THE USE OF NYLON BAGS TO CHARACTERISE THE POTENTIAL DEGRADABILITY OF FEEDS FOR RUMINANTS

T J Kemp ton

Department of Biochemistry and Nutrition, University of New England, Armidale, NSW 2351, Australia

. The nylon bag technique is a simple means of obtaining estimates of potential degradability of supplements and feedstuffs for ruminants. Rate of disappearance of test material from the bags is particularly sensitive to the basal diet or the cannulated animal. Inclusion of values for fractional clearance of undigested food residues from the rumen into calculation of degradability provides estimates of rate of degradation of the various components of the test material which more closely approximate true degradability of the material in the rumen.

Key words: Review, nylon bags, rumen, degradability

The nylon bag technique has been used for many years to provide estimates of the rate and extent of disappearance of feed constituents from the rumen (Quin et al 1938; Rodriguez 1968; Mehrez & 0rskov 1977). Under certain dietary and production conditions, ruminant diets must be supplemented with forms of rumen non-degraded dietary protein (by pass protein) to increase the efficiency of nutrient utilisation and hence production (Kempton et al 1977). Thus there is a need for a technique to quantitate the potential degradability in the rumen of commercially available supplements. Although accuracy of the technique is influenced by certain factors, it provides a relatively simple means of grading supplements in terms of potential degradability.

Factors affecting the accuracy of the nylon bag technique

The technique involves suspending 4 - 6 nylon bags each containing a known weight (5g) of sample, on nylon string in the rumen of sheep or cattle fitted with appropriate rumen cannulae. Bags are removed from the rumen at known intervals over the following 24 to 72 h, depending on the nature of the sample, and then washed under tap water. Bags are oven dried (70^0 for 24 h) and degradability is normally assessed from disappearance of dry matter (DM) and protein from the bag with time Comparison of results from different experiments is complicated to some extent by differences in bag size, porosity of bag material, preparation of test sample and time of incubation in the rumen. The major source of variation however is associated with the composition of the basal diet and level at which it is fed to the animals.

Pore size of the bag material and fineness of grinding: Pore size of the material used for the manufacture of nylon bags apparently has no significant effect on DM disappearance from bags during a 72 h incubation (Rodriguez 1968). Furthermore, there was no detectable effect of particle size of the test material (dried lucerne) on DM disappearance (Rodriguez 1968). In studies in this laboratory, the rate of degradation of lucerne chaff when either chopped (0.5-1cm length) or ground (40 mm sieve) was not significantly different (13 h half time for DM disappearance for both samples, Kempton and Hiscox, unpublished). With grains however, cracking the glumes increases degradability and with protein meals, degradability increases with reduction in particle size (Mohamed and Smith 1977).

Effect of washing: The standard procedure is to wash the bag under tap water until the water is clear, which takes approximately 5 minutes per sample, although the method and time of washing apparently has no appreciable influence on coefficient of variation of DM disappearance (Van Keuren et al 1962; Mehrez and 0rskov 1977).

Diet and between animal variation: The basal diet of cannulated animals has a major effect on DM disappearance. For instance, half time for DM disappearance from rice hulls is considerably less in sheep given a diet of chopped lucerne chaff in comparison with sheep given a diet of liquid molasses and 100 g wheaten chaff (Table 1). There was no significant effect of between sheep variation on DM disappearance in this study, although a small amount of variation was associated with between sheep and between sampling days differences in the studies of Mehrez and 0rskov (1977)

Table 1:
Half time (hours) for disappearance of dry matter from alkali treated rice-hulls when suspended in nylon bags in the rumen of sheep given diets of either chopped lucerne or liquid molasses (Kempton and Gupta, unpublished observation).

Treatment	Diet		Significance of difference between diets
	Lucerne	Molasses	
Rice hulls + 3% NaOH	228	1180	P<0.01
Rice hulls + 3% NH_3	874	3340	P <0.05

0rskov and Hovell (1978) demonstrated similar between diet differences for rate of degradation of chopped hay when placed in the rumen of Zebu cattle given diets of either chopped sugar cane or pangola hay. DM disappearance was 18% lower in bags incubated in the rumen of animals given the chopped cane diet after 40 h incubation. Similarly in sheep, the rate of disappearance of protein from the various plant protein meals was faster when the meals were incubated in the rumen of sheep given a grass based diet as compared with a whole barley diet. The faster rates of degradation on the grass diet would mostly result from faster rumen turnover on the grass diet compared with the straw diet. The rate of disappearance of an animal protein meal (fishmeal) however was not different in sheep given the whole barley or dried grass diet (Ganev et al 1979). It is important therefore that the cannulated animals are given standard rations when used to assess the rates of degradation of most feed materials. Between animal variation can be overcome by replicating the measurements in not less than three animals.

Practical aspects

Rumen cannulae in sheep should be approximately 40 mm in diameter to facilitate insertion and removal of bags. Ideally, a maximum of six bags can be placed in the rumen of sheep at any time, although considerably more can be used in cattle. Bags are suspended in the rumen on nylon string with a free length inside the rumen of between 20-25 cm. The strings often entwine and make removal of the initial bags difficult, although this can be overcome if the strings are covered with thin plastic tubing.

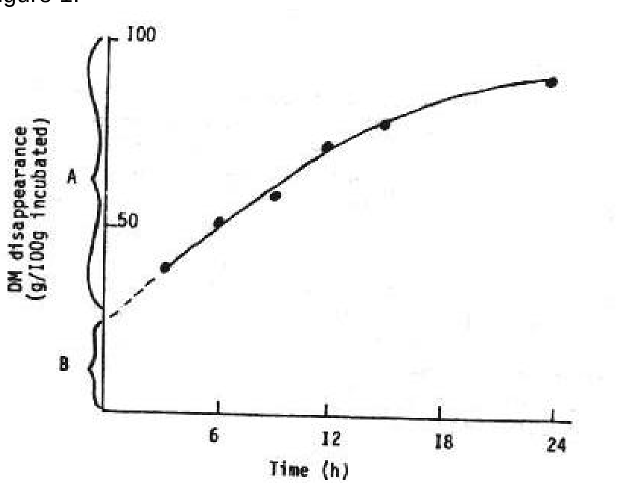
Calculation and interpretation of results

Disappearance of material from the rumen is the sum of material degraded by microbial fermentation and material of suitable particle size washed from the rumen. Disappearance of material from nylon bags over time is therefore an estimate of degradation by microbial activity. The relation.be.tween DM or protein disappearance (D) from bags over time (t) as shown in Figure 1 can be described by the equation:

$$D = A + B\,(1-e^{-ct}) \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 1$$

where A is the proportion of material present in the bag at zero-time, B is the proportion of material immediately soluble in rumen fluid and c is the rate of degradation.

Figure 1:



Disappearance of dry matter from rolled barley suspended in nylon bags in the rumen of sheep given grass based diets, over time (from Mehrez and Ørskov 1977)

Disappearance of DM or protein calculated from this equation is overestimated however because the nylon bag prevents movement of unfermented sample particles from the rumen. 0rskov and McDonald (1979) therefore treated soya bean meal with sodium dichromate (after Uden et al 1979) to render sample completely insoluble in the rumen. Rate of passage of treated meal from the rumen was then calculated by first order dilution principles from rate of disappearance of chromium in samples of rumen contents after a single injection of treated meal. Effective degradation of material at any time (t), which includes rate of passage of undigested residues from the rumen is given by the equation:

$$\text{Effective degradation} = A + \frac{Bc}{c+k}\,(1-e^{-(c+k)t}) \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 2$$

where k is the fractional rate of outflow of meal from the rumen (hours)(after 0rskov and McDonald 1979). Measurements of proportion of soya bean meal disappearing from nylon bags as calculated from the equations given above are given in Table 2.

Table 2:
Percentage disappearance of soya bean meat protein from nylon bags in the rumen of sheep (from Orskov and McDonald

Incubation time (t)	Protein disappearance, %		Effective degradation, (%) **	
	Measured	Fitted *	Restricted feeding	Ad libitum Feeding
3	38	37	36	36
6	51	51	47	46
9	59	62	55	53
15	79	77	64	61
24	89	89	69	65
∞		100	71	66

* calculated from equation 1
** calculated from equation 2

The potential degradability of soya bean meal protein (given by the asymptote at time infinity) was apparently 100% when predicted from equation 1, or 66-71% from equation 2. Therefore, unless a value for fractional clearance of undigested meal from the rumen is included in calculations of degradability, potential degradability after 24 h incubation may be overestimated by at least 20 %. Values for fractional clearance of undegraded residues will vary both with levels of feeding (0rskov and McDonald 1979) and nature of the basal diet given to the animals. To obtain realistic estimates of potential degradability therefore, a measurement of particle turnover in the rumen at the time of nylon bag measurements is necessary. For most purposes however, measurements of liquid turnover in the rumen (using Cr-EDTA as a non-absorbable liquid flow marker) may provide values for rumen turnover suitable to complete the model for rumen degradability of feed materials.

$$DR = Ae^{-ct} \quad \ldots\ldots \quad 3$$

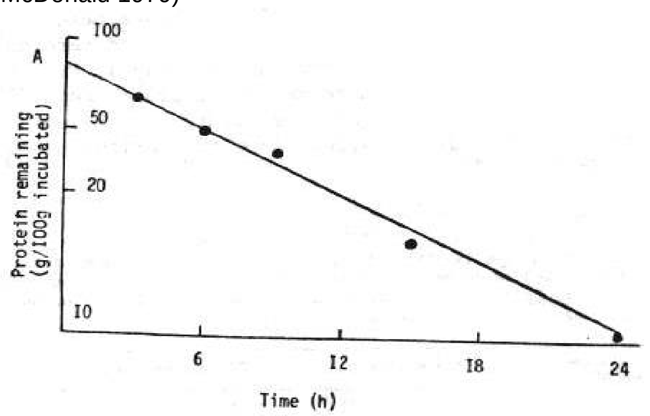
where DR is the proportion of material remaining in the bag at any time (t). The DR-time relationship, plotted on semi-logarithmic co-ordinates (Figure 2) is described by the equation:

$$\ln (DR) = \ln A - ct \quad \ldots\ldots \quad 4$$

In this relationship, A is the proportion (80%) of the material in the bag at zero-time which is degraded at a rate given by the slope (c) of the exponential component. The proportion of material which is readily soluble in rumen fluid (B) is given by 100-A (ie 20%). The soluble component can also be physically determined from the DM loss after washing the material in a nylon bag. Time taken for half the material in the pool described by the exponential component to be degraded ($T_{1/2}$) is given by the equation:

$$T_{1/2}(h) = \frac{0.693}{c} \quad \ldots\ldots \quad 5$$

Figure 2:
Proportion of soya-bean meat protein remaining in the nylon bags, suspended in the rumen over time (from Orskov and McDonald 1979)



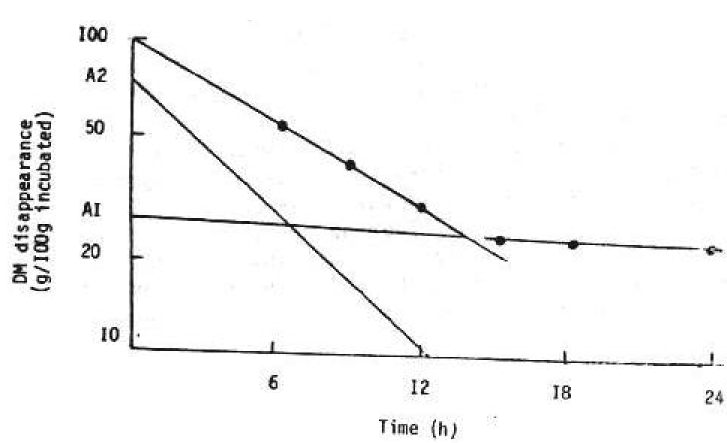
Thus for soya bean meal in Figure 2, 20% of the protein was soluble in rumen fluid and the T1 for disappearance of the remaining 80% of the protein was 8 h. This suggested that the material was completely degraded in the rumen. Some materials however are not completely degraded in the bag such that A+B < 100 and the ln (DR) - time relationship is not linear. This indicates that the material contains several pools of DM or protein which are degraded at different rates, and that material remaining in the bag (DR) is theoretically described by the sum of these components, ie

$$DR = \sum_{i=i}^{n} A_i e^{-cit} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad 6$$

where n is the number of components and i is the component identification.

For example, disappearance of DM in rolled barley(from Mehrez and Orskov 1977) when plotted on semi-logarithmic co-ordinates suggests there are two identifiable pools of DM with different rates of degradation (Figure 3).

Figure 3:
Proportion of dry matter in rolled barley remaining in nylon bag when suspended in the rumen of sheep given grass based diets over time (calculated from data presented by Mehrez and Orskov 1977)



The more slowly degraded pool (1) is described by the final rectilinear component of the curve from 15 h through 24 h. The proportion of DM in the original material degraded at that rate described by the terminal exponent is given by the zero-time intercept (Al). The more rapidly degraded pool (2) is then described by the exponent from 3 h to 13 h. Since both pools of DM are degraded simultaneously, it is necessary to subtract the terminal exponent from the earlier exponent (a process known as "peeling"). Pool size (A2) and $T_{1/2}$ for DM disappearance from the more rapidly degraded pool (2) is given by the exponent through the peeled values (see Figure 3). Values for pool size and $T_{1/2}$ for DM and protein disappearance from the various pools in rolled barley are given in Table 3.

Table 3:
Pool size (%) and half time (hours) for dry matter and protein disappearance from rolled barley suspended in nylon bag. in the rumen of sheep given a grass based diet. The values were calculated by multi-exponential analysis (equation 6 in the text) from the data presented by Mehrez and Orskov (1977)

Component	Dry matter		Protein	
	Pool size (%)	$T_{l/2}$(h)	Pool size (%)	$T_{l/2}$(h)
1	27	77	33	53.0
2	73	45	88	5.7

These results suggest there are two identifiable pools of DM and protein in rolled barley with measurable rates of degradation and that there was no pool of DM or protein immediately soluble in rumen fluid. That the total pool of protein exceeds 100% suggested considerable contamination of the undigested residues with microbial protein. Only "trace amounts of DAPA" were detected in the sample (Mehrez and Orskov 1977), although the reliability of DAPA as a marker of microbial protein is questionable (Siddons et al 1979). In most other studies however, values for size of the total protein pool in various meals suggest that microbial contamination of undegraded residues is of minor importance.

Application of exponential analysis to estimates of protein disappearance from soya bean meal calculated to include fractional clearance of undegraded residues from the rumen (Table 2) identifies three pools of protein. Apparently 40% of the soya bean meal protein is degraded relatively slowly ($T_{1/2}$ 5.5h), 35% is degraded more rapidly ($T_{1/2}$ 2 5.5 h) and 25% is immediately soluble. By comparison, analysis of estimates of soya bean protein disappearance uncorrected for undigested particle outflow indicates 80% of the protein was degraded at $T_{1/2}$ 8 h, and 20% of the protein was soluble.

Exponential analysis of the relationship between proportion of material remaining in the bag with time therefore identifies the major pools of material with different rates of degradation which are characteristic of that particular supplement under the specified feeding conditions. Inclusion of values for fractional clearance of undigested residues into the calculations would provide estimates of degradability which more closely approximate true degradability of the material in the rumen.

Practical application of the nylon bag technique

The nylon bag technique has been used to provide comparative estimates of degradability of feedstuffs used as supplements or components of the basal diet for ruminants (Table 4).

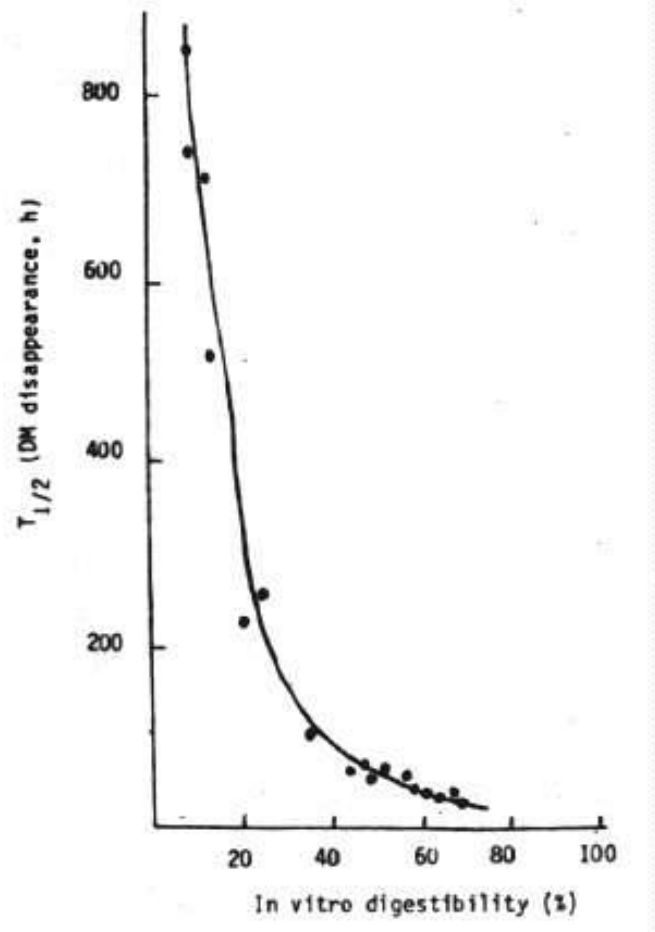
Table 4:
Pool size (%) and half time (hours) for dry matter disappearance of material from that pool for various feedstuffs suspended in nylon bags in the rumen.

Test	Pool I			Pool 2		Soluble Pool	References
	Diet	Pool	$T_{1/2}$	Pool	$T_{1/2}$		
Supplements							
Barley	Grass	73	4.5	27	100	0	Mehrez and Orskov 1977
Soyabean	Barley	70	24.0	-	-	30	
	Grass	70	9.5	-	-	30	Ganev et al 1979
Groundnut	Barley	76	16.0	-	_	24	Ganev et al 1979
	Grass	52	4.5	30	22	18	Ganev et al 1979
Sunflower	Barley	75	32.0	-	-	25	Ganev et al 1979
	Grass	30	5.5	45	54	25	Ganev et al 1979
Fishmeal	Barley	70	29.0	-	-	30	Ganev et al 1979
	Grass	66	54.0	-	-	34	Ganev et al 1979
Cottonseed	Barley Straw	78	13 5				Kempton & Hiscox (unpublished)
Fishmeal	Barley Straw	90	20.5				Kempton & Hiscox (unplublished)
Maize	Barley Straw	60	17.0				Kempton & Hiscox (unpublished)
Sorghum	Barley Straw	70	18.0				
Feedstuffs							
Legume	Pasture	28	55.0				Archer & Kempton (unpublished)
Grass	Pasture	40	65				
Barley	Lucerne chaff	90	157				Kempton & Gupta (unpublished)
Bagasse	Lucerne chaff	82	105				
Lucerne chaff	Lucerne chaff	65	55				
Rice hulls	Lucerne chaff	95	1400				

Supplements in which the major proportion of the DM or protein is undegraded in the rumen would therefore typically have a small soluble pool and a large relatively slowly degraded pool (pool 1). Since pool size and rate of degradation are very sensitive to the dietary condition of the animals, it is necessary to assess the degradability of the supplements in animals given the diet to which this supplement is to be added.

The potential digestibility of feedstuffs by ruminants can be predicted from the half time for DM disappearance of the material in nylon bags. For instance, low quality feeds including rice hulls, bagasse and barley straw were treated with various alkalis and then either placed in nylon bags in the rumen of sheep given lucerne chaff, or incubated in vitro (Tilley and Terry 1963). From the relationship between in vitro digestibility (%) and $T_{1/2}$ DM disappearance (Figure 4), materials with a potential digestibility greater than 40% have a $T_{1/2}$ DM disappearance of less than 50.

Figure 4
Relationship between in vitro digestibility of fibrous material aud the half time for the dry matter disappearance of that material suspended in nylon bags in the rumen of sheep given lucerne chaff (Kempton and Gupta, unpublished observations).



On the basis of the relation in Figure 4, the nylon bag technique has been used to quantitate rate of degradation of clover and grass pastures consumed by grazing lambs. Samples of oesophageal extrusa were collected from lambs grazing either lucerne or phalaris/fescue swards and the material placed in nylon bags in the rumen of animals from which the sample was collected. Since the first bags were not removed until after 19 h incubation, values for analysis of the terminal exponent only are given in Table 4. These preliminary observations suggest there was a considerably larger pool of slowly degraded DM in the grass diet (40%) compared with the legume diet (28%).

Conclusions

The nylon bag technique provides a useful means of evaluating the rate of degradation and potential degradability of feedstuffs and supplements. The technique can be used in field situations to assess the digestibility of forage consumed by grazing animals. The major sources of variation about measurements of degradability are associated with between diet and between animal variation; the between animal variation can be sufficiently reduced by replicating the measurements in not less than three animals. Provided samples are homogeneous, preparation of fibrous dietary material has little effect on degradability. Degradability of protein meals and grains however increases progressively with decrease in particle size. Bag size, porosity of the bag material and and method of washing are easily standardised and of comparatively minor importance. Analysis of the relationship between the proportion of material remaining in the bag with time by compartmental analysis provides information about the size of the identifiable degradable and non-degradable components. The soluble component is also that proportion of the material that can be physically removed from the bag by washing. Inclusion of estimates for the fractional clearance of unfermented residues of the sample from the rumen provide realistic estimates of the degradability of the material in the rumen under the specified {ceding condition.

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Appendix

Recommended nylon bag technique for comparative estimates of degradability of supplements.

1. *Bag*: 15 x 8 cm, uniform pore size, double sewn or heat bonded.

2. *Sample preparation*: homogeneous and ground to a constant particle size (1 mm sieve is adequate)

3. *Sample weight*:
   - low density fibrous material - 3 g
   - protein meals, grains - 5 g

4. *Incubation time*:
   - rapidly degraded meals 8-12 h
   - moderately digested meals and forages 24 h
   - low digestible roughages 48 h

5. *Animals and diets*: 6 bags per animal with not less than 3 animals if using sheep standard diet typical of that which is to be supplemented with the test material

6. *Washing the bag:* 5 minutes under running tap water dry at 70° C

7. *Soluble component*: wash a sample of material in a nylon bag under tap water

8. *Calculation* : plot the disappearance of material - time relationship on semilogarithmic coordinates and fit the terminal exponent to the curve. Use the value measured for the proportion of soluble material in the sample to set the zero-time intercept of the curve. If more than one component "peel" the exponents and fit the second exponential. Calculate proportional size and $T_{1/2}$ for disappearance for each pool of material.

9. *Rumen turnover*: If necessary, inject 200 mg CrEDTA/kgDM into the rumen and measure water turnover from the disappearance of Cr from rumen fluid with time.