

## **RECENT DEVELOPMENTS IN LABORATORY METHODS FOR THE ASSESSMENT OF RUMINANT FEEDS**

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### **Summary**

This pamphlet presents a brief overview of the role and methods used for current conventional feed analyses, followed by a description of more recent techniques and their possible application to less developed country feeding systems. Chemical assays are increasingly being applied to the evaluation of anti-nutritive factors particularly tannins, although more research is required before the effects of tannins are better understood and become predictable. The gas production laboratory digestibility assay is being used for research purposes to provide information on rates of degradation, interactions between feeds and other complex areas, where earlier techniques can be inadequate. Near infra-red spectroscopy (NIRS) is a technique increasingly being used for rapid analysis and quality control. It appears to be particularly suitable for industrial applications and is used to evaluate silages for ruminants. In the longer term it may find applications in tropical ruminant feeding systems.

### **Introduction**

Laboratory methods have for many years been used to help define animal feeds, assess their nutritive value and provide data for the prediction of animal performance. This pamphlet is designed to give an overview of laboratory methods in current use, and particularly where newer techniques are being researched and increasingly applied. It will concentrate on ruminant feeds in tropical less developed country feeding systems, but draws on experiences of other situations. It is intended primarily for scientists in less developed countries with knowledge of livestock nutrition, to inform and stimulate greater awareness of laboratory methods. More detailed information can be obtained from the scientific literature which for the sake of simplicity has been quoted selectively in this review. BSAS (1998) contains a wide range of review articles and scientific papers on this topic.

Feeds are analysed to give information about their value as sources of nutrients. Where possible feeding trials are used to obtain such information, but feeding trials are time consuming, require relatively large quantities of feed and are expensive to conduct. In practice, due to these constraints, feeding trials can be used to provide only a fraction of the information required to evaluate complex diets and alternative feeding options. Certain chemical assays have long been used to give a general picture of the nutrient composition of feeds, and laboratory methods have been refined and extended in more recent years. This has been in recognition of the limitations of the conventional methods together with the development of new techniques. Crude fibre assays have been replaced by acid detergent fibre (ADF) and neutral detergent fibre (NDF) assays. More recent developments have included assays for tannins, *in vitro* gas production and near infra-red spectroscopy (NIRS). This pamphlet will give an overview of these newer methods and their applicability to tropical feeding systems.

## Role of feed evaluation in smallholder feeding systems

Table 1 indicates where feed evaluation may help provide information to farmers.

**Table 1 Role of feed evaluation in developing improved feeds and feeding systems**

What farmers know	What farmers don't know	Role of feed evaluation in providing new information
feeds available	potential use of improved, exotic or unfamiliar feeds	<ul style="list-style-type: none"> <li>- assess improved feeds (selection during breeding programmes, treated feeds)</li> <li>- identify role/use of new feeds within existing feeding systems</li> <li>- compare new with existing feeds to assess potential benefits</li> </ul>
approximate performance of livestock on available feeds	how to improve performance	<ul style="list-style-type: none"> <li>- diagnose nutrient imbalances in diets</li> <li>- identify feed combinations which improve the nutrient balance of the diet</li> </ul>

The type of information which would be useful to farmers will vary considerably from place to place, but would often include answering the questions listed below.

- What feeds need supplementation?

This may require an evaluation of basal diets to see if there are major deficiencies in protein, minerals and/or digestible energy.

- What supplement should be used?

If basal diets have particular deficiencies, then supplements should obviously be supplying the nutrients which are deficient. Information on the adequacy of existing supplements and the potential of alternatives would be useful.

- How much supplement should be given with particular roughages?

Information on the response to supplementation is required to optimise levels of supplementation. Such information will indicate if existing supplementation levels are sub-optimal (insufficient or excessive), and help identify roles of new supplements.

- What feed mixtures, from those available, will give the best performance?

In complex feeding systems a very wide range of feeds may be in use. There may be scope to use improved feed mixtures to provide a better balance of nutrients and also to reduce the intake of anti-nutritive factors.

## Feed evaluation and nutrient requirements of animals

For ruminants, current feed evaluation systems generally separate nutrients into energy and protein and try to estimate the supply of energy and protein to the animal (for example see AFRC, 1993, for the United Kingdom (UK) metabolisable energy (ME) and metabolisable protein evaluation system). Feed evaluation requires not just information on the composition of feeds, but also on how available the feed is to the animal when consumed. For ruminants the situation is particularly complicated due to the role of rumen micro-organisms in digesting feed components and so altering the nutrients available to the ruminant animal itself. This has stimulated the development of laboratory (otherwise called *in vitro*) digestibility methods which seek to mimic and predict the digestion processes which take place in ruminants.

Current feed evaluation systems have their limitations (for example see Tamminga, 1993) and are of limited applicability to many tropical feeding systems. Laboratory analyses are linked to the evaluation system used to interpret the data. Developments in feed evaluation systems, analytical techniques, increased understanding on how animals digest and utilise nutrients, and how feed components can affect animals will all affect the types of laboratory feed evaluation techniques in use.

A detailed consideration of feed evaluation systems is outside the scope of this pamphlet, but it is worthwhile noting that these systems are generally designed to service the needs of intensive livestock production systems found in industrialised countries. Supplementary feeds are purchased and fed to give the required levels of milk and/or meat production. The cost of purchased feeds is a major cost of production. Therefore, achieving commercially-optimal levels of feeding is of considerable importance. In many tropical farming systems livestock are kept for multiple purposes; milk and meat production may be less important than draught power and manure. There may be little purchase of feed. Advice on how to achieve the best results from the available resources is more usually the primary consideration.

Tropical feeds are often of lower quality than most temperate feeds; low digestibility and low protein contents are common constraints (Leng, 1990). In tropical feeding systems a priority is to ensure that there are no deficiencies of microbial nutrients (particularly protein) in the rumen, so that the microbes can grow and degrade the feed fibre as quickly and extensively as possible. In intensive temperate feeding systems this is usually a much less important consideration as nutrient availability is very much higher. Hence, it is usual to assume that feeds do not interact with one another, and the nutritive value of a diet is assumed to be the sum of the nutritive values of its components. This assumption can be invalid in tropical feeding systems where diets may not be balanced, particularly in periods of feed scarcity.

## **Conventional chemical analyses**

### *Proximate composition and fibre*

The composition of the feed in terms of its fundamental components (proximate composition), moisture, crude protein, fat, ash, crude fibre and the residue (nitrogen free extract) has been used as a starting point for evaluating feeds since the nineteenth century. Other nutrients, such as minerals, may also be of interest and can be

analysed in the laboratory. These methods provide useful information and are widely used, but their limitations are also very apparent.

Proximate analyses divide carbohydrates into two components, the crude fibre fraction which was originally intended to represent the indigestible component and the nitrogen free extract which included soluble sugars (and other components). However, ruminants (or more specifically rumen micro-organisms) are capable of digesting some of the fibre fraction. Crude fibre is in any case not a particularly good indicator of the component indigestible by monogastric animals. Modifications aimed at characterising the carbohydrate more effectively by partitioning it into a poorly-digested cell-wall component and a readily digested non-structural component were proposed by Van Soest (1967). The assays used are acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF). ADF represents the crude lignin and cellulose fractions of plant material, but also includes silica. NDF consists essentially of lignin, cellulose and hemicellulose and is regarded as a measure of the plant cell wall material. The determination of ADF is useful for forages as there can be a good statistical correlation between this and digestibility. NDF can be a useful indicator of intake.

These assays are now widely used for forages. However, in many cases chemical composition alone can be a poor indicator of animal parameters such as intake and *in vivo* digestibility. For example, Khazaal and Ørskov (1993) found a significant relationship between NDF and intake for ten hays, but no other relationships between composition and either *in vivo* digestibility or intake. Non-structural carbohydrates are composed of sugars and starch, which can be a complex and variable mixture depending on the nature of the feed. More detailed analysis of non-structural components is relatively complex and, therefore, not generally included in routine feed analysis. The characterisation of carbohydrates is a problem area which requires more research (BSAP, 1993).

### *Crude protein*

Crude protein (nitrogen content x 6.25) together with a value for the digestibility has been widely used to estimate protein supply to ruminants. Indigestible protein nitrogen in forages can be estimated from the nitrogen content in the acid detergent fibre fraction (ADIN). More recently, the digestible protein fraction has been divided into protein degraded and used by rumen microbes (which is in turn digested by ruminants) and that digested directly by the animal. This is to allow for the loss of protein nitrogen associated with the production and subsequent digestion of microbial biomass. Appropriate data are usually obtained using the nylon bag technique (Ørskov and McDonald, 1979), which is described in greater detail below.

### ***In vitro* digestibility**

#### *Tilley and Terry method*

Tilley and Terry (1963) described a two-stage method for predicting the *in vivo* digestibility of good quality temperate roughages, in which a feed sample is digested for 48 hours in buffered rumen liquor taken from rumen-fistulated animals, followed

by pepsin digestion in an acidic solution. Fibre digestion by rumen microbes had been completed within 48 hours (although this is not the case for poor quality tropical roughages), but the residue contained unchanged feed protein and microbial protein. To digest these materials, 48 h incubation in acid pepsin solution was used. Digestible dry matter was estimated from the weight of the residues and hence the weight of dry matter disappearance during the incubation, following the two-stage digestion. The method was initially calibrated using samples of temperate grasses, clover and lucerne, and has since been calibrated against various forages. The *in vivo* digestibility of tropical grasses was less accurately predicted, although predictions could be improved by using suitable standards of known digestibility (reviewed by Minson, 1998). For non-forages *in vitro* digestibilities are generally lower than *in vivo* digestibilities. High fat contents and high protein contents appear to lead to more marked underestimation of *in vivo* digestibility. When used to predict the *in vivo* digestibility of mixed oak and alfalfa diets, Nastis and Malechek (1988) found the Tilley and Terry technique to be an inaccurate predictor. It was concluded that separate regression equations are required for shrub and tree foliage, possibly due to the influence of tannins.

The Tilley and Terry method has been widely adopted due to its relative simplicity and the usefulness of the data provided. The major limitations of the Tilley and Terry method are that it is less accurate for tropical feeds, possibly due to the slow rates at which poor quality roughages are digested and the effects of anti-nutritive factors. However, there has been relatively little published work on calibrating the method for tropical feeds. Rates of degradation are probably particularly important in determining the nutritive value of poor quality tropical roughages, but this has been little investigated. The Tilley and Terry method was designed to measure the end point of *in vitro* digestion, although it measures a rather arbitrary intermediate point for slowly-digested feeds. It can be used to obtain kinetic data by using multiple batches but this is labour intensive.

#### *Neutral cellulase method*

Due to the cost and undesirability of keeping rumen-fistulated animals, and the variability in activity of rumen fluid, attempts have been made to develop purely enzymic digestibility assays. Fungal neutral cellulase is available commercially and has been used with some success to mimic the action of rumen microbes in degrading fibre, although the properties of different enzymic preparations can vary considerably. Jones and Hayward (1975) described a method which involved the pre-treatment of herbage with acid pepsin then treatment with a range of cellulase preparations. Acid pepsin pre-treatment was found to increase the correlation between neutral cellulase digestibility (NCD) and *in vivo* digestibility in sheep compared with treatment with neutral cellulase alone or neutral cellulase followed by acid pepsin. This was perhaps surprising as it is a reversal of the physiological sequence. A cellulase preparation from *Trichoderma viride* was found to have sufficient and appropriate activity for degrading herbage fibre.

More recently, a modified procedure which includes neutral detergent cellulase followed by digestion using the enzyme gammanase (MAFF, 1993) has been used for compound feeds. Although the predicted *in vivo* digestibility derived from this method is less accurate than that from the Tilley and Terry method, unlike that assay

it does not require access to animals and, therefore, is the assay of choice for some feed compounders and other organisations. Research on the use of enzymic preparations is continuing, but the activity of the enzyme preparation is critical and needs adequate calibration and standardisation.

### **Nylon bag (*in sacco*) method**

Although essentially an *in vivo* technique, the nylon bag method has characteristics in common with the purely laboratory *in vitro* methods and is, therefore, included in this brief review. It involves the incubation of feed samples in small, closed nylon bags in the rumens of fistulated animals and measurement of material lost from the bags. The method can be used to give end-point degradability data, or by incubating bags for different lengths of time, can give data on the rate of feed degradation. It can be used to measure the rumen degradation of dry matter (or organic matter) and, by weighing and analysing the residues, different components of the feed. Ørskov and McDonald (1979) first described the use of this method to estimate the protein degradability of feeds in the rumen. It has become widely used for such measurements which are required by the metabolisable protein feed evaluation system.

While the Tilley and Terry and neutral cellulase methods are usually used to produce end-point digestibility estimates, the nylon bag method is most usually used to obtain rate data on the rumen degradability of feeds. The analysis and interpretation of rate data is more complex, but potentially much more useful than end-point data. Parameters from the nylon bag technique have been correlated with *in vivo* digestibility, dry matter intake, digestible dry matter intake and growth rate. The nylon bag method has become widely used, although there are a number of practical limitations. A major one is the wide laboratory to laboratory variation in nylon bag data. A ring test involving 23 laboratories in 17 European countries revealed appreciable variabilities in the mean protein degradabilities measured by the nylon bag technique (Madsen and Hvelplund, 1994). Variations between laboratories were mainly associated with differences in sample preparation and processing, and in the bags used for the incubations. The repeatability of the crude protein assay was also poor. The ranking of the test feeds was, however, very consistent between laboratories. Thus, without considerably improved standardisation, the reliability of the absolute figures obtained from this method are open to doubt, but relative data from a single source appear to be robust.

Other limitations are the need to measure or assume outflow rates; data which are time consuming to collect and, generally, not available for less developed country feeding systems where feeding levels can be very low. Hence, in practice, outflow rates often have to be assumed. Microbes can adhere to feed samples giving rise to under-estimation of protein degradation. The reliability of the method for evaluating feeds with a large, soluble component or feeds of fine particle size is doubtful, as components of samples are lost from the bags without necessarily being degraded. Some feeds may give variable proportions of fine particles when ground, giving rise to variable over-estimates of degradability. Anti-nutritive factors may be washed out of feeds and diluted to neutralise their effects (Preston, 1995). The method also requires relatively large numbers of rumen-fistulated animals.

The nylon bag technique has, however, been shown to be a potentially useful predictor of animal growth, intake and digestibility. The technique has proved to be versatile and applicable to a wide range of feeds. Prediction equations, some of which include composition as well as nylon bag parameters, have been reported in a range of studies. Larger pools of data are probably required to facilitate the development of robust prediction equations. The method does not require sophisticated laboratory facilities, not even a reliable supply of electricity. For this reason its use has been advocated in less developed countries (Osuji *et al.*, 1993; Preston, 1995).

## **More recently developed laboratory techniques**

### **Tannins**

#### *Importance of tannins*

For many tropical feeds additional types of assays are potentially useful. This is particularly the case for forage legumes, and tree fodders more generally, as many contain anti-nutritive factors which can modify (and usually reduce) the nutritive value of the feed. Makkar (1993) and Paterson (1993) have reviewed the significance of anti-nutritive factors in fodder trees. Tannins are the most widely occurring of the anti-nutritive factors, being present in most tree fodders and forage legumes, and this pamphlet will concentrate on them.

As the name suggests, the word tannin was originally used to describe plant extracts used to tan animal skins into leather. The important biochemical property of tannins is their ability to bind to protein and form insoluble complexes. They are implicated in reducing the digestibility of feeds, particularly the protein content. General descriptions of the chemical structures can be found in reviews such as Mangan (1988), Jansman (1993) and Kumar and D'Mello (1995). Tannins are generally poorly defined chemically and are found in complex mixtures in many plants. All tannins are polyphenolic compounds, although not all polyphenols have the protein-binding properties of tannins. Tannins can be divided into two chemical types, hydrolysable and condensed tannins. Additionally, polyphenols of lower molecular weight than the tannins (and not having tannin-like properties) are commonly found.

#### *Measurement of tannins*

To match the range of tannins, there is a very diverse range of chemical and biochemical assays which has been used to measure the tannin contents of plants. Several methods use the chemical properties of tannins in colorimetric assays. These assays vary considerably in specificity. Their properties and merits have been reviewed by Hagerman and Butler (1989). The most widely-used colorimetric assays are given below:

#### General measurements of tannins (total phenols)

- Folin Denis assay
- Prussian blue assay

#### Measurements of condensed tannins

- Vanillin assay

### Acid butanol assay

There are several published procedures and modifications to these assays. Based on the recommendations of Hagerman and Butler (1989), the Prussian blue assay using the Price and Butler (1977) procedure and the acid butanol assay by the Porter *et al.* (1986) procedure are used at the Natural Resources Institute. These assays are usually used to measure polyphenols which have been extracted in solvent, often aqueous acetone. However, the acid butanol method is also used to measure non-extractable tannins. Again, there are various procedures but they involve the treatment of a residue (after extraction) with acid butanol. Terrill *et al.* (1992) have developed a method which sequentially extracts free, protein-bound and fibre-bound condensed tannins.

Biochemical assays can be used to measure the activity or capacity of tannins to bind and precipitate protein. This is really a measurement of tannin activity and it has been suggested by Hagerman and Butler (1989) that these types of assays give better indicators of the biological activity of tannins than chemical assays. Again there are several procedures available. The radial diffusion assay of Hagerman (1987) does not require sophisticated equipment, making it potentially suitable for use in less developed countries.

More sophisticated chemical techniques such as high performance liquid chromatography (HPLC) have been used to identify particular tannins, especially lower molecular weight polyphenols (Mueller-Harvey and Reed, 1992), but also for separating tannins of different molecular weight (Rigaud *et al.*, 1993). The detailed analysis of the higher molecular weight tannins is, however, very challenging.

Considering the volume of literature on tannin assays, there is comparatively little on the effectiveness of the different assays as indicators of anti-nutritive effects. This is because such trials are difficult to conduct. The nutritive value of tanniniferous feeds is due to all its components and isolating the effect of tannins is not straightforward. There are few tannins which can be readily obtained and added to feeds as experimental models. With such a diverse group of compounds there is a considerable risk that model compounds may be of limited value. Tannin-binding agents, such as polyethylene glycol (PEG), have been used to inactivate tannins thus giving indications of what effects the tannins are having. Equations have been developed to predict protein and dry matter digestibility of tanniniferous forages in deer, using protein precipitation as an indicator of tannin activity (Robbins *et al.*, 1987; Hanley *et al.*, 1992). There is evidence indicating the general applicability of protein precipitation assays and the Prussian blue total phenols assay (Wood and Plumb, 1995), although neither of these can distinguish between condensed and hydrolysable tannins. However, there is no clear consensus as to which assays are the most reliable indicators of anti-nutritive effects.

The analysis of tannins, and the incorporation of such data into a feed evaluation system presents many challenges, and is the topic of research in many countries (see Brooker, 2000, for the findings of a recent workshop on the topic). There may be advantages in using *in vitro* measurements of feed digestion which are affected by tannins. However, tannins may have direct toxic effects on livestock which are presently poorly understood.



## Gas production method

The two *in vitro* methods and the nylon bag method described above all depend on measuring the nutrients left in a residue after degradation. Degradation is estimated by subtracting the nutrients which have disappeared from the original nutrients. There is an underlying assumption that all soluble components which are not recovered in the residue are degraded. Kinetic measurements are made of the degradation of the insoluble fraction only, not of the feed as a whole. In contrast, the gas production method measures a product of degradation; gas produced during the fermentation of the feed by rumen micro organisms. The standard assumptions are not applicable, although there are others which apply uniquely to gas production techniques.

### *Menke method*

Menke *et al.* (1979) first described a method where the gas evolved during fermentation by rumen microbes was collected and used as a measure of the extent of fermentation. Feed samples were incubated in a buffered medium containing fresh rumen fluid to provide rumen microbes; essentially very similar to the first stage of the Tilley and Terry method. However incubations were conducted in large glass syringes to trap the gas evolved, where as in the Tilley and Terry method gases are allowed to escape to the atmosphere. Blummel and Ørskov (1993) modified the technique to monitor gas production at regular intervals up to 72 h incubation. Gas production data were analysed using the same equation used to interpret nylon bag data (Ørskov and McDonald, 1979), using gas produced up to 6 h of incubation as an indicator of parameter (a), the rapidly fermented fraction. A range of regression equations linking gas production, various compositional parameters and ME or net energy-lactation have been reported by Menke and Steingass (1988).

The main products of carbohydrate fermentation by rumen microbes are volatile fatty acids (VFAs: acetic, propionic and butyric acids), the gases carbon dioxide and methane, and microbial biomass. Carbon dioxide is also released from the bicarbonate buffer used for this method as the VFAs are neutralised, hence giving an indirect indicator of VFA production. VFAs accumulate in the fermentation medium and can be analysed. The amount of gas produced per amount of carbohydrate (glucose) degraded depends on the fermentation pathways used by the rumen microbes. Non-carbohydrate components, such as protein, may produce little gas when they are fermented. Additionally, ammonium formed by the degradation of protein can combine with carbon dioxide to form ammonium bicarbonate which stays in the medium, hence reducing the amount of carbon dioxide released as gas. Some of the carbon (and other elements) of the feed will also be incorporated into microbial biomass, and so will not be released as gas. Thus the quantity of gas produced per gram of feed sample fermented can vary.

### *Theodorou method*

Theodorou *et al.* (1994) described a gas production method which used a pressure transducer to monitor the production of gas. The method is essentially the same in principle as the Menke method, the key difference being that instead of using glass

syringes, sealed bottles are used. At selected times, the gas pressure inside the bottles is measured using the pressure transducer and the gas produced is removed, the volume measured and the gas discarded. By sequential measurement of the gas produced, curves of cumulative gas production against time can be constructed. A range of equations has been used to interpret the data in terms of rate constants and end points. As yet there is little consensus on which equations are the most appropriate. The method was proposed as a simple method of investigating fermentation (degradation) rates and a ranking tool for feeds on the basis of their *in vitro* fermentability.

As a relatively new technique which, until recently, has not received widespread attention, there are only a limited number of examples of the practical uses of gas production methods. Some of the potential applications of particular relevance to less developed countries are:

- screening and ranking feeds
- estimations of digestibility and feed intake
- investigation of interactions between feed mixtures
- as an experimental tool to investigate the fermentation of feeds and feed components
- to provide rate parameters for use in computer models of rumen function
- reduction or avoidance of the use of rumen-fistulated animals.

Nsahlai *et al.* (1994) have distinguished between 23 *Sesbania* accessions (varieties) using *in vitro* gas production and chemical composition. Siaw *et al.* (1993) ranked leaves of 20 accessions from multi-purpose trees of six genera, using both the nylon bag and gas production methods. The two methods agreed on the relative positions of leaves of high degradability/fermentability, but they failed to agree on the relative positions of the forages of lower degradabilities. Williams *et al.* (1996) have distinguished between rice straws of different cultivars, grown at different altitudes and harvested at different seasons using the gas production technique. The above studies did not establish if the *in vitro* rankings truly reflected the relative performance of animals given these feeds. More recently, Wood *et al.* (1998) found that differences in gas production (and chemical composition) in leaves of different *Gliricidia sepium* provenances were small compared to site-related differences. Feeding trials had similarly indicated few differences in the nutritive value of the leaves. Dryhurst and Wood (1998) demonstrated that the Theodorou gas production technique was responsive to nitrogen supplementation in a way consistent with earlier laboratory work.

The gas production technique may have the potential to replace some or all of the existing *in vitro* digestibility and nylon bag techniques, and can be used in combination with other feed evaluation techniques to assess nutritive value. To date, the gas production technique appears to be useful as a ranking tool for feeds, but it is unclear to what extent the technique is sensitive to anti-nutritive factors in feeds, and whether the *in vitro* ranking accurately reflects relative animal performance.

#### *Advantages and limitations of gas production techniques*

The major advantages of the technique are the capacities to monitor fermentation non-destructively and to measure the degradation of soluble material. Alternative *in vitro* digestibility assays and the nylon bag technique consist of single or multiple

incubations where the residue is recovered to give a single value for each incubation. In the gas production technique each incubation is monitored non-destructively at regular intervals, so that the full fermentation process can be measured. The method is particularly suitable for obtaining data on degradation rates. Gas production is potentially considerably less expensive to perform than other techniques, especially in industrialised countries where automated gas production systems could replace more labour-intensive techniques. Gas production is the only *in vitro* technique suitable for the study of the degradation of soluble materials; important when considering supplements such as urea-molasses blocks. Potentially, the technique could be used for estimating microbial protein production and for investigating interactions between feeds. Another advantage is that VFAs accumulating in the incubation medium can be analysed to investigate the VFA production profiles.

One disadvantage of gas production techniques is that the units of the data generated, volumes of gas, are not readily comprehensible. Other *in vitro* techniques generate data as dry matter disappearances which are regarded as *in vitro* digestibilities without further transformation, although prediction equations should be applied to derive more accurate estimates of *in vivo* digestibility. Menke *et al.* (1979) developed equations to equate gas production to ME and digestible organic matter, but similar equations have not as yet been developed for the Theodorou method.

### **Near infra-red spectroscopy (NIRS)**

Near infra red spectroscopy (NIRS) is a new technique which is being used increasingly in the food and feed industry, and elsewhere, for quality control and feed analysis. NIRS is based on the irradiation with infra-red light of organic materials which selectively absorb the energy in the near infra-red wavelength region. The intensity of the absorbency is proportional to the concentration of a specific chemical bonding or nutrient in the sample. Samples can have very complex NIR spectra, but the advent of sensitive equipment and inexpensive powerful computers to analyse the data has increasingly allowed NIR spectra to be calibrated against conventional wet chemistry techniques and other properties of the sample. The key advantages of the technique were initially seen as its speed and cheapness (once calibrated), as no complex sample preparation or wet chemistry is required. More recently, it has also been shown to be more reliable than some conventional wet chemistry techniques and can also be used to monitor complex properties which are difficult to measure by alternative techniques.

NIR has been used for measuring chemical composition, *in vitro* digestibility, *in vivo* digestibility and metabolisable energy. NIR has now become the method of choice in the UK for the prediction of organic matter digestibility of silage, performing better than wet chemistry techniques (Deaville and Baker, 1993). De Boever *et al.* (1993) reported that NIRS was less accurate than *in vitro* methods of estimating the metabolisable energy content of compounded feeds, but was more reliable than depending on literature values (which is a common commercial practice). The technique has considerable potential as a provider of a detailed but rapid evaluation of feeds.

The major disadvantages of the technique are that the cost of NIR spectrometers is high and it requires detailed calibration before the data produced can be interpreted.

The technique is, therefore, particularly suited to evaluating limited ranges of feeds where it is practicable to obtain sufficient reference data to calibrate the spectral data. These factors mean that the technique appears much better suited to intensive feeding systems, which generally use a limited number of feeds, than to extensive tropical livestock production where diets are complex and very variable seasonally.

## Conclusions

Laboratory methods for evaluating feeds are developing as newer techniques become available and feed evaluation systems become more sophisticated. However, there is still a considerable dependence on nineteenth century chemical techniques which are tried and trusted, but have considerable limitations. Generally, developments have been aimed at livestock production in industrialised countries, but there has also been more work on approaches of particular relevance to less developed countries. Progress has been limited by significant gaps in the current understanding of the effects of tannins on ruminants and of the digestion of poor quality feeds, as well as uncertainties on the most appropriate laboratory techniques to use. More strategic and applied research is required to further develop the science and application of tropical feed evaluation and laboratory techniques.

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## Bibliography

AFRC (1993) Energy and protein requirements of ruminants. An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. Published by CAB International, Wallingford, UK. 159pp.

BLUMMEL, M. and ØRSKOV, E. R. (1993) Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Animal Feed Science and Technology*, **40**: 109 - 119.

BROOKER, J.D. (2000) Tannins in livestock and human nutrition: proceedings of an international workshop, Adelaide, Australia May 31 - June 2, 1999. Canberra. ACIAR Proceedings No 92. 171pp.

BSAS (1998) In vitro techniques for measuring nutrient supply to nutrients. *Occasional Publication No 22*, British Society of Animal Science. Deaville, E. R., Owen, E., Adesogan, A. T., Rymer, C., Huntington, J. A. and Lawrence, T. L. J. (Eds.) 348pp.

BSAP (1993) Characterisation of feeds for farm animals. *British Society of Animal Production Workshop Publication No1*. British Society of Animal Production, Edinburgh, UK. 45pp.

- DEAVILLE, E. R. and BAKER, C. W. (1993) Spectral interpretations in relation to feed characterisation. Paper 3 In: *NIR Spectroscopy: Developments in Agriculture and Food. Proceedings of the International Conference, 12 October 1993, Birmingham, UK*. Published by ADAS Feed Evaluation Unit, UK.
- DE BOEVER, J. L., COTTYN, B. G., VANACKER, J. M. and BOUCQUE, C. H. V. (1993) Recent developments in the use of NIRS for evaluating compound feeds and raw materials for ruminants. Paper 1 In: *NIR Spectroscopy: Developments in Agriculture and Food. Proceedings of the International Conference, 12 October 1993, Birmingham, UK*. Published by ADAS Feed Evaluation Unit, UK.
- DRYHURST, N. and WOOD, C. D. (1998). The effect of nitrogen source and concentration on *in vitro* gas production using rumen micro-organisms. *Animal Feed Science and Technology*, **71**: 131 - 143.
- HAGERMAN, A. E. (1987) Radial diffusion method for determining tannin in plant extracts. *Journal of Chemical Ecology*, **13**: 437 - 449.
- HAGERMAN, A. E. and BUTLER, L. G. (1989) Choosing appropriate methods and standards for assaying tannin. *Journal of Chemical Ecology*, **15**: 1795 - 1810.
- HANLEY, T. A., ROBBINS, C. T., HAGERMAN, A. E. and McARTHUR, C. (1992). Predicting digestible protein and digestible dry matter in tannin-containing forages consumed by ruminants. *Ecology*, **73**: 537-541.
- JANSMAN, A. J. M. (1993) Tannins in feedstuffs for simple-stomached animals. *Nutrition Research Reviews* **6**: 209 - 236.
- JONES, D. I. H. and HAYWARD, M. V. (1975) The effect of pepsin pre-treatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase solutions. *Journal of the Science of Food and Agriculture*, **26**: 711 – 718.
- KHAZAAL, K. and ØRSKOV, E. R. (1993) A comparison of gas production during incubation with rumen contents *in vitro* and nylon bag degradability as predictors of the apparent digestibility *in vivo* and the voluntary intake of hays. *Animal Production* **57**: 105 - 112.
- KUMAR, R. and D'MELLO, J. P. F. (1995) Anti-nutritional factors in forage legumes. p 95 - 133. In: *Tropical legumes in animal nutrition*. D'Mello, J. P. F. and Devendra, C. (Eds) CAB International, Wallingford, Oxon OX10 8DE, UK 338pp.
- LENG, R. A. (1990) Factors affecting the utilization of “poor-quality” forages by ruminants particularly under tropical conditions. *Nutrition Research Reviews* **3**: 277 – 303.
- MADSEN, J. and HVELPLUND, T. (1994) Prediction of *in situ* protein degradability in the rumen. Results of a European ringtest. *Livestock Production Science* **39**: 201-212.

- MAFF (1993) Prediction of energy value of compound feedingstuffs for farm animals. Booklet 1285, MAFF Publications, Alnwick, UK.
- MAKKAR, H. P. S., 1993. Antinutritional factors in foods for livestock. pp 69 - 85. In: *Animal Production in Developing Countries*. Occasional Publication No 16, British Society of Animal Production. Gill, M. Owen E, Pollott G E and Lawrence T L J (Eds.), 243 pp.
- MANGAN, J. L. (1988) Nutritional effects of tannins in animal feeds. *Nutrition Research Reviews*, **1**: 209-231.
- MENKE, K. H. and STEINGASS, H. (1988) Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development* **28**: 7 - 55.
- MENKE, K. H., RAAB, L., SALEWSKI, A., STEINGASS, H., FRITZ, D. and SCHNEIDER, W. (1979) The estimation of the digestibility and energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agricultural Science, Cambridge*, **93**: 217-222.
- MINSON, D. J. (1998) A history of *in vitro* techniques. pp 13 – 19 In: *In vitro techniques for measuring nutrient supply to nutrients*. Occasional Publication No 22, British Society of Animal Science. Deaville, E. R., Owen, E., Adesogan, A. T., Rymer, C., Huntington, J. A. and Lawrence, T. L. J. (Eds.) 348pp.
- MUELLER-HARVEY, I. and REED, J. D. (1992) Identification of phenolic compounds and their relationships to *in vitro* digestibility of sorghum leaves from bird-resistant and non-bird-resistant varieties. *Journal of the Science of Food and Agriculture*, **60**: 179 - 196.
- NASTIS, A. S. and MALECHEK, J. C. (1988) Estimating digestibility of oak browse diets for goats by *in vitro* techniques. *Journal of Range Management*, **41**: 255 - 258.
- NSAHLAI, I. V., SIAW, D. E. K. A. and OSUJI, P. O. (1994) The relationship between gas production and chemical composition of 23 browses of the genus *Sesbania*. *Journal of the Science of Food and Agriculture*, **65**: 13-20.
- ØRSKOV, E. R. and MCDONALD, I. (1979) The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. *Journal of Agricultural Science, Cambridge*, **92**: 499-503.
- OSUJI P, O., NSAHLAI, I. V. and KHALILI, H. (1993) Feed Evaluation. International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia, 36pp.
- PATERSON, R. T. (1993) Anti-nutritive factors. Use of trees by livestock No 4. Natural Resources Institute, UK, 20pp.
- PORTER, L. P., HRSTICH, L. N. and CHAN, B. G. (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, **25**: 223 - 230.

- PRICE, M. L, and BUTLER, L. G. (1977) Prussian blue assay for total phenols. *Journal of Agricultural and Food Chemistry* **25**: 1268 - 1273.
- PRESTON, T. R. (1995) Tropical animal feeding. A manual for research workers. FAO Animal Production and Health Paper No. 126. FAO, Rome, Italy. 124pp.
- RIGAUD, J., ESCRIBANO-BAILON, M. T., PRIEUR, C., SOUQUET, J-M. and CHEYNIER, V. (1993) Normal-phase high-performance liquid chromatographic separation of procyanidins from cacao beans and grape seeds. *Journal of Chromatography*, **654**: 255 - 260.
- ROBBINS, C. T., HANLEY, T. A., HAGERMAN, A. E., HJELJORD, O., BAKER, D. L., SCHWARTZ, C. C. and MAUTZ, W. W. (1987) Role of tannins in defending plants against ruminants: reduction in protein availability. *Ecology*, **68**: 98-107.
- SIAW, D. E. K. A., OSUJI, P. O. and NSAHLAI, I. V. (1993) Evaluation of multipurpose tree germplasm: the use of gas production and rumen degradation characteristics. *Journal of Agricultural Science, Cambridge*, **120**: 319-330.
- TAMMINGA, S. (1993) Characterisation of feeds for farm livestock: ruminants. pp13 – 15. In: *Characterisation of feeds for farm animals*. British Society of Animal Production Workshop Publication No1.
- TERRILL, T. H., ROWAN, A. M., DOUGLAS, G. B. and BARRY, T. N. (1992) Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture*, **58**: 321 - 329.
- THEODOROU, M. K., WILLIAMS, B. A., DHANOA, M. S., MCALLAN, A. B. and FRANCE, J. (1994) A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology*, **48**: 185-197.
- TILLEY, J. M. A. and TERRY, R. A. (1963) A two-stage technique for the *in vitro* digestion of forage crops. *Journal of the British Grassland Society*, **18**: 104-111.
- VAN SOEST, P. J. (1967) Development of a comprehensive system of feed analysis and its application to forages. *Journal of Animal Science*, **26**: 119 – 128.
- WILLIAMS, B. A., CHUZAEMI, S., SOEBARINOTO, VAN BRUCHEM, J., BOER, H. and TAMMINGA, S. (1996) A comparison of ten rice-straw varieties grown at two different altitudes during a wet and a dry season, using the *in vitro* cumulative gas production technique. *Animal Feed Science and Technology*, **57**:183-194.
- WOOD, C. D. and PLUMB, V. E. (1995). Evaluation of assays for phenolic compounds on the basis of *in vitro* gas production by rumen micro-organisms. *Animal Feed Science and Technology*, **56**: 195-206.

WOOD, C. D., STEWART, J. L. and VARGAS, J. E. (1988). Genetic variation in the nutritive value of *Gliricidia sepium*. 2. Leaf chemical composition and fermentability by an *in vitro* gas production technique. *Animal Feed Science and Technology*, **75**(2): 125 - 143.