

## THE USE OF THE NYLON BAG TECHNIQUE FOR THE EVALUATION OF FEEDSTUFFS<sup>1</sup>

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The history of evaluation of the nutritive value of feedstuffs is reviewed, leading into a description of the development of the artificial fibre bag technique. The type of material and construction of the bag are described in detail and preparation of the samples and incubation times are also discussed. Applications of the technique to a study of the rumen processes; degradation of protein supplements; degradation of roughages and forages and application in the Tropics are also discussed.

Key words: Review, nylon bags, ruminal degradability, protein supplements

The history of the development of methods for the assessment of the value of feedstuffs for animal production is a long one. In the early attempts in Europe feeding trials were used, and workers also tried to predict the nutritive value of feedstuffs by the extraction of the "solubles" with water, alkali, ether and alcohol. By the early nineteenth century scientists in several European countries were publishing tables of the nutritive value of feedstuffs, and were developing methods upon which many of our current techniques are based. (For excellent reviews see Tyler 1975; Blaxter 1980).

As knowledge increased, the early methods were modified and developed, in order to improve the reliability with which laboratory techniques could be used to predict nutritive value to the animal. Although highly developed laboratory procedures are now available (for example, acid detergent fibre, and *in vitro* digestion), the modifications which have been introduced have often simply attempted to mimic the *in vivo* processes. For the evaluation of feedstuffs, *in vivo* techniques are nearly always preferred. The use of the artificial fibre bag, which will be discussed here, has the advantage of giving a very rapid estimate of the rate, and extent, of the degradation of the feedstuff in the functioning rumen, without necessitating any procedure more complicated than simple weighing.

Quin et al (1938) used the fibre bag technique to investigate the digestion of feeds in the rumen of cannulated sheep. They used cylindrical bags composed of a very fine natural silk. Subsequent workers have used artificial fibres for the bags (Erwin and Elliston 1959; Johnson 1966; Rodriguez 1968a). The artificial fibre bag (dacron bag, nylon bag, rumen bag) technique, provides a powerful tool for the initial evaluation of feedstuffs and for improving our understanding of the processes of degradation which occur within the rumen. This paper will describe the techniques we are currently using at the Rowett Institute and will suggest some ways in which we think the technique could usefully be employed in the tropics. However, it must be remembered that the technique has limitations as well as advantages.

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There are three important limitations. Firstly, since the sample is confined within the bag it is not exposed to any breakdown due to chewing and rumination. Secondly food would normally be able to leave the rumen once broken down to a suitable particle size. Thirdly, it must be remembered that, strictly speaking, what is actually measured is the breakdown of material to a size small enough to leave the bag and not necessarily a complete degradation to simple chemical compounds. The results must therefore be treated with due caution, and, in general, be used as qualitative indicators of general principles.

With these reservations, there are examples of ways in which the technique can be used to make measurements of a more quantitative nature. One example is the technique developed at the Rowett Institute for the study of protein degradation in, and outflow from, the rumen (Ørskov and McDonald 1979). This technique will be discussed in more detail in a later section of the paper. We propose first to describe the methodology.

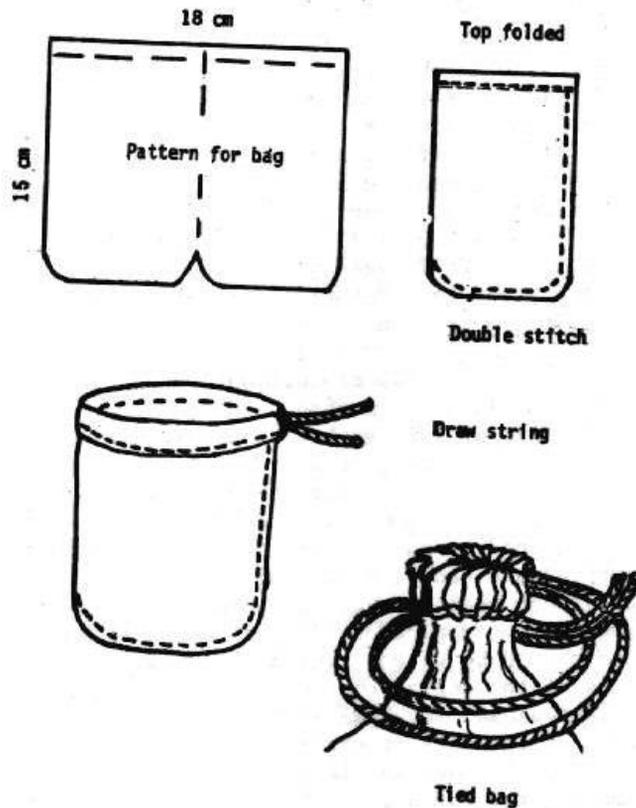
### Materials and Methodology

Fibre bags: Various materials have been used in the construction of the bags. Quin et al (1938), as already mentioned, utilized fine silk, while Schoeman et al (1972) and Mehrez and Ørskov (1977) used Dacron material obtained from an old parachute. Early workers rightly placed a great deal of emphasis on the pore size of the bag material, since this regulates the passage of solid particles from the bags. Rodriguez (1968a) reported that bag materials with 1680, 2303 and 2550 holes/cm gave similar values for the disappearance of dry matter from the bags. Materials with pores of 20 $\mu$  and 35 $\mu$  have been found to give smaller dry matter losses than from bag materials with 53 $\mu$  pores (Uden et al 1974), and Van Miellen and Ellis (1977) considered 10 $\mu$  to be the maximum pore size if loss of solids were to be prevented. HS013 (nylon filter cloth currently being used at the Rowett Institute has a pore size of 12 $\mu$  square. The important point is that the same material should be used in any one trial, and that the bags should be well washed between trials - in order to ensure the pores are cleaned out. If possible the bags should be checked periodically under a microscope (to examine the pores), and the type of material used should be described.

The optimum size of bag has been investigated by a number of workers (Rodriguez 1968a; Mehrez 1976). The optimum size is essentially a compromise between the two opposing factors. On the one hand the necessity to have the bag large enough relative to the sample size used, so as to ensure that rumen fluid can easily enter the bag and mix with the sample. On the other hand there is the necessity to have a bag small enough to be easily withdrawn through the rumen cannula. The effect of sample size will be discussed later on, but we use a bag of about 140 x 90 mm when laid flat. The bottom corners should be rounded (so as to prevent any of the sample being trapped), and the bag can be closed either by tying or with a simple draw string (Figure 1). These bags can easily be withdrawn through a cannula of 40 mm internal diameter, and with care through a cannula of 30 mm internal diameter (the former dimension is preferred).

The material for the bags is cut out using a soldering iron (this prevents fraying) and is sewn with a double line of sewing (Figure 1) using a polyester thread. If necessary the bag can be colour coded with a nylon tag or draw string.

Figure 1:  
Nylon bag as now used at the Rowett Institute (Ceagana design)



*Treatment and preparation of samples:* Preparation of the samples for incubation is critical as they should represent, as far as possible, the materials as they would appear in the rumen had they been consumed by the animal. Ideally, masticated ingesta from animals fitted with an oesophageal cannula can be collected (Bailey 1962), but in practice the use of a laboratory hammermill fitted with a 2.5 - 3.0 mm screen for dry feeds is adequate. Alternative methods for the reduction of particle size, such as chopping, cutting, rolling and grinding, may have to be utilized if the above mentioned methods prove to be unsuitable.

Fresh forages and succulent feeds present problems, particularly when attempting to describe in a quantitative way the form of sample preparation. Minor and Hovell (1979) found the method of preparation of banana leaf to be important. These workers used a domestic homogeniser to break the structure of the leaf open. The procedure used was to cut the leaf into 20-30 mm squares. Small quantities of cut leaf were then homogenised for 20 - 30 seconds - sufficient to break the structure of the leaf open, but not to pulp the leaf into a puree. A similar technique was used with fresh grasses and other forages, and with experience a sample of uniform appearance can be produced. Any juice expelled is soaked up by the fragmented forage. The preparation of resin forage is an area that requires further work for it is difficult to describe quantitatively, and the effect of degree of fragmentation has not been measured.

Whatever procedure is chosen it is important that a consistent method is established within each laboratory.

Comminution of dry feeds is rather easier to define (by type of mill and screen size). Erwin and Elliston (1959) found that the fineness of grinding (not defined) of the sample had less effect on the disappearance of dry matter as the period of incubation was increased. This is to be expected, since a decrease in the particle size will increase the surface area per unit weight of substrate. This increase may affect the initial rate of degradation, but not necessarily the final extent of degradation. Van Keuren and Heinemann (1962) found no differences between samples (dried grasses, clover and alfalfa) when ground through a 20, 40 or 60 mesh screen (Wiley Mill). Lawrey (1969) found no differences in dry matter losses with forages ground through 4, 3, 2 or 1 mm screen sizes, although passive losses of materials through the pores of the bag occur with the use of 1 mm screen (Payne et al 1972). However, such losses are easily corrected for, as will be described later.

Since the relative rate of degradation of different samples is important information, the method used within a laboratory must be standardised, and be as well described as possible. The sample incubated should be reasonably homogeneous - Mehrez (1976) found that he improved the precision of his data (using rolled barley) if he removed any uncracked grains from the sample. The way in which the sample is prepared may be defined by the purpose of the incubation. For example, protein supplements can have very different particle sizes as with fishmeal and soyabean meal. If the purpose of the study is to compare the degradation of the supplements as fed to the animal, then incubation of the samples should be without further processing. If, on the other hand the study is of the degradation of the protein from these two sources, then the fishmeal and soyabean meal should be further processed so as to eliminate differences in particle size.

*Sample size:* A reduction in degradability was observed by many workers as the sample size, for a given bag size, was increased (for example Tomlin et al 1967; Mehrez and Ørskov 1977. The smallest amount of sample necessary may be defined as that which will provide adequate material for analysis after incubation (for example, for Nitrogen), or possibly by the precision of the balances available for weighing the bag and sample. The amount of sample incubated in the bag will also depend on the bulk density of the prepared sample. We have found generally that about 2 g air dry ground straw, 3 g good hay or dried grass, 5 g of concentrate (e.g. barley, protein supplements) and 10 - 15 g of fresh herbage are suitable for the size of bag that we use.

*Position in the rumen:* Balch and Johnson (1950) reported that a more rapid digestion was obtained when the bags were incubated in the ventral rumen sac of cattle, although later work by Erwin and Elliston (1959) and Rodriguez (1968b) showed that the position of bags in the rumen had little or no effect on the degradation of the various feeds. No reduction in the variability in DM disappearance between bags has been shown by attaching weights so as to anchor the bags in the ventral sac of the rumen, but Rodriguez (1968b) found that variation between bags was reduced when the bags were attached to 50 cm of string rather than to 30 cm. He suggested that the longer string allowed greater movement of the bags within the rumen of the steer, and thus minimised the effects of variations in the rumen environment.

*Incubation time:* Much of the published data relate to experiments in which the workers tended to incubate bags for only a few different times, and attempted to relate dry matter losses from the bags to the apparent digestibility of the feedstuff. We are now more interested in measuring the rate of degradation, which requires a number of measurements of degradation after different times. The total time for complete degradation will vary with the material being incubated, and hence the intermediate times chosen will also vary. As a rough guide: concentrates require 12-36 hours, good quality forages 24-60 hours, poor quality roughages 48-72 hours. These are the times required to reach, or nearly reach, the asymptote (potential degradation).

*Replication:* Mehrez and Ørskov (1977) found that the greatest source of variation in the disappearance of DM from the bags was the between sheep component (6.2% of the mean), followed by that of between days (4.9%). The least variation was found between the bags (3.3%) incubated together and withdrawn at the same time. They suggested that the use of one bag, two days (i.e. a repeat measurement) and three sheep was a reasonable combination. The variance of the mean was given by 
$$\frac{V_B + (b + V_D) + (b \times d \times V_S)}{b \times d \times s}$$
 when  $V_B$ ,  $V_D$ ,  $V_S$  and  $b$ ,  $d$  and  $s$  were the percent

variation of the means, and number of bags, days and sheep respectively. If the number of sheep used was reduced to two, this increased variation to a greater extent than could be compensated for by increasing the number of bags to four, as is shown in Table 1, which was calculated from their values for  $V_B$ ,  $V_D$  and  $V_S$  given above.

Table 1:

Estimated variance of the mean dry matter disappearance using the rumen bag technique for various numbers of bags (b), days (d) and sheep (s) (Mehrez and Ørskov 1977)

Replicates			n* (b x d x s)	Variance of Mean (% of mean)
bags	days	sheep		
1	2	3	6	3.43
2	1	3	6	4.25
4	1	2	8	5.96
2	2	2	8	4.74
1	2	4	8	3.19
4	2	2	16	4.53

\*n is the number of incubations for each time

*Number of bags incubated:* In cattle which generally can have much larger rumen cannulae than sheep, the number of bags incubated at one time can be greater than with sheep (12, Balch and Johnson 1950; 20, Miles 1951). With sheep, Mehrez and Ørskov (1977) found it preferable to incubate no more than five bags in the rumen at the same time, in order to avoid the difficulties in their removal from the rumen. We now use up to nine bags in sheep, since most of the cannulae are now of 40 mm internal diameter. We have assumed that, up to this point, the main constraint is the removal of bags from the rumen, and not interaction between bags within the rumen.

The tendency for bags to clump together can be minimised by introducing the bags individually and slightly varying the length of string allowed free, or by tying the bags in a line.

*Diet of the animal:* The diet can have a pronounced effect on the rate of degradation of the material being incubated; for example, animals given diets with a high proportion of concentrate will have reduced cellulolytic activity in the rumen. The diet chosen for the animal used will obviously depend on the purpose of the experiment, as will be shown in the section on applications of the technique.

*Incubation procedure used at the Rowett Institute:* The bags are made from nylon filter cloth (HS 013, from Henry Simon Ltd., Special Products Division, P O Box 31, Stockport, Cheshire, England SK3 0RT) as already described. They are then tied closed either with the draw-string or by the use of polypropylene twine. More than one bag may be attached to the same string, as long as they are spaced to avoid interference, and the top bag is at least 25 cm from the cannula top in sheep, and 40 cm in cattle. The string(s) is (are) then attached to a wire loop inserted through the cannula top and the bags are pushed well into the rumen. Identification of the bags on removal is often difficult, and is aided by colour coding of the strings. On removal from the rumen, the tied bag is held by the neck and vigorously shaken in a bucket of water. The tie string is then cut (or drawstring loosened) in order to clear debris trapped by the folded material, and the bag and contents rinsed under running water until the wash water is clear.

The bag material should be cleaned by rubbing between the finger and thumb. (A good guide to the sample and bag being clean is when the water from a slowly running tap will run out of the bag as quickly as it enters.) The bags are then dried to a constant weight at 60-70°C, and percent dry matter loss calculated.

Part of the weight loss may be due to a simple solubilisation of constituents of the sample, and also to the loss of very fine particulate material which can be removed by washing. This is very easily corrected for by preparing additional samples and soaking these control bags in water, and then washing and drying normally. This is important, for we have found losses of up to 20% with dried grass and finely ground sugar cane bagasse, and up to 60% from sugar cane. The bags are then thoroughly washed (in soap and water) and inspected for tears or holes before re-use.

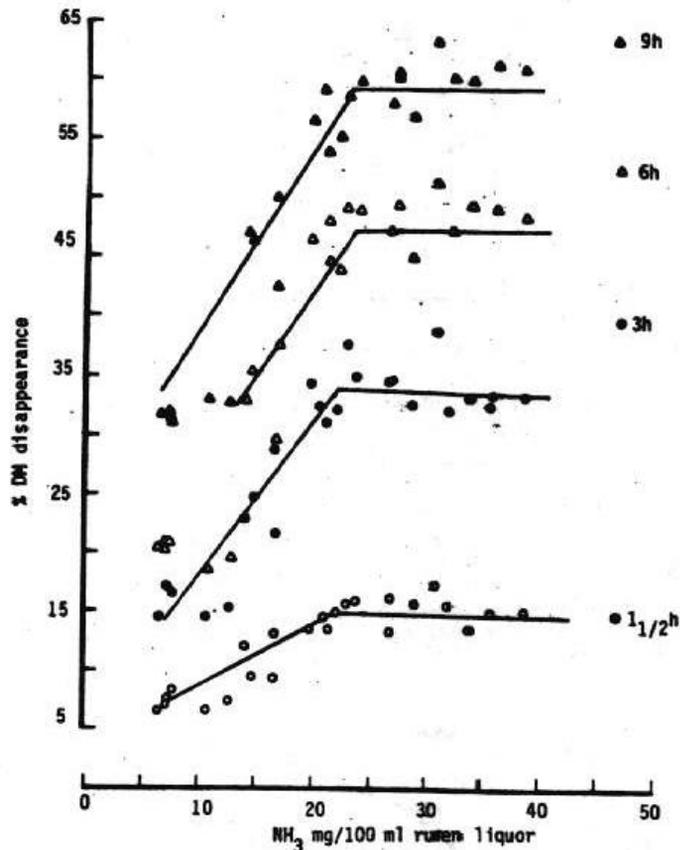
### Applications of the technique

The rumen bag may be used to explore many features of the degradation processes that occur within the rumen. Not only is it a powerful tool for indexing the relative degradabilities of feedstuffs, it may also be used to improve our understanding of the processes of rumen fermentation.

*Study of rumen processes:* It is possible to vary the factors within the bag, or within the rumen. Thus the animal can be fed a constant diet, and the effect of manipulating the feedstuff incubated in the bag can be studied (type of feedstuff, or the effect of processing or special treatments of the feed). Alternatively, the conditions within the rumen can be varied (basal diet offered to the animal, and conditions within the rumen), and a standard material incubated in the rumen in order to study the effect of these factors on the rates of degradation. Some examples of these types of application are given below.

Mehrez et al (1977) varied the amount of urea added to a whole barley diet (from 0 to 10 g/kg diet), and then incubated rolled barley grains in bags in the rumen of animals given these diets. They found that the disappearance of dry matter from the bags was greatest and plateaued at rumen ammonia levels of about 24 mg/100 ml rumen fluid (Figure 2), and suggested that levels below this would result in a reduction in the efficiency of fermentation.

Figure 2:  
Effect of DM disappearance of  $\text{NH}_3$  concentrations at 1 1/2 ("), 3 (!), 6 ( $\Delta$ ) and 9 (\*) h of incubation (Mehrez and Ørskov 1977)



Schoeman et al (1972) used the technique to study the effect of formaldehyde on the degradation of a variety of protein samples. They then measured the hydrolysis of the sample (after incubation in the rumen bag) in vitro using acid pepsin and by combining the two measurements were able to derive estimates of the value to the animal of proteins treated with different levels of formaldehyde. Some of their data are summarised by Figure 3. A feature of interest in this work was the additional data provided by the hydrolysis of the incubated residues with pepsin, which showed that with four of the five proteins tested, the amount of protein made available to the animal was improved by treatment with formaldehyde, while for fishmeal the amount was actually reduced.

Figure 3:  
Loss of nitrogen from protein concentrates treated with formaldehyde and incubated in nylon bags in the rumen of sheep (Adapted from Schoeman et al 1972)

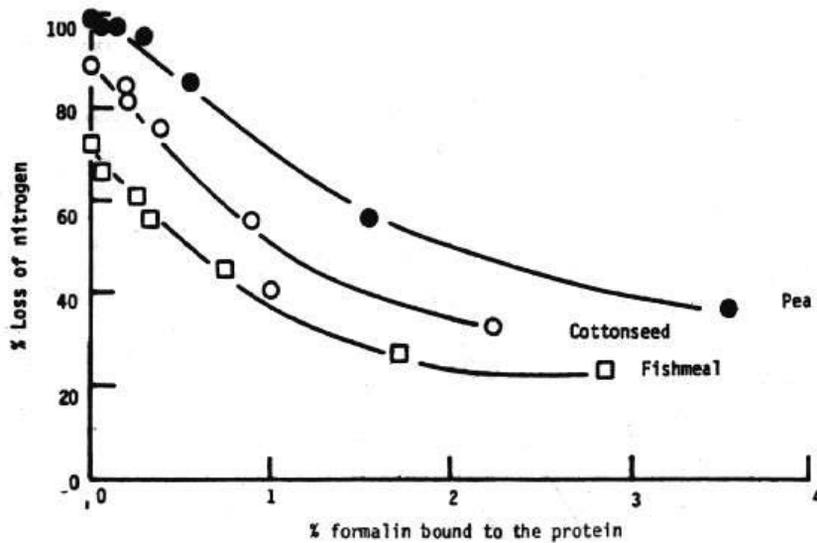
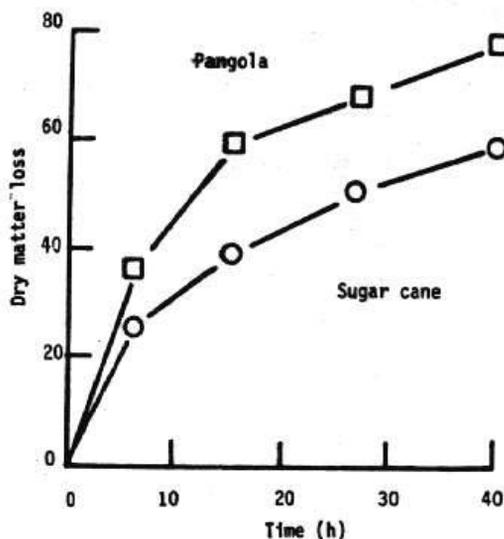


Figure 4:  
The rumen digestion (from nylon bags) of Scottish hay incubated in the rumen of cattle given sugar cane (○) or Pangola hay (□) (Ørskov and Hovell 1978)



Ørskov and Hovell (1978) incubated a sample of Scottish hay in the rumen of cattle given either pangola hay or sugar cane, and showed degradation of the hay to be less in the rumen of the cane fed animals (Figure 4)

Ørskov et al (1978) studied the effect of fat on rumen digestion. It was known that fat reduced fibre digestion (Brooks et al 1954), and the rate of cellulose digestion (Kowalczyk et al 1977). What was uncertain was whether the cause was due to an

inhibition of the microbial population, or a protective coating being formed on the fibre. Ørskov et al (1978) sprayed tallow onto dried grass and then either gave sheep the tallow mixture as their diet and incubated dried grass samples in the rumen, or gave the animals untreated grass and incubated the fat treated grass in the rumen. The results, given in Table 2, demonstrate the main effect of the fat to be on the microbial population in the rumen and on cellulolytic activity, and not due to the protective coating of the fat on the grass.

Table 2:  
The effect of fat (tallow) on the disappearance of grass dry matter from samples incubated in the rumen of sheep

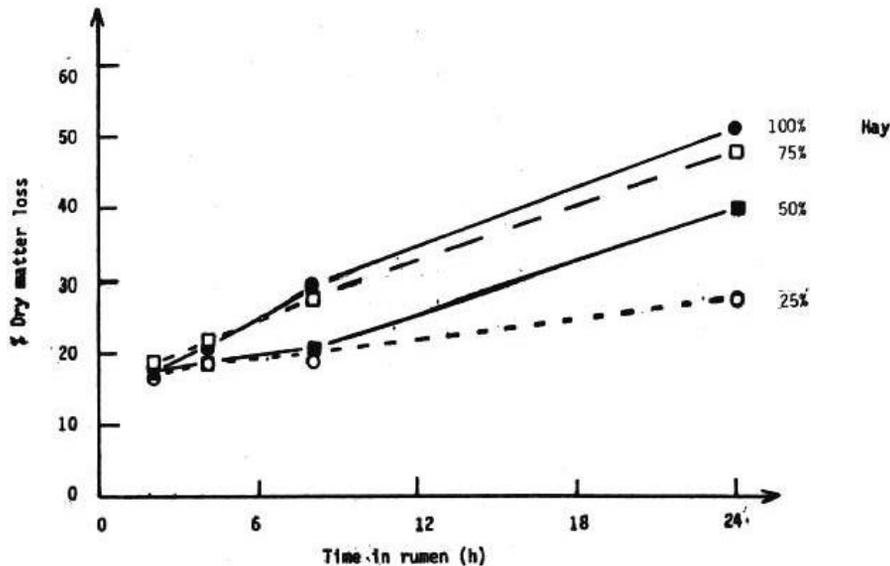
Level of fat (g/kg grass)	Loss of dried grass dry matter (mg/g) from 6-24 hr	
	A	B
0	324	327
50	331	329
100	320	314
150	334	255
SE mean	17	30

A: animals given untreated grass, treated grass incubated

B: animals given treated grass, untreated grass incubated

We currently have a programme of investigations at the Rowett Institute to use the rumen bag technique to study cellulose digestion. It is well known (Belch and Campling 1962) that the rate of digestion of cellulosic material is an important factor affecting the voluntary food intake. It is therefore important to determine whether the rate of cellulose digestion is normal for the fibre in question, or whether it could be increased by some manipulation of the diet. The nylon bag technique provides an excellent tool for such studies. It is known for instance, that the amount of starchy feed given can seriously inhibit the rate of cellulose digestion. (see Ørskov 1976). An example of such inhibition is illustrated from results of Mould (unpublished observations), where the rate of disappearance of hay from the nylon bags was compared when the animals were fed on hay alone, or on diets consisting of hay and either 25 or 75% of a processed concentrate. It can be clearly seen (Figure 5) that the rate of hay digestion is well below the potential, particularly when 75% was given. Having identified the problem, it is then possible to seek the solutions. For example, by measuring the extent to which the inhibition of the rate of cellulose digestion can be minimised by maintaining a higher rumen pH, by changing the frequency of feeding the concentrate, or by decreasing the rate of solubilization of the concentrate by changing its method of processing.

Figure 5:  
Effect of supplementing a hay diet with pelleted barley on DM disappearance of ground hay from nylon bags (Mould, unpublished observations)



Degradation of protein supplements: When coupled with estimations of turnover rates of rumen digesta, the rumen bag technique also offers the possibility of obtaining quantitative estimates of the true degradability within the rumen, and has been applied with some success at the Rowett Institute with protein-rich concentrates (see Ørskov and McDonald 1979).

In the first stage, a variety of protein sources were incubated in the rumen of sheep and cattle, and the degradation curves as percentage losses of dry matter and nitrogen plotted as the dependent variable, and time in hours as the independent variable (Figure 6). In this case, the percentage of material degraded 'p' after a time 't' hours may be described by the equation:

$$p = a + b(1 - e^{-ct}) \quad (1)$$

when a, b and c are constants (Ørskov and McDonald 1979).

Thus we have several terms:

p = the actual degradation after time 't'

a = the intercept of the degradation curve at time zero.

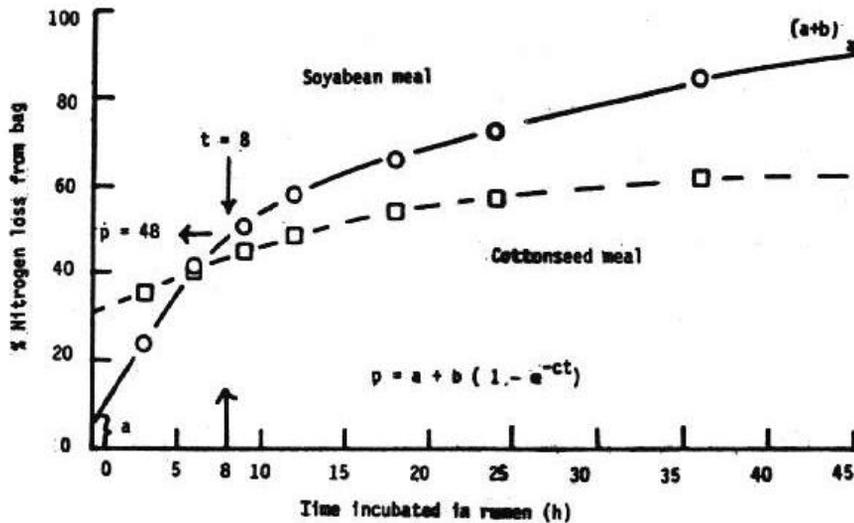
This represents the component of the protein degraded rapidly relative to the degradation of the component described by  $b(1 - e^{-ct})$ .

b = the potential degradability of the component of the protein which will, in time, be degraded.

c = the rate constant for the degradation of 'b'.

The total degradability of the sample is given by a + b which obviously cannot exceed 100. It follows that  $100 - (a+b)$  represents the fraction which will appear to be undegradable in the rumen. Some typical degradation curves for proteins are demonstrated in Figure 6.

Figure 6:  
Loss of nitrogen of protein supplements incubated nylon bags in the rumen of sheep  
(Hughes-Jones 1979)



If 'a' is positive, then there is a component which is degraded rapidly and/or a component which is soluble, or fine enough to escape from the bags simply by soaking and washing. Whether 'a' represents rapid degradation, or simply washing losses, can be determined with control bags which are simply soaked in water and then washed and dried in the normal way. When a negative value for 'a' is obtained this means that there has to be an initiation period for degradation to start (termed the lag phase).

Once an estimate of degradation rate has been made, then it can be linked to an estimate of outflow from the rumen - with the assumption that the particle size of the protein supplement is such that further diminution of particle size will have little or no influence on the outflow rate (Ørskov and McDonald 1979). The procedure is as follows: A sample of the supplement is made undegradable using sodium dichromate (Ganev et al 1979) and the animal dosed with a known amount of this chromium labelled protein. The outflow of the chromium is estimated in the normal way by taking the rumen samples at intervals and estimating chromium concentration. Then when 'f' is the fraction of chromium remaining in the rumen after time 't' hours, then

$$f = e^{-kt}$$

The effective degradability (P) of the protein can then be calculated from the description of its degradation (equation 1), and from the outflow of the protein from the rumen (equation 2), as:

$$P = a + \frac{bc}{c + k}$$

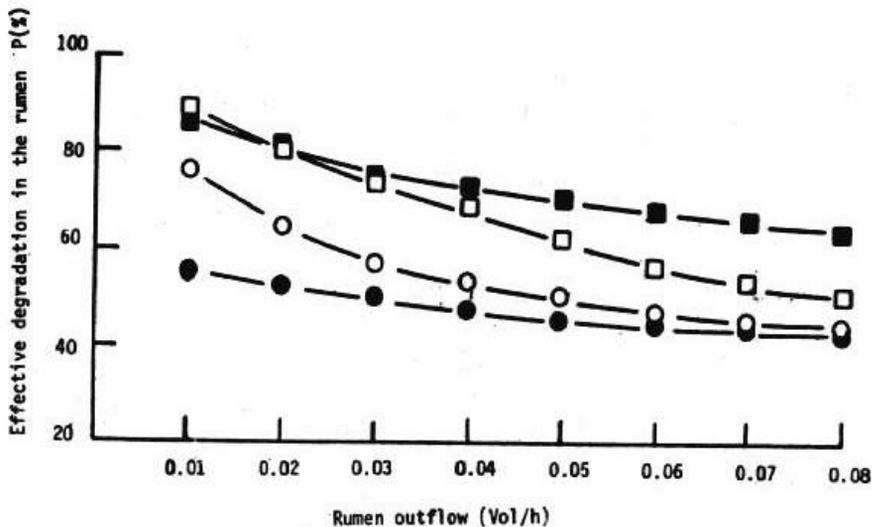
Thus we have three terms:

*The Degradation Rate:* This is the rate at which potentially degradable protein is broken down in the rumen, and is described by 'c'. A half-life for the material which can be degraded is given by  $\frac{\ln 2}{c} = \frac{0.6931}{c}$  hours.

*The Potential Degradability.* This is given by  $a + b$  and represents the amount of protein which can be dissolved and degraded within the rumen given sufficient time.

*The Effective Degradability.* This is given by  $P$  (equation (3)). It represents the amount of the protein which will actually be degraded in the rumen, and is defined by the time for which the protein is present in the rumen. ' $P$ ' is variable, and the lower the turnover rate of the rumen digesta, the greater will be the value of ' $P$ '. The effect of outflow rate on degradability is shown in Figure 7 (Hughes-Jones 1979). It can be seen that the effective degradability ' $P$ ' can vary enormously as outflow from the rumen changes. The proportion of the protein available for post-ruminal digestion is given by  $100 - P\%$  and it should be remembered that this may not necessarily be completely digestible by the animal.

Figure 7: Effective degradation ( $P$ ) of fishmeal ( $\square$ ), cottonseed meal ( $\blacksquare$ ), soyabean meal ( $\circ$ ) and meat and bone meal ( $\bullet$ ) corrected for rumen outflow (Hughes-Jones 1979)



The constants  $a$ ,  $b$  and  $c$  are calculated by an iterative least squares procedure which requires a small computer (Ørskov and McDonald 1979). However a good approximation of the value may be arrived at by fitting a curve by eye to the data points, and calculating the constants  $a$ ,  $b$  and  $c$  by simple algebra. Four examples are given in Figures 6 and 8 and Table 3. Data from actual experiments were used and a comparison made between the computer calculated values.

Thus when  $p = a + b(1 - e^{-ct})$

then:  $e^{-ct} = \frac{a + b - p}{b}$

The process for calculating the values is then as follows:

1) Fit a curve by eye in order to obtain the intercept, ' $a$ ' (by extrapolation to time 0). The value from the soya curve was estimated as 6%.

Table 3:

Comparison for the values of a, b and c (see text page 204) calculated by least squares and estimated from hand drawn curves fitted by eye (Figures 6 and 8).

	Calculated by least squares†			Estimates values (see text)			Values used to calculate c estimated from Figures 6 and 8)		
	a ± SE	b ± SE	c ± SE	a	b	c	a+b	t <sub>1</sub>	p <sub>1</sub>
Soya bean meal ‡	9.5 ± 5.2	77.8 ± 4.5	0.080 ± 0.015	6	86	0.084	92	8	48
Cotton seed meal 1	29.2 ± 0.9	36.5 ± 0.9	0.063 ± 0.006	30	33	0.063	63	8	43
Sugar cane bagasse 2	2.6 ± 9.0	48.6 ± 7.9	0.049 ± 0.012	4	47	0.048	51	20	33
Sugar cane bagasse (Na OH) *2	-25.7 ± 14.8	117.8 ± 12.6	0.064 ± 0.009	-17	105	0.027	88	20	44

† See Ørskov and McDonald (1979)

‡ Value P<sub>1</sub> at time t<sub>1</sub> estimated from curve fitted by eye

\* Sugar cane bagasse created with 8% NaOH

1 Data from Hughes-Jones 1979)

2 Dee. from Hovell et al (1980)

2) Select a time 't', at a point on the curve where values for p are still changing rapidly with time. (This is the most sensitive area of the curve and will give the best estimate of c). We chose a time t, of 8 hours for the soya curve. We then estimated that at 8 hours, the degradability p was 48% (from the degradation curve fitted by eye).

3) The asymptote a + b (the maximum digestibility of the soya) was estimated to be 92%.

4) From 2 and 3 above

$$a = 6 \text{ (from 2)}$$

$$a + b = 92 \text{ (from 3)}$$

Then

$$b = 86$$

5) Thus, at a time t, of 8 hours,

$$e^{-8c} = \frac{6 + 86 - 48}{86} = 0.5116$$

$$\text{the natural logarithm of } 0.5116 = -0.6702$$

Then,

$$-8c = -0.6702$$

$$8c = 0.6702$$

$$c = \frac{0.6702}{8} = 0.0838$$

The value calculated by the computer was 0.0796 + 0.0150 and thus our 'estimated' value compares well with the value calculated by computer. The natural logarithm of a number may be calculated by multiplying the logarithm to base 10 by 2.3026.

*Degradation of roughages and forages:* The same general principles as described above can be applied to the study of the degradation of forages within the rumen. The simplified model used for the protein supplement (namely that the protein consists of three components, 'a' 'b' and  $1-(a+b)$ ) may not always apply. There is however, a considerable amount of very useful information which can be gained, even if only by tabulating the amount of material degraded after two or three time intervals. Some forages may contain very considerable amounts of a water soluble component (for example the sugar in sugar cane), and the inclusion of a control in the form of bags containing a sample simply soaked in water and washed will help in interpretation of the data.

An example is given by the data on sugar cane bagasse in Figure 8. Figure 9 shows the same data corrected for the very fine material which could be washed out of the bag. This was an average of 20.7% with the untreated bagasse, and 3.9% with the treated bagasse. The degradation curves are obviously of the same shape, and the intercepts of the corrected curves demonstrate clear lag phases. The figures also show how the asymptote was reached after about 50 hours. Washing losses due to soluble material or very fine material may be distinguished by soaking a sample in water, then filtering, using a filter paper.

The rumen bag technique has been used for many years to study the degradation of forages (Fine et al 1958; Hopson et al 1963). Generally there was an attempt to correlate the values obtained (usually after a set period) with measurements obtained from digestibility trials and also from a variety of laboratory techniques (Neathery 1972). However, the digestibility of a feedstuff is defined by the potential degradability of the material, the rate of degradation of this potentially degradable fraction, and its residence time in the rumen (ie its effective degradation) plus digestion after the rumen fermentation in the hind gut.

Figure 8:  
Loss of dry matter from samples of sugar cane bagasse incubated in nylon bags in the rumen of sheep (Hovell et al 1980)

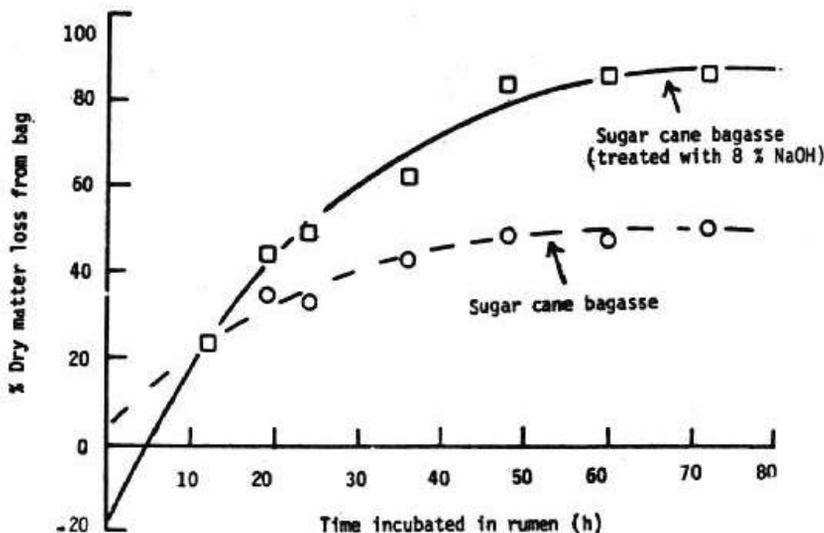
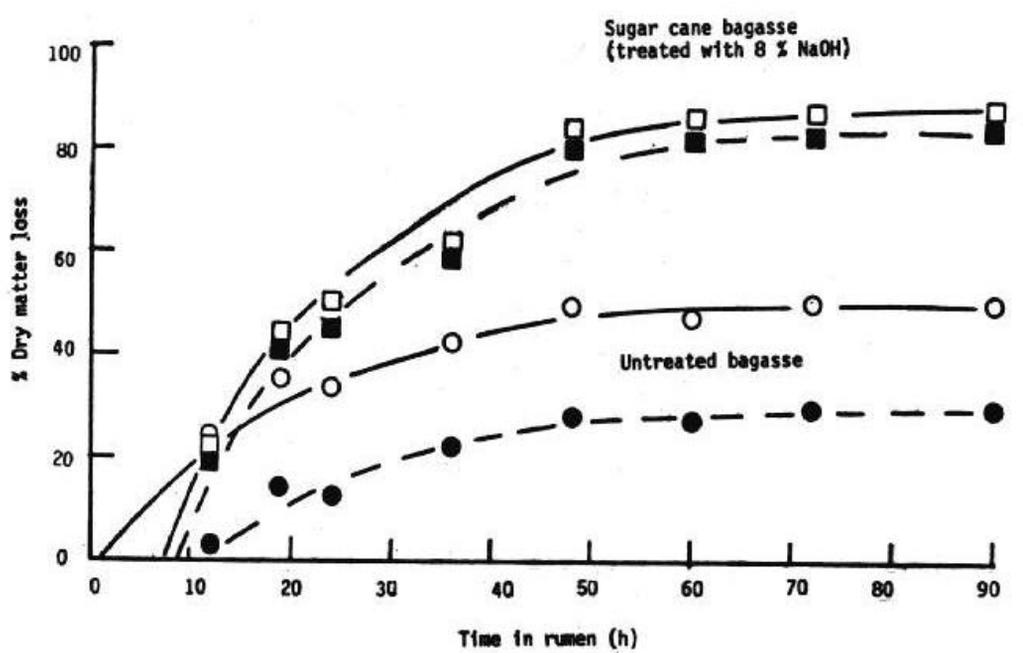


Figure 9:  
 Degradation of sugar cane bagasse. Losses uncorrected (—) or corrected for washing losses (- - -) (Hovell et al 1980)



Cellulosic materials have to be degraded by micro-organisms, which occur largely in the rumen, and as with proteins the outflow rate from the rumen will determine what the effective degradation will be. The effective degradation may not be the full potential degradation, although with cellulosic materials degradation is probably rapid relative to the possible outflow rate of the non-degradable fraction. This is because the non-degradable fraction normally has to be broken down physically to a size small enough so as to be able to leave the rumen. However, it is better to attempt to define some form of degradation curve for the material under test, and to make comparisons in this way. The importance of a standard method of sample preparation must again be stressed.

Dried samples have the advantage that the materials can be stored preparation for testing is easier, and repeat determinations can be made with the same material. However the process of drying, whether in the sun or oven, does make changes to the material - in particular to the more rapidly degraded carbohydrates, and possibly to some proteins as well. Some examples of trials using fresh material (carried out in the Dominican Republic) are illustrated by Figures 10 and 11. These are from the data of Santana and Hovell (1979). In Figure 10 the degradation of the leaves of sweet potato and of banana is compared (three trials with sweet potato and two with banana). Clearly the degradation of the sweet potato leaf is much more rapid and more complete than that of banana. With the degradation of the stems however (Figure 11), differences between sweet potato and banana were far less apparent.

Figure 10:  
 Degradation of fresh sweet potato leaves ( $\circ$   $\square$   $\nabla$ ) and fresh banana leaves ( $\bullet$   $\blacksquare$ ) incubated in the rumen of cattle. (Different symbols represent different trials) (Santana and Hovell 1979)

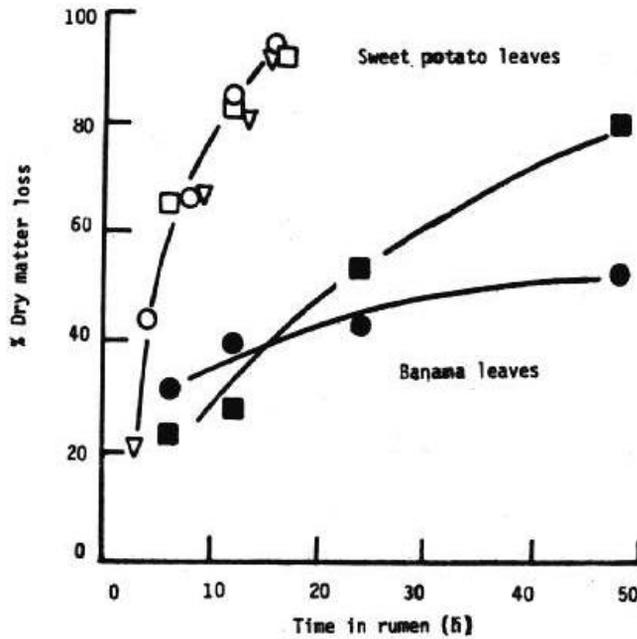
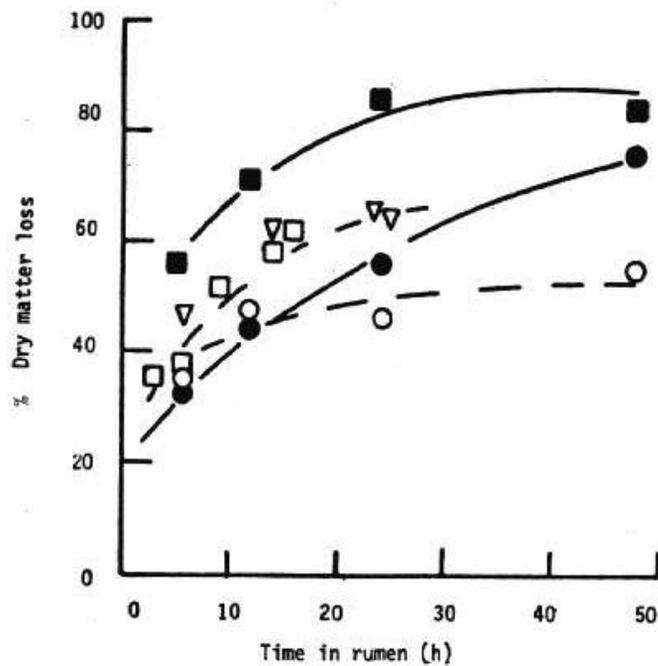


Figure 11:  
 Degradation of fresh sweet potato ( $\circ$   $\square$   $\nabla$ ) and fresh banana stems ( $\bullet$   $\blacksquare$ ) incubated in the rumen of cattle. (Different symbols represent different trials) (Santana and Hovell 1979)



The samples were prepared with a domestic homogeniser, as described earlier, and some of the variation between trials may have been due to slightly different standards of preparation. Even so, these were very simple trials provided a great deal of information about the relative degradabilities of these two plants, and of the relative values of the leaves and stems.

*Application of the technique in the Tropics:* The simplicity of the technique as well as its direct relevance to the value of the material under test make the rumen bag technique a very powerful tool. Rumen fistulation is not a difficult technique, and simple materials may be used to make the cannula (Rowe 1979).

*Degradation of protein supplements:* Proteins are a particularly valuable resource, and it is important that they are used to best effect. Information as to their relative degradabilities and outflow rates from the rumen when used in tropical diets is required. Accumulation of this type of information will ensure that protein supplements are allocated on the basis of their flow rate from the rumen with a given type of diet. The consistency of the quality of protein supplements also needs to be checked (for example quality be affected by heat damage during preparation).

*Evaluation of forages:* A great deal of information is required on forage. Little is known of the relative degradabilities of the wide range of tropical forages and browses available or potentially available. Information on the degradabilities of different forages, of the variation between species and varieties, of the differences between different parts of the plant and the effect of maturity on degradability, will help towards a better understanding of the potential value of the forages, and their selection. For example, the very low degradability of sugar cane bagasse (Figure 9) suggests that the low and slow degradability of this material is an important constraint in limiting the voluntary intake of sugar cane by cattle. The high degradability of sweet potato suggests that this will be a very good material. An exciting development would be if it were possible to link forage degradability to rumen outflow of undegraded materials. The approach may not be the same as that described for protein concentrates since the forages have to be degraded and comminuted to a size small enough to permit outflow. Unfortunately all markers in use at the moment have characteristics which raise doubts as to their suitability for this purpose.

*Evaluation of By-Products and feedstuff processing:* The technique is very well suited for the initial evaluation of any by-products which could become available. It can also test the effectiveness of any physical, biological or chemical processing which might improve the value of the product.

*Understanding of rumen processes:* The rumen bag technique is particularly effective in this role and will have a valuable part to play in unravelling the most important constraints in tropical feeding systems, and elucidating the effect of and sugar dietary constraints and interactions. Some possible areas are, rapidly degradable carbohydrates (for example those in molasses cane), urea supplementation or forages low in nitrogen, interactions between protein supplements and basal diets, and the relationships between degradability, digestibility and voluntary food intake.

The rumen bag technique is not new. What is new is the better recognition of the potential of the technique as a tool in nutritional research. A great deal of very valuable information may be gathered simply by measuring dry matter disappearance. This can in some cases be augmented by also measuring nitrogen losses - however the researcher must remember how easy it is to generate a huge number of samples with this method. It has both simplicity and low cost (once the animals are acquired

and cannulated). It has the potential, provided the limitations are remembered, to improve our understanding of the fermentation processes which occur in the rumen, and the value of feedstuffs as substrates for these processes.

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