a sheep at a particular time. To obtain the total amount present at a particular time the ordinates are summated. For explanation see text, this page.

The total ballast is measured in terms of units of the day's faecal output, that is, if the day's faecal output is 400 g the ballast or indigestible dry-matter content of the digestive tract is 1260 g. As with equations (5) and (6), a mathematical approach can be used, entailing Heaviside superposition. The steady-state and transient equations, though comparable, are more complicated than equations (5) and (6). In Fig. 8, which was, in fact, calculated from the steady-state equations rather than obtained by graphical analysis, the weight of ballast in the digestive tract of sheep given the three foods at the highest levels of feeding is shown. It will be noted that there was considerable variation over the 24 h. In Table 8 data relating to the amount of ballast present in the digestive tracts of the sheep immediately before feeding are given. These data show that immediately before they are given their next meal, sheep fed long material carry in their digestive tracts food residues equivalent to 2.6 days' food intake. Sheep given the cubes made from the same material after grinding it finely carry less than half this amount.

Table 8. Predicted amount of 'ballast' (indigestible dry matter) present in the digestive tract of sheep immediately before they were given a meal, expressed as multiples of the daily excretion of dry matter

| Amount and form of dried grass given daily | Amount of ballast (multiples of daily excretion of faecal dry matter) |
|--|---|
| 1500 g long | 2.57 |
| 1500 g medium-ground and cubed | 1.51 |
| 1500 g finely ground and cubed | 1.14 |

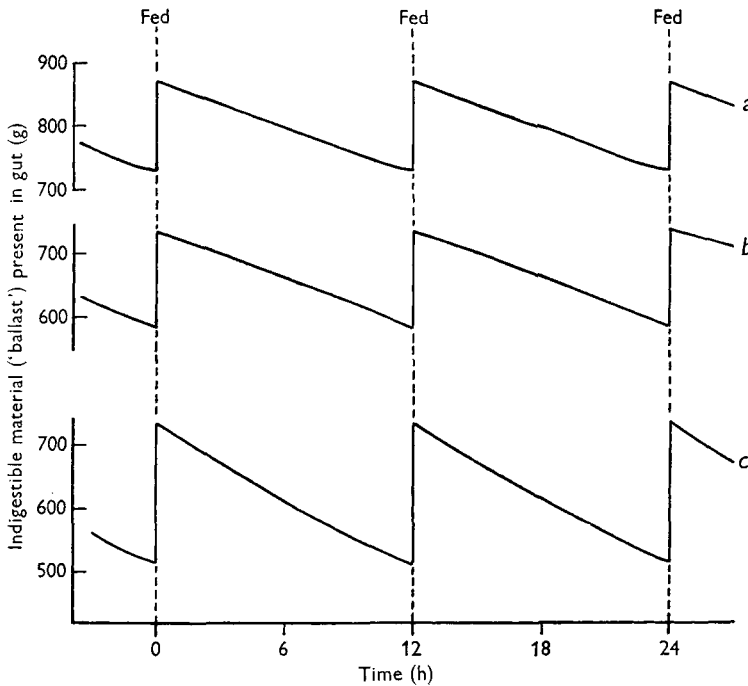


Fig. 8. Diurnal variation in indigestible dry-matter content of the digestive tract ('ballast') of sheep determined from equations describing the steady state as obtained by superposition of food residues from previous meals as shown in Fig. 7. In these sheep fed twice daily, at each meal-time the 'ballast' increased by an amount equivalent to half the 24 h faecal output. *a*, sheep given daily 1200 g long dried grass; *b*, sheep given daily 1200 g medium-ground and cubed dried grass; *c* sheep given daily 1500 g finely ground and cubed dried grass.

Estimation of gut contents in terms of dry matter

The above approach measures the contents of the digestive tract in terms of indigestible residues. This is a biased estimate of the space occupied by feed, since a meal when first ingested contributes the whole of its weight to the gut contents, not merely the weight of potential indigestible material that it contains. As digestion proceeds so the weight of this single meal falls, until the remainder is expelled as indigestible material. This suggests that the contents of the gut can be estimated in terms of dry matter, here denoted 'fill', by weighting each meal, not only by the length of time it spends inside the animal (the function $1 - R(t)$), but also by the inverse of its digestibility—its indigestibility or $1 - D(t)$.

If one considers a pattern of meals given at times θ which are prior to time t , each meal being given in amount $g(\theta)$ measured in terms of dry matter, then the amount of dry matter in the digestive tract at time t will be

$$K(t) = \int_{-\infty}^t g(\theta) \{1 - R(t - \theta)\} \{1 - D(t - \theta)\} d\theta. \quad (12)$$

This equation states that to find the weight of dry matter the digestive tract contains at a particular time it is necessary to sum all previous intakes of food, each being weighted by two functions the numerical values of which are determined solely by the time which has elapsed between the ingestion of the meal and the time under consideration. Fig. 7 showed the superposition of the function $1 - R(t)$. To find the dry-matter 'fill', each particular value of $1 - R(t)$ is multiplied by the corresponding value of $1 - D(t)$ and by $g(\theta)$.

Table 9. *Estimates of the dry-matter content of the digestive tract of sheep in relation to time after feeding, obtained by application of the discrete form of equation (12) (see above), together with experimental observations made by Dr D. P. Cuthbertson, in which the gut contents were weighed at slaughter*

| Time after feeding (h) | Predicted dry-matter content (g) of whole digestive tract of sheep given: | | Actual dry-matter content of digestive tract determined at slaughter* (g) |
|---------------------------|---|--|---|
| | 1500 g long dried grass | 1500 g medium-ground and cubed dried grass | |
| 0 | 1359 | 1110 | 1860 |
| 2 | 1177 | 919 | 1690 |
| 4 | 1037 | 778 | 1920 |
| 6 | 929 | 668 | 1390 |
| 8 | 844 | 579 | 1510 |
| 10 | 773 | 518 | 1390 |
| 12 | 720 | 465 | 1050 |

* Eighteen sheep were slaughtered to provide these values.

Such calculations were made for the three highest levels of feeding with 0.15 as a value for h in the expression for $1 - D(t)$, and the results given in Table 9 show that the 'fill' of the gut fell with time after feeding, and that the maximal and minimal 'fill' were both greater for the long ration than for the cubed ration. When the experiments were completed, the sheep concerned were given as much of the long dried grass or cubes as they would consume. Appetite failed in sheep given long material when they were consuming 1800 g daily. With both types of cubes, much higher food intakes—up to 2400 g daily—were obtained. These findings suggest that the space-filling attributes of the ration determine appetite to a considerable extent, and that an index of this extent is given by the 'fill' of the digestive tract as measured above.

That the results obtained by a mathematical analysis of passage curves are in accordance with actual results is shown in the last column of Table 9. This column shows the mean results obtained in a slaughter experiment with sheep and were kindly provided by Dr D. P. Cuthbertson of the Rowett Research Institute. The sheep concerned were about 40% heavier than ours (57 kg compared with 40 kg) and they

received more food, including both hay and concentrates. The total dry matter in the digestive tract, however, was also approximately 40% greater than the amounts calculated from the equations for sheep given long dried grass, which further suggests that the method gives meaningful results.

The water content of the faeces

Ewing & Smith (1917) assumed that more rapid passage of food led to an increase in the water content of the faeces. This was certainly true of the present experiments, as shown in Table 10.

The differences due to the effect of grinding increased with increasing nutritional plane. This is understandable if, as is usually accepted, the lower part of the colon is the water-absorbing area of the gut, since at low nutritional levels food residues would spend longer there before expulsion. Clearly, the physical form of the food and the level of feeding have considerable effect on this aspect of the water economy of the animal.

Table 10. *Mean dry-matter content of the faeces of sheep given rations of dried grass*

| Form in which dried grass was given | Amount of dried grass given daily (g) | Mean dry-matter content of faeces (%) |
|-------------------------------------|---------------------------------------|---------------------------------------|
| Long | 600 | 42.3 |
| | 1200 | 38.6 |
| | 1500 | 37.4 |
| Medium-ground and cubed | 600 | 41.6 |
| | 1200 | 39.7 |
| | 1500 | 35.4 |
| Finely ground and cubed | 600 | 41.5 |
| | 1200 | 33.7 |
| | 1500 | 31.2 |

DISCUSSION

The results of the experimental study of the effect of processing on the digestibility of dried grass and its passage through the digestive tract, discussed already, show that changes in the physical form of a food can modify its passage through the digestive tract, and that the time relationships of this passage determine its digestibility. The effect of processing on the overall nutritive value of the grass cannot be assessed from the present results, but complete determinations of energy metabolism, which permit such computations, have been made with a further series of sheep given the same three materials (Blaxter & Graham, 1955*b*).

The statistical and mathematical analysis of the data needs some comment, since the approach is rather novel. Firstly, the analysis of the faecal weights shows that end-period errors arising from the irregularity of excretion are a major cause of error in the determination of digestibility coefficients, provided that the analytical methods employed have a high precision.

Analysis of passage curves was undertaken in order to provide information on the rate of production of faeces. The equation describing the passage of food was initially

arrived at from kinetic considerations and it fitted the data well. At this stage it may be regarded as purely descriptive and not necessarily representing underlying physiological mechanisms, since no tests to show that the constants k_1 and k_2 do, in fact, refer to rates of transfer of digesta between the compartments of the tract specified have been made. No assumptions of the physiological validity of its constants are, in fact, necessary in the development of the superposition of the derived functions $1 - R(t)$ and $dR(t)/dt$ which follows. The only assumption made is that the relation between the digestibility of a particle of food and its length of sojourn in the digestive tract is an exponential approach towards a limiting digestibility. This assumption is an approximation, as pointed out on p. 84, but its utility is apparent from the reasonable concordance of the estimates of h given in Table 7.

The results obtained using this approach show that the rate of production of faeces before expulsion usually is not constant, but that it depends on the frequency of feeding and the passage of the food. Foods which pass quickly and are given at infrequent intervals give rise to considerable variation in rate; foods which are given at frequent intervals and pass slowly give relatively stable rates. From this it follows that in the conduct of digestibility trials, frequent feeding at equal intervals of time is desirable, and that a departure from such a regimen, even for a single day, will result in changes in the rate of faeces production and hence errors in the weights of collections of the faeces in subsequent days. The results show also that a preliminary period of 10 days is usually sufficient to annul effects of a previous ration, and to ensure that collections of faeces are representative of the food given.

The determination of the total dry-matter content of the gut from a consideration of passage curves and the time relationships of digestion, indicate that gut 'ballast' and 'fill', and their diurnal variation, can help to explain appetite variation, more particularly the low appetite for low-quality roughages and the high appetite for cubed concentrates. It appears reasonable to suppose that appetite for food will fail at a particular distention of the alimentary tract, of which the dry-matter content or 'fill' is an index. Foods which are highly digestible and which pass rapidly through the tract must necessarily lead to less distension, and consequently a greater intake can be attained. That neither the dry-matter content of foods nor the amount of indigestible residues they produce in a 24 h period is a good measure of their ability to cause satiety in ruminants probably reflects the fact that neither attribute measures the distension of the tract. In accounting for satiety in ruminants, other factors must be considered also. Thus we have already pointed out (Blaxter & Graham, 1955*a*) that appetite in sheep appears to fail before the point of maximal gross energetic efficiency of food utilization is reached. Whether this merely reflects aspects of food passage or is an independent factor is not known.

SUMMARY

1. Eighteen experiments with six sheep were designed to study the effect of the method of preparing dried grass and the level of feeding on its digestibility and its passage through the digestive tract. The same sample of dried grass was given in the

long state, and as two types of cubes, one made from medium-ground, the other from finely ground material.

2. The cubes of dried grass passed through the digestive tract more quickly and had a lower digestibility than the long material. Increasing the feeding level resulted in an increase in the passage of food, and a fall in its digestibility. The latter effect was most marked with the cubes made from the finely ground material. It is concluded that the method of preparation modified the rate of passage of food through the gut and that this rate was the determinant of its digestibility.

3. The results were used to study aspects of the design of digestibility trials and related problems. The error attached to a collection of faeces was found to be almost independent of the length of the collection period and the amount of ration given, and it is concluded that a large part of the variation is referable to an 'end-period' error.

4. From a mathematical analysis of the passage curves in which it was found that a simple kinetic equation fitted the results well, and from the subsequent manipulation of this equation, 'steady-state' equations were obtained which permitted estimation of diurnal variation in faeces production. 'Transient' equations which allowed estimation of the length of preliminary period necessary in digestibility trials were also obtained. These equations showed that frequent feeding at regular intervals would minimize errors in digestibility trials.

5. Analysis of the relation between the digestibility of food and its passage through the gut indicated that the former could be predicted from the latter. The constant describing the time course of digestion showed that about 70% of the digestive process was completed in 10 h for the particular grass used.

6. The amount of indigestible residues ('ballast') and the total amount of dry matter present in the gut ('fill') at various times after feeding were obtained by superposition of functions derived from the equation describing the passage of food. At equal levels of intake the digestive tracts of sheep given long material contained more 'ballast' (indigestible dry matter) and more 'fill' (dry matter) than those of sheep given cubes. These results for 'fill' were compared with those obtained in slaughter experiments, with good agreement. It was concluded that the maximal appetite of animals for food is determined to a considerable extent by the food residues present in their digestive tracts. Animals given highly digestible food which passes through the tract rapidly have larger appetites than those given foods of low digestibility which pass slowly.

In this work we have been greatly helped by Dr H. Ruben of the University of Manchester, who has been concerned with one of us (K. L. B.) in mathematical studies of energy transfer in the dairy cow. Though the results are here expressed graphically, they were, in fact, arrived at algebraically. In this, application of Dr Ruben's finding that Dirac δ -functions and Heaviside's superposition theorem could be used to solve similar problems in the nutrition of the cow was of considerable help.

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Effect of continued administration of cortisone and ACTH on vitamin C levels in guinea-pig adrenals*

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It is well known that an injection of ACTH gives rise to a transient fall in the concentration of adrenal ascorbic acid (Sayers, Sayers, Lewis & Long, 1944); this action forms the basis for a standard assay procedure in which the hormone is administered to hypophysectomized rats (Sayers & Sayers, 1946). Since one important effect of ACTH is to stimulate the production of corticosteroids by the adrenals, it may be asked whether cortisone itself can be shown to have any corresponding action on vitamin C levels, in the adrenals or in other tissues. It was to investigate this question that the experiments to be described were conducted. We have previously found that one common effect of cortisone with ACTH was that, when repeatedly injected, it brought about the remarkable increase in liver weight observed in guinea-pigs and in rabbits (Hughes, Harris, Constable & Bland, 1952; Bland, Constable, Harris &

* Read in part before The Nutrition Society on 7 May 1955 (Constable, Harris & Hughes, 1955).