Laboratory techniques appropriate for evaluating ruminant feeds for less developed countries, with particular reference to the potential use of in vitro gas production methods

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Prologue

The following review has been prepared as background to project A0316. The review was prepared as part of this project, and to take account of the most recent developments it has been extensively updated towards the end of the project. **It has tried to avoid reviewing the outputs of this project (which are covered elsewhere in the Final Technical Report),** although the ideas expressed have been developed during the course of the project and a certain amount of overlap is inescapable. It has been written in a format intended to facilitate future publication, probably after updating in the light of the outputs of A0316 and related projects (e.g. on intake) within the Livestock Production Programme.

1 Purpose of this review

Recent years have seen several developments in the fields of nutritive value assessment and livestock research in less developed countries (LDCs). New systems for predicting the protein supply to ruminants, the Metabolisable Protein system, have been developed in the United Kingdom and elsewhere. New techniques such as the gas production technique and Near Infra Red (NIR) spectroscopy are receiving considerable research interest, and increasingly practical use, as tools to evaluate aspects of feed quality. New approaches are used particularly where existing techniques have proved inadequate, such as for evaluating silages where NIR spectroscopy is becoming the method of choice. NIR, however, requires extensive calibration against in vivo or laboratory data before it can be interpreted and may be far more suitable for feeding systems where limited numbers of different feeds are used (for example most temperate feeding systems). The gas production technique has the potential to provide data on the rates (kinetics) of fermentation (although, to date, the use of the method in this way has been limited). Being based on a biological process, it may be suitable for evaluating diverse and poorly characterised feeds (typical of tropical feeding systems).

These developments have been made primarily to service the needs of the livestock industries of the industrialised countries, but nevertheless have implications for feed evaluation in LDCs. There has also been an increasing recognition of the fact that the needs of smallholder farmers in LDCs do not always mirror those of farmers in industrialised countries. Thus, while some existing approaches may be adaptable to support the development of feeding strategies that are appropriate for smallholders, some aspects of their production objectives may require more novel approaches.

The purpose of this review is to consider the role of in vitro (laboratory) methods of assessing feeds, with particular emphasis on the potential role of one of the new techniques mentioned above, the in vitro gas production technique. Theodorou and Brooks (1990) reported that the gas production method was a useful tool which could provide precise data relating to the fermentation kinetics of ruminant feeds and rank feeds with respect to their in vitro fermentability. The method was seen by Theodorou and Brooks (1990) as a lower cost alternative to the conventional nylon bag technique which provides similar information, as well as using fewer rumen-fistulated animals making it desirable for improved animal welfare. Additionally, the method was also seen by NRI as being potentially suitable for studying interactions between feeds and the effects of anti-nutritive factors on rumen microbes, factors of particular importance for tropical feeds and feeding systems.

The emphasis will be on its role in feed evaluation as it relates to investigating and developing feed resources and feeding systems for LDCs. The review will consider the most common constraints imposed by the available feed resources and feeding systems, then outline briefly the conventional approaches to feed evaluation and their usefulness for tropical feeds and feeding systems. . The gas production method will then be described and its possible role discussed. The review will then widen the discussion to whether conventional feed evaluation is appropriate to the investigation of LDC feeding systems and suggest approaches which may be more suitable. The review will finally draw conclusions on the issues considered and make recommendations on future work on this topic.

2. Existing feed evaluation

2.1 The role of feed evaluation in feeding systems used in industrialised countries

Livestock production in industrialised countries is generally intensive and aimed at producing directly marketable products such as meat, milk, hides and animal fibre (e.g. wool). In intensive production systems, feeds produced on farm are often supplemented with purchased feeds, and the cost of feed is an important factor in the overall cost of producing livestock products. Temperate feeding systems used on different farms are usually very similar to each other in a particular region and use a relatively limited number of feeds. Individual feeds do not usually vary greatly in their nutritional characteristics, which has facilitated the use of literature nutritive values obtained from animal feeding trials and laboratory analysis. The role of conventional feed evaluation is to provide data for quantitative predictions of animal production using specific feeds within an established feeding system. This information can be used for quality control, to introduce new feeds, for feed rationing and for investigating feed problems. Feeds may be evaluated by using literature values for their composition; simple laboratory analyses and in vitro determinations may be conducted. The data obtained are interpreted within the context of an appropriate feed evaluation or rationing system. Current feed evaluation systems for ruminants are separated into those for energy and for protein although more recent systems seek to encompass the interdependence of energy and protein in determining levels of performance. Conventional feed rationing systems are generally designed to meet fixed requirements for given outputs of product, meat and milk (Tamminga, 1995) i.e. the system is usually driven by outputs of product rather than inputs of feed. Accordingly, the

consituent mathematical relationships of rationing systems generally predict inputs required to achieve defined outputs, and may no longer be optimum if manipulated to predict levels of outputs from fixed inputs (Elston and Glaseby, 1991). The system uses the nutritive value of the feed when fed as part of a balanced diet and nutritive values of feeds in a diet are assumed to be additive, thereby facilitating the use of linear programming approaches to least cost rationing. In temperate feeding systems energy is normally the first limiting factor in the supply of nutrients (AFRC, 1993).

The objectives of livestock keepers in industrialised countries are largely concerned with maximising the economic return in the production of meat and milk. Feed availability and quality can be a constraint, particularly when fresh green pasture is unavailable, but diets are generally free from nutrient imbalances. Other restraints in industrialised countries may be related to production quotas fixed by systems of state subsidies.

2.2 Chemical analysis of feeds

Since the middle of the 19th century, scientists in temperate industrialized countries have attempted to develop analytical systems to characterise the value of feed to livestock. They include measurements of the chemical composition of the feed and its digestibility (INRA, 1989). One of the most popular set of analyses is the proximate analysis of feed, which was devised about 100 years ago by two German scientists, Henneberg and Stohmann (McDonald et al., 1988). This system of analysis divides the feed into six fractions, as shown in Table 1

Table 1: Components of different fractions in the proximate analysis of foods.

Fraction	Component
Moisture	Water (and volatile acids and bases if present).
Ash	Essential elements: Major: Ca, K, Mg, Na, S, P, Cl. Trace: Fe, Mn, Cu, Co, I, Zn, Si, Mo, Se, Cr, F, V, Sn, As, Ni. Non-essential elements: Ti, Al, B, Pb.
Crude protein	Proteins, amino acids, amines, nitrates, nitrogenous glycosides, glycolipids, B-vitamins, nucleic acids, urea, ammonia.
Ether extract	Fats, oils, waxes, organic acids, pigments, sterols, vitamins A, D, E, K
Crude fibre	Cellulose, hemicellulose, lignin
Nitrogen free extract	Sugars, fructans, starch, pectins, organic acids, resins, pigments, water soluble proteins.

Source: McDonald et al. (1988)

The chemical composition of a feed is routinely used as a rapid and economical method for partially characterising the feed, and predicting digestibility and other measures of the nutritive value, provided that appropriate equations are available. Such equations are based on statistical association and causal relationships between the content of analysed constituents and feed quality (Van Soest, 1982). No single compositional parameter can adequately predict nutritive value across a range of feeds, although combining the results from several analyses may improve its prediction (Vadiveloo and Fadel, 1992).

2.3 Analysis of the carbohydrate component

In the proximate analysis system, the carbohydrate component of feed is partitioned between two fractions; the crude fibre and the nitrogen-free extract. When the sum of the amounts of moisture, ash, crude protein, ether extract and crude fibre (expressed in g/kg) is subtracted from 1000, the difference is designated the nitrogen free extract (NFE). The crude fibre fraction contains cellulose, lignin and hemicellulose, but not necessarily all of these materials are present in the feed; a variable proportion of them is contained in the nitrogen-free extract, depending upon the species and the stage of growth of the plant material. The complexity of the NFE is illustrated by the constituents shown in Table 1. The crude fibre was intended originally to provide a measure of the indigestible part of the food, but quite a large part of it may in fact be digested by ruminants (McDonald et al., 1988). The division of carbohydrate between NFE and CF has proved to be of limited use in predicting the extraction of nutrients by the animal (Van Soest, 1982).

This scheme has been critically reviewed and refinements have been developed which include further partition of the main components. The main modification aims to characterise the carbohydrate component more effectively by partitioning it into structural and non-structural pools. These are the acid detergent fibre (ADF), acid detergent lignin (ADL) and the neutral detergent fibre (NDF). This approach was proposed by Van Soest (1976). ADF represents essentially 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 particularly useful for forages as there can be a good statistical correlation between this and digestibility (McDonald et al., 1988). NDF can be a useful indicator of intake. However, in many cases chemical composition alone can be a poor indicator of parameters such as intake and in vivo digestibility. For example, Khazaal and Ørskov (1993) found a significant ($P<0.05$) 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. Analysis of non-structural components is relatively complex and therefore not generally included in routine feed analysis.

2.4 Feed evaluation: energy

Energy is required for the maintenance and productive outputs of animals. Energy is released as feeds are oxidised. The total amount of energy in a feed is termed the Gross Energy (GE). However, in practice not all of this energy is available to the animal as a result of losses in faeces, urine and as methane. When these losses are deducted from the GE, the amount of energy that may potentially be assimilated by the animal is obtained. This is termed the Metabolisable Energy (ME). ME may be used by the animal but the processes involved are not completely efficient, and the efficiency of utilisation for maintenance and production are different. When the feed ME values are multiplied by the appropriate efficiency factor, the energy actually assimilated by the animal for production (of meat, milk etc.) and maintenance (of basal metabolic functions) is obtained. This is termed the Net Energy (NE). From these theoretical considerations various systems have been devised to describe the amount of energy

which ruminants can obtain from a feed. This process is complicated by the fact that the efficiency of utilisation of ME (by definition calculated at maintenance) is affected by the level of feeding (because of reduced rumen retention time as the level of feeding increases) so correction factors must be included.

The fermentable metabolisable energy (FME) is a measure of the ME available to rumen microbes, which is defined as the total ME less the ME in fat and fermentation acids which microbes are unable to utilise (but which can be utilised by the ruminant). FME supply determines the extent of microbial biomass production in the rumen, assuming other nutrients are not limiting (this is described in more detail below). There are several texts which give a fuller description of the energy evaluation system, for example Van der Honing and Steg (1990), McDonald et al. (1988), Ørskov and Ryle (1990), AFRC (1993).

Feed rationing systems combine feed evaluation with estimates of nutrient requirements. Such systems use empirical equations which relate energy measurements made during animal feeding trials to laboratory measurements of feed quality. The oldest feed rationing system is the Total Digestible Nutrients (TDN) system where TDN = digestible organic matter (DOM) + 2.25 x digestible ether extract. This system does not take into account energy lost as methane and in the urine, losses which tend to be relatively high with roughage feeds. It has been widely used, particularly in the USA, but more complex systems based on ME or NE have become increasingly popular due to their greater accuracy. These have been developed as techniques for measuring energy exchanges (calorimetry using respiration chambers) have become widely used. To quote Ørskov and Ryle (1990) "...many respiration chambers were established in many parts of the world and, with them, almost as many feed evaluation systems!". The Metabolisable Energy system used in the United Kingdom and elsewhere may be implemented with direct measurements of feed ME values or using predicted values derived from equations which relate contents of digestible components (crude protein, fibre, lipids, nitrogen-free extract) to measured ME values (reviewed by Van der Honing and Steg, 1990). A range of equations linking ME or NE to composition and in vivo or in vitro digestibility measurements have been developed for various uses (reviewed by Van der Honing and Steg, 1990; Thomas, 1990; AFRC, 1993). Digestibility data from in vivo experiments exist for many temperate feeds, but estimates of in vivo digestibility based on in vitro digestibilities may be used where in vivo data are not available or where literature data may not adequately represent the particular feed samples being evaluated. The Tilley and Terry (1963) and enzymic procedures are commonly used to measure in vitro digestibility, although NIR methods using reference samples of known in vivo digestibility have become increasingly popular in the UK feeds industry in recent years.

2.5 Feed evaluation: protein

Protein is required for the normal functioning, milk and fibre production, and growth of ruminants. However, rumen microbes also require protein to allow them to perform their usual function of degrading feeds. Crude protein (CP) content, conventionally taken as nitrogen x 6.25, is used as the simplest, quantitative indicator of the protein content of feeds. Protein is degradable to yield nitrogen in the form of ammonia, and rumen microbes are able to re-synthesise protein to varying extents from ammonia

nitrogen. However, it must be noted that protein can be degraded to yield amino acids and peptides which may be utilised directly by rumen microbes, and the response to these may not be the same if the nitrogen is available as ammonia.

The digestibility of the protein of some feeds has been determined by animal feeding trials, so an estimate of Digestible Crude Protein (DCP) can be obtained by multiplying actual CP contents with literature digestibility coefficients. This approach is used with some success for concentrates used in temperate countries, but not for roughages where variability is greater and CP contents lower (McDonald et al., 1988). The following empirical equation has been used for grasses, hays and silages:

$$DCP\ (g\ kg^{-1}\ DM) = CP\ (g\ kg^{-1}\ DM) \times 0.9115 - 36.7$$

Measurements of the protein transactions in ruminants increasingly distinguish between protein degraded in the rumen to become available to the ruminant as microbial protein, or lost as ammonia, (rumen degradable protein, RDP) and protein digested post ruminally (undegradable dietary protein, UDP). Rumen degradable protein can be sub-divided into quickly degradable protein (QDP) and slowly degradable protein (SDP). Some SDP may escape degradation in the rumen and be digested post-ruminally, particularly at higher rates of passage.

The Metabolisable Protein (MP) system, recently adopted in the United Kingdom, estimates the amount of rumen degradable protein which is captured by rumen microbes, which in turn depends on the available energy (FME) assuming that energy supply, rather than protein supply, is the primary limiting nutrient. The estimation of the supply of protein depends on the measurement of the amounts of protein degraded in the rumen (AFRC, 1992; 1993). In vivo measurement of protein degradation requires animals with duodenal cannulae and is of limited accuracy. The nylon bag (in sacco) technique described by Ørskov and McDonald (1979) has therefore become the most widely used technique for providing working estimates for protein degradability studies (van Straalen and Tamminga, 1990). Kandylis and Nikokyris (1991) produced an extensive list of published nylon bag protein degradabilities for mainly temperate feeds. The mobile nylon bag technique is used to estimate intestinal digestion of protein due to difficulties in making in vivo measurements (for example, see Frydrych, 1992). A major assumption of the nylon bag techniques is that feed disappearing from the bag is degraded. The Acid detergent insoluble nitrogen (ADIN) assay is used as an indicator of the protein which is not degraded in either the rumen or lower gut (AFRC, 1993). While these techniques are the most useful currently available, they have their limitations both in terms of ease of use and accuracy (McDonald et al., 1988; Webster, 1992).

The French protein evaluation system (called the PDI system) similarly seeks to predict the protein supply to the animal. It assigns two protein values to each feed, a lower value when the feed is fed alone and a higher one indicating the potential value when the feed is associated with a suitable complimentary feed. These values are the sum of the feed protein undegraded in the rumen plus either the microbial protein synthesised when energy and nutrients are not limiting (called the PDIN) or the microbial protein synthesised from the energy available in the rumen when degraded nitrogen and other nutrients are not limiting (called the PDIE). When calculating the protein supply to

the animal (PDI value) the PDIN and PDIE values of all the ingredients of the diet are summed separately (PDIN and PDIE values must not be added together) and the actual PDI value is the lower of the two values.

For a fuller description of the MP system see AFRC (1992; 1993), and see Webster (1992) for a critical review of it. The French PDI system has been described by Vérité and Peyraud (1989).

2.6 Feed intake

Feed intake is a critical factor in temperate feeding systems as it can be a primary constraint to nutrient supply. Systems aimed at predicting the intake of forages have been devised, for example the Fill Unit system used in France (Dulphy et al., 1989). Fill values for forages have been estimated from animal feeding trials. Different fill values are used for different ruminants (sheep, cattle and lactating dairy cows), the fill values being inversely related to voluntary dry matter intakes. Fill values of forages are assumed to be additive. The Fill Unit system also estimates the substitution rate of forages for concentrates when forage intake alone is inadequate to supply the animal's requirements. Estimates were derived using data from extensive feeding trials. It was found that the substitution rate of concentrate for forage was negatively related to the magnitude of the difference between the energy of the forage fed alone and the energy requirements of the animal.

2.7 Formulating rations

In many temperate feeding systems, the diet is based on forages such as fresh grass pasture and hay, grass silage or other silages when fresh pasture is not available. Ruminants are usually offered a maximum amount of the forages grown on the farm and the minimum amount of concentrates necessary to meet the nutrient requirements of the animal in order to minimise feed costs. Ration formulation involves the estimation of the nutrient requirements of the animal, prediction of forage dry matter intake and the nutrients this provides. Then, by subtraction, the additional nutrients to be provided by the concentrate are estimated. Concentrate ingredients can then be selected and balanced to provide the required nutrients at lowest cost (for example formulating rations using the French feed evaluation systems is described by Jarrige, 1989).

3 Feed evaluation in LDC ruminant feeding systems

3.1 Constraints and opportunities in LDC ruminant feeding systems

The constraints to livestock production in LDCs are generally very different to those of intensive livestock production systems. Livestock keeping is often closely integrated with other agricultural activities, and hence may have multiple objectives. Production may be on a subsistence basis, so there may be little potential to purchase feeds from external sources. The feeding systems are very strongly influenced by feed availability rather than product outputs, unlike feeding systems used in intensive livestock production where feed supplements are often purchased from external sources.

Case study: semi-arid East Africa (Tanzania)

Jonsson et al. (1993) have recently described livestock production systems in the Babati district of northern Tanzania which is in many ways typical of semi-arid East Africa and where the above general constraints are very much in evidence. In the wet season cattle graze on lush green grasses which have a crude protein content of 8 to 10%, adequate for maintenance and some production. This is the season of peak milk production. However, in the dry season grasses senesce and their crude protein falls to 4 to 6%. The cattle lose weight, and if there is a drought large numbers perish.

Indigenous browse trees and shrubs are used to supplement the grass for dry season cattle feed, using feeds such as *Acacia tortilis* pods and a range of leaves. Trees and shrubs provide goat feed throughout the year. Herbaceous forage legumes are not commonly cultivated by farmers; the leguminous fodder tree *Leucaena leucocephala* has been widely planted, mainly on farm boundaries, but has been severely damaged by pysllid beetles. The majority of farmers do not have sufficient fodder for their animals and in the dry season the feed situation can be critical. Maize stovers and other locally produced by-products are used in the dry season, but the nutritive value of such feeds is generally poor. Most farmers purchase small amounts of sunflower seed cake or cotton seed cake, and other by-products such as beer brewing waste and maize bran as dry season supplements. However, the supply of purchased supplements tends to be unreliable, prices are high and they are only available in the towns. There is, therefore, an urgent need to evaluate a range of dry season fodders, such as tree fodders, which farmers can grow to provide a local and reliable source of supplement.

The objectives of farmers and the problems they have in feeding their livestock can be very diverse. For example milk and/or meat production may be a primary objective, but it may secondary to draught power and manure production. For example, Tanner et al. (1996) indicated that manure production appeared to be a high priority for some farmers in an intensive crop producing farming system in Indonesia. Feeding constraints may be seasonal, as can be demand for draught power, therefore farmers' priorities can also vary seasonally. These can vary from boosting milk production in times of feed plenty to survival in times of drought. These factors can affect the specific problems to which feed evaluation can be applied as a tool for identifying solutions. Hence, information on supplementation to try to balance protein-deficient straw based diets may be the priority in some farming systems, estimating the nutrient supply to animals to predict meat or milk production may be appropriate in others.

A major aspect of research into LDC feeding systems is trying to improve the balance of nutrients supplied from what are generally limited resources. The full potential of a feed will only be achieved as part of a balanced diet. Leng et al. (1992) indicated that growth and milk production were often only 10% of the genetic potential of the animal in LDCs due to imbalances in nutrients, disease and parasitism, and climatic conditions. However, nutrient imbalances appeared to be a primary constraint and one which potentially could be improved considerably by improved feeds and feeding strategies.

These constraints can be in the form of:

- low feed quality
- unbalanced availability of nutrients in feeds
- inadequate feed quantity
- seasonality of feed supplies
- conflicts with other resource uses
- lack of access to feeds.

Feed shortages are often seasonal, for example in semi-arid zones feeding animals during the dry season is a regular problem for farmers. In intensively cultivated regions, grazing and labour may be restricted during seasons when crops are being cultivated.

Case study: forest/agriculture system, Nepal

Thapa (1994) has described the farming systems used in the mid-hills of Eastern Nepal. The zone lies between 300 to 3000m in altitude, with some 80 to 90% of the annual rainfall falling during the May to September monsoon season. Climate and soil conditions can vary considerably depending on altitude and site, even over small distances. This complex and variable environment has led to a complex mosaic of land use. Here crop production, animal husbandry and forestry are components of integrated farming systems. Most farmers have some cattle, goats and buffalo. Ruminant livestock are important sources of farm income and dietary protein for the local population. However, they also play an essential role in providing draught power and manure for crop production. Chemical fertiliser is considered prohibitively expensive and is not readily available.

Livestock feeds are generally in limited supply during the dry season, so tree fodders are widely used to feed to ruminants to alleviate these shortages. There is a large number of tree species used by farmers in this zone, some 129 reported by Thapa (1994), of which 90 were used as fodder. Rice straw and other crop residues are stored for use as a dry season feed, however crop residues are generally protein deficient and very fibrous. Tree fodders are used as supplements to these poor quality feeds. Feed shortages are seen as a constraint to livestock production, which in turn can restrain crop production.

Various approaches have been investigated and/or adopted aiming at improving the diets available to ruminants by including feeds relatively high in protein to supplement roughages, giving diets which are more balanced in terms of protein and carbohydrate. These have included the use of forage legumes and tree fodders, treatment of staws with urea, urea supplementation, fodder storage, redistribution of feeds, supplements such as oil seed cakes and other agricultural by-products. Such approaches help to maximise the intake and digestibility of roughages and improve animal performance i.e. maximise the efficiency of use of scarce feed resources. Even in situations where meat and milk production is not the primary objective of the farmer, improved nutrition will generally help the animals survive during times of feed shortage and facilitate reproduction.

In more humid regions there can be similar seasonal feed shortages but a potential to sustain a higher intensity of livestock production. A wide diversity of fodders, including tree and shrub leaves and seed pods, are used. For example, Blair (1990)

listed some 270 species from 74 genera as being of potential value as fodders. Devendra (1992) listed common trees used as animal feeds such as acacias, calliandra, erythrina, ficuses, sesbania, gliricidia and leucaena. While leucanea and gliricidia have been relatively well researched, for many of even the more common species there is little or no quantitative data on their contribution to animal production. The trees used are often unimproved indigenous types. While their value is certainly not being underestimated, there must be potential to select for improved varieties of indigenous species, selectively introduce exotic species and develop improved feeding practices based on diverse feed resources.

3.2 Information on the nutritive value of feeds which LDC farmers require

The table below indicates where feed evaluation may help provide information to farmers.

Table 2 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	- assess improved feeds (selection during breeding programmes, treated feeds) - identify role/use of new feeds within existing feeding systems - compare new with existing feeds to assess potential benefits
approximate performance of livestock on available feeds	how to improve performance	- diagnose nutrient imbalances in diets - identify feed combinations which improve the nutrient balance of the diet

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), help identify roles of new supplements.
- What feed mixtures, from those available, will give the best performance?

In complex feeding systems, such as that described in Nepal above, 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.

3.3 Evaluation of feed resources

Different types of feeds can have different compositions and provide different nutrients to a diet. The distinction between roughages and supplements has been made above. In tropical feeding systems feed resources can be very diverse. Preston (1995) categorised feeds into five types and listed the information considered as being useful in assessing whether a resource should be used as feed. The five categories were:
Category 1 - feeds high in fibre and low in nitrogen e.g. crop residues such as cereal straws and stovers.
Category 2 - feeds high in fibre and relatively high in nitrogen e.g. tree fodders, animal excreta.
Category 3 - feeds low in fibre and low in nitrogen e.g. by-products of sugarcane.
Category 4 - By-products low in fibre and high in nitrogen e.g. oil seed cakes.
Category 5 - By-products high in oil and fibre, low in nitrogen e.g. by-products from African oil palm.

The different categories of feeds are sources of different nutrients, therefore feed evaluation should be tailored to evaluating the nutrients which particular feed categories provide to the diet. The following information will be required to assess the potential value of these resources (adapted from Preston, 1995):
- The digestibility of the organic matter (for categories 1,2 and 5).
- The availability of nitrogen for micro-organisms (category 2, rather than category 3 as suggested by Preston, 1995).
- The by-pass protein for ruminants (category 2, rather than category 3 as suggested by Preston, 1995).
- The amino acid balance (category 2 and 4, particularly for monogastric feeds).
- How to protect the protein and oil for feeding to ruminants (categories 4 and 5).
- Does the product contain secondary plant compounds and how can these be neutralised? (category 2 in particular, possibly other categories as well).

To this list could be added a prediction of voluntary intake, which in many situations may be a primary limitation on animal performance for livestock fed high fibre feeds.

The information will be suitable for examining how the resource, if suitable as a feed, can best be used in feeding systems with other locally produced resources. This will include consideration of the complimentarity and interactions between feeds. Evaluation of feeds in isolation is unlikely to be appropriate in LDC feeding systems.

4 Relevance of current evaluation techniques to LDC feeding systems

4.1 Overview of limitations

Although chemical analysis of feeds has seen some refinements, the essentially 19th century approach of partitioning feeds into components which can be related to nutritional parameters is still in use. This in itself indicates the utility of the system. However, it also indicates that the conventional approach has been unable to develop to overcome the deficiencies which have become evident in existing techniques.

The limitations of current feed evaluation techniques in temperate feeding systems have been discussed in a recent workshop (BSAP, 1993). Some of the more important limitations are the inability to take account of interactions between feeds, lack of accurate prediction of feed intake and a general lack of flexibility and, at times, accuracy. Conventional fibre assays may not adequately reflect the considerable diversity in composition and nutritive value of different carbohydrate fractions, but more detailed analysis by conventional methods is too costly for routine use. There are also further limitations which apply more to feeding systems in LDCs. Current methods do not specifically take into account the effects of anti-nutritive factors. Most temperate feeds do not contain factors of this type, but they can be important in many tropical feeds. In general the limitations are omissions rather than fundamental flaws in the theoretical framework. Another class of limitations relates to the ability of currently available techniques to provide accurate estimates of the parameters needed. This will be discussed in more detail below.

Feeds are normally evaluated in vivo or in vitro under conditions which are not deficient in major nutrients. However, in LDC feeding systems diets are often unbalanced and deficient in protein. While this does not alter the general validity of the ME and MP systems outlined above, it does mean that the nutritive value of roughages evaluated under conventional in vitro (or in vivo) conditions may overestimate the actual contribution of the feed under on-farm conditions (if digestibility and intake are limited by a shortage of dietary protein or other nutrients).

4.2 Interactions between feeds

Although feed evaluation systems generally assume that feeds do not interact, it has been known for a long time that such interactions (otherwise known as associative effects) can occur. Forbes et al. (1933) measured the net availability of metabolisable energy (ME) of maize meal in fattening steers and found that it differed markedly according to the basal ration on which the animal was fed. If maize was added to a ration of oat straw, corn meal and molasses, a higher efficiency was obtained than if it was added to a ration of alfalfa hay, linseed meal and bran. The latter was in turn higher than that found when maize was added to a basal ration of timothy hay. Hamilton (1942) demonstrated the depression of the digestibility of cellulose by the addition of starch or glucose to the diet. It occurred when starchy grains were added to roughage diets, particularly those low in nitrogen content. Watson (1945) gathered evidence to show that, when mixtures of different feedingstuffs are given to animals, the apparent digestibility of the mixture was not necessarily the same as the weighted sum of the apparent digestibilities of its components. This phenomenon was called

'associative digestibility'. Although well documented, the mechanisms of these interactions between feeds were (and in many ways still are) poorly understood and difficult to predict.

In 1962, Blaxter, when outlining the basis of the new ME system, acknowledged the associative effects of feedstuffs both in ME and in digestibility and proposed an explanation. He hypothesised that the associative effects on ME found by Forbes et al. (1933) arose from the different type of fermentation produced when maize was used to supplement the different rations. The depression in cellulose digestibility found by Hamilton (1942) could be accounted for by the depletion in soluble nitrogen (and possible other essential nutrients) in the fluid phase of the rumen resulting from the rapid growth of starch-fermenting organisms. This had a consequent negative effect on the cellulolytic flora. In a similar way, the addition of protein-rich material could cause an increase in the apparent digestibility of a low nitrogen roughage. One of the fundamental assumptions in the new ME scheme was that the values for individual feeds could be added together to obtain the metabolizable energy value of whole rations (principle of additivity). This implied a rejection of all associative effects on the digestibility of the feed. Blaxter argued that, although these effects existed, their magnitude was usually not large, particularly if time was allowed for an equilibrium to be established between the microflora in the gut and the change in diet. They were not considered likely to limit the usefulness of the scheme in practice. Since then, the prevalent feeding systems based on ME, both in Europe and the USA, have assumed additivity of ME (and digestibility of all other nutrients) in ration formulation. Nevertheless fermentable carbohydrate, protein, nitrogen and minerals can stimulate substantial and important interactions which affect digestibility and intake in vivo (reviewed by Oldham and Emmans,1990).

4.3 Studies on feed interactions

There have been several studies using temperate feed mixtures, with a range of responses observed. Interactions between energy and protein for a range of mixtures of maize silage and red clover were measured in sheep (Margan *et al.*, 1994). Positive interactions were observed for voluntary ME intake, digestibilities of energy, nitrogen and cell wall organic matter, and energy and nitrogen balances. It was hypothesised that the more efficient use of dietary energy and nitrogen could be attributed to improved microbial synthesis of rumen-degraded clover nitrogen and/or synchronous absorption from the duodenum of non-ammonia nitrogen from the clover and undegraded maize starch from the silage. Glenn (1989) found interactions between mixtures of lucerne (*Medicago sativa*) and orchardgrass (*Dactylis glomerata*) which increased rumen microbial protein synthesis and tissue nitrogen retention. The author suggested these results indicated synergism in rumen fermentation of fibre and nitrogen from mixtures of the two plant species.

Negative interactions on the digestibility of fibre were found between mixtures of tyfon (*Brassica campestris*) and hay fed to sheep (Cassida et al., 1994). The inhibition of fibre digestibility (NDF, ADF and cellulose) was attributed to the likely reduction in rumen pH caused by the readily fermentable carbohydrates. Reid et al. (1987) studied the effect of feeding mixtures of two grass hays, orchard grass (*Dactylis glomerata*) and perennial ryegrass (*Lolium perenne*), and two legumes, alfalfa (*Medicago sativa*)

and red clover (*Trifolium pratense*), individually and in different combinations to growing lambs. For all combinations a small, negative interaction was observed in dry matter digestibility. A positive interaction was found in dry matter intake. Moss *et al.* (1992) measured the interactions, in vivo and in vitro, of a range of mixtures of grass silage:straw, grass silage:maize silage, and maize silage:lucerne silage. For the mixtures of maize:lucerne and maize:grass, negative interactions were found for the digestibility of the organic matter (OMD) that were suggested to be due to the starch in the maize inhibiting fibre digestion. However, the inhibition was less marked with mixtures of maize:lucerne. The authors suggested that this was possibly due to the higher buffering capacity of lucerne compared to the grass. The negative associative effects found for the grass:straw mixture were considered to be due to the lack of readily fermentable carbohydrates limiting the utilization of the soluble nitrogen in the mixtures.

While both positive and negative interactions have been found between temperate feeds, work in tropical regions has tended to concentrate on supplementing roughages with protein sources to obtain positive interactions. Osuji et al. (1995) described how the relatively protein-rich fodder from Sesbania increased the digestibility and rate of passage of protein-deficient teff straw. Positive interactions in in vitro NDF digestion were observed when the legume *Aeschynomene americana* was combined with mature grasses of low protein content (Brown et al., 1991). The magnitude of the associative effects was similar to the increase in in vitro NDF digestion of pure grass when rumen fluid from a donor animal fed low quality hay plus supplementary nitrogen was used, suggesting that *Aeschynomene americana* provided soluble and/or degradable nitrogen to the in vitro fermentation. The fermentation kinetics were studied and it appeared that the likely mode of action of the positive interactions was a reduced lag time in the initiation of fibre digestion. Interactions were found at 48 hours but not at 96 hours of incubation.

Ben Salem et al. (1996) studied the supplementation of wheat straw with spineless cactus (*Opuntia ficus indica* var. *inermis*). Spineless cactus was considered to be a good source of readily available carbohydrates and had a protein content two and a half times higher than that of wheat straw. Spineless cactus decreased the dry matter and NDF disappearance from nylon bags incubated in the rumen. Intake of straw was increased with increasing cactus supplementation (to 600 g DM per head per day), apparent digestibilities also tended to increase. The increase in digestibilities was explained by an improved rumen nitrogen supply as well as the availability of readily fermentable carbohydrate. The (perhaps surprising) reduction in celluloytic activity was suggested as being related to increased numbers of protozoa reducing the numbers of rumen bacteria.

4.4 Mode of action of feed interactions

As can be seen above, several authors have hypothesised about the likely mode of action of feed interactions. Minson (1990) suggested that 'synergism' or positive associative effects would only occur where one species is deficient in an essential nutrient, which is provided by the second forage. This could occur between nitrogen-rich and nitrogen-deficient feeds, but also between feeds rich and deficient in rapidly fermentable carbohydrate. Ørskov (1995) has suggested that provision of easily

fermentable fibre could stimulate the fermentation of less degradable fibre by stimulating microbial activity and increasing the number of microbes in the rumen.

Negative associative effects have also been reported in the literature, most commonly the depression of fibre digestion by the addition of a feedstuff rich in highly fermentable starch or sugars. This has been explained by the depletion of soluble nitrogen due to the rapid growth of amylolytic organisms using highly fermentable carbohydrates as an energy source. This shortage of nitrogen causes a reduction in the growth rate of cellulolytic organisms and therefore the cellulose digestibility is decreased (Blaxter, 1962; Garnsworthy and Cole, 1990). Rapid fermentation can also cause a fall in rumen pH, which again can inhibit cellulolytic activity. Mould et al. (1983) demonstrated that both mechanisms could operate together. Chesson and Forsberg (1989) highlighted the importance of the form of the fermentable carbohydrate and Oldham (1984) stressed the amount and form of dietary crude protein in determining the type and scale of associative effects. As for the effect found when high concentrations of fat in roughage diets decrease the digestibility of fibre in the rumen, Devendra and Lewis (1974) suggested that this may be due to physical coating of the fibre by the fat, toxic effects of fat on rumen microbes, surfactant effects of fatty acids on cell membranes or the formation of insoluble cation soaps.

Hence, while progress has been made in describing and explaining some associative effects, they are not predictable using current feed evaluation techniques. Generally these effects have been investigated in vivo in the absence of established in vitro methods. In LDC feeding systems, negative interactive effects associated with high fat contents are not encountered very frequently as fatty feeds are not common. High levels of very fermentable carbohydrate are also not commonly found in LDC systems as cereal grains are rarely used as ruminant feeds. However, supplementation of fibrous protein-deficient feeds (such as crop residues) with protein sources (such as tree fodders) is very common. Tree fodders could fulfill two roles as supplements to crop residues, stimulating microbial fermentation by providing both degradable protein and easily fermentable carbohydrate. Optimising this type of interaction is potentially an important focus in the development of improved feeding systems.

4.5 Intake

Apart from the nutrient content and digestibility of feeds, a major factor in determining the performance of animals is the amount they actually eat. This depends on animal as well as feed factors. The size, species and breed of animal; amount of feed offered to the animal, which can be restricted in LDC feeding systems in times of shortage; the form in which the feed is offered e.g. chopped, mixed etc.; husbandry practices, e.g. the time animals are given to graze, are all important factors in determining feed intake. However, the nature of the feed can also greatly influence intake.

The factors which control voluntary feed intake of ruminants are complex and not fully understood (reviewed extensively by Forbes, 1995). Palatability of the feed may be a factor. Palatability certainly affects the choice of feeds, although it is unclear to what extent intake is affected when there is no choice (unless the feed is completely unpalatable). Rumen fill can be important in intake control: as the rumen is filled with undigested feed, intake is inhibited. This appears to be the major factor controlling

intake of roughages which are digested slowly and to a relatively limited extent. For more rapidly and completely digestible diets chemostatic sensory signals appear to be important. These two mechanisms appear to be additive rather than mutually exclusive. Nutrient demand is an additional factor which can affect intake.

Roughages which are degraded slowly stay in the rumen longer than more digestible feeds. There is, therefore, a relationship between digestibility and feed intake, at least for roughages. Therefore some attempts have been made to predict the intake of roughages from nylon bag and in vitro digestibility assays as described in Sections 5 and 6. A mathematical model to predict daily DMI and account for effects of energy supplementation on forage intake has been developed based on the contribution of various feeds to ruminal fill (Hyer et al., 1991a). The model correctly predicted effects of energy supplementation and forage composition on forage intake (Hyer et al., 1991b). However, the model failed to predict the increased forage intake typically observed with protein supplementation, suggesting that it was insufficient for intake prediction in protein-limiting situations. Indeed, none of the existing models for predicting voluntary intake are able to consider interactions between nutrients from different feeds in the diet (Ingvartsen, 1994).

Leng (1990) has suggested that increased intake in response to protein supplements under tropical conditions is due to an increased efficiency of utilisation of digested nutrients which in turn reduces heat stress as the inefficient oxidation of nutrients to produce waste heat is reduced.

4.6 Anti-nutritive factors

Some plants have apparently developed defense mechanisms to protect themselves from herbivores; the accumulation of compounds which deter herbivores is one such mechanism. These compounds can be toxins, but also include anti-nutritive factors which have less severe short term effects on the animals which consume them. There are a wide range of anti-nutritive compounds which have equally diverse mechanisms of action (reviewed by Kumar and D'Mello, 1995). These compounds include tannins, cyanogens, saponins, non-protein amino acids, alkaloids, coumarins, phytohaemagglutinins and oxalate. A detailed consideration of these is beyond the scope of this review. As tannins are widely found in ruminant diets used in LDCs, these will be described briefly below. However, non-tannin anti-nutritive factors are certainly important in some feeds.

Tannins (sometimes called polyphenols due to their chemical structures) are found widely in plant material, in both leguminous and non-leguminous species. Of most significance to ruminant feeds is that many, if not nearly all, species of forage and browse legumes contain tannins. Tannins are not restricted to tropical feeds; lotus, sainfoin and other temperate species have been found to contain condensed tannins (Waghorn et al., 1990). Tannins are not confined to just trees and shrubs. Makkar (1993) lists 28 agro-industrial by-products which might be useful as feeds. 19 of these by-products, including various seeds and seed cakes, have been shown to contain tannins. Cassava leaves, a potentially useful forage, contain condensed tannins (Reed et al., 1982). Sorghum grain and stover can also contain tannins (Mueller-Harvey and

Reed, 1992). Therefore many forages, several crop residues and by-products of considerable importance as livestock feeds in LDCs contain tannins.

The effects of tannins on ruminants have been reviewed by Makkar (1993), Hagerman and Butler (1991) and Kumar and D'Mello (1995), and on monogastrics by Jansman (1993). The major effects can be summarised below:
a) It has not been conclusively demonstrated that tannins reduce feed intake, although they do affect feed selection.
b) Tannins impair feed conversion efficiency.
c) Tannins reduce apparent digestibility of protein and, to a lesser extent, energy.
There are a range of mechanisms by which tannins exert these effects. Tannins can affect the mouth feel of a feed by binding to salivary proteins, hence reducing palatability. By binding to feed components, particularly protein, it can reduce the availability of the feed to rumen microbes. Under some circumstances this could have a positive effect on animal performance, as it could potentially protect protein in the rumen making it available for digestion in the lower gut, hence potentially improving the overall efficiency of protein utilisation by the animal. Tannins may also directly inhibit rumen microbes. Tannins, and/or their breakdown products, can also be absorbed by the ruminant where they can have direct toxic effects.

Current feed evaluation may take into consideration effects listed in (c) above, if the techniques used are suitably sensitive to the effects of tannins on protein and dry matter digestibility. For example, in vitro digestibility assays may be useful indicators of the nutritive value of tanniniferous feeds while fibre and crude protein determinations are likely to be of limited value. However, there is little information on the effects of anti-nutritive factors on feed conversion efficiency and, at present, no framework for including such information in feed evaluation systems.

4.7 Suitability of current feed evaluation methods for ruminant feeds for LDCs

Preston (1995) stated that the levels of production achieved in tropical feeding systems can be considerably less than the level predicted by conventional feed evaluation. It is widely accepted that the roughages commonly fed in LDCs are slowly degraded in the rumen, yet there may be no socio-economically viable alternatives. However, Leng et al. (1992) stated that "it has been a major misconception throughout the scientific literature that low productivity of ruminants fed on forages is a result of the low energy density (i.e. low digestibility) of feeds... low productivity in ruminants on forages results from inefficient utilisation of the feed because of deficiencies of critical nutrients in the diet". While noting that lack of nitrogen supply to rumen microbes and the animal itself depresses feed intake, Hogan (1996) suggested that restricted intake and digestibility of roughages will remain a constraint even when the protein supply deficiencies have been overcome.

Environmental differences between temperate and tropical regions, notably the effect of heat stress on ruminants in the tropics, have been suggested as making the feed requirements and standards developed for temperate countries incorrect under tropical conditions. Leng (1990) has even questioned how useful as a practical tool the concept of ME is under tropical conditions, given the range of physiological conditions of animals, management regimes used and climatic conditions. In particular, under

tropical conditions ruminants show a greater response to protein supplementation than under temperate conditions (Leng, 1990; Preston, 1995). Leng et al. (1992) reported that by balancing roughage based diets, animal growth can improve by 2-3 fold and the efficiency of animal growth by as much as 6 fold over previous estimates. Kaiser and Weniger (1995) reported an overall reduction of protein digestibility under heat stress related to thermoregulation; maintenance energy requirements also increase. Solis et al. (1991) found that growth of Pelibuey lambs in tropical southern Mexico had higher energy requirements than generally reported for sheep under temperate conditions, which may relate to environmental and/or genetic differences. It is certainly the case that the equations developed to predict animal performance from feed evaluation data are based on feeding trials conducted on temperate feeds under temperate conditions. It is a matter for debate whether there are fundamental problems with the ME system framework, or whether more appropriate equations need to be developed.

Ørskov (1995) noted that the most common constraints which reduce the rumen fermentation of fibrous feeds are deficiencies in protein and to a lesser extent sulphur, the number of bacteria in the rumen fluid to invade newly ingested feed, antimicrobial factors and low rumen pH. In LDCs the types of energy concentrates which can cause low rumen pH values are rarely fed. Many ruminant diets in LDCs are not balanced, particularly at times of feed shortage such as in dry seasons. Deficiencies in sulphur, phosphorus and magnesium are not uncommon in tropical regions, but ensuring an adequate protein supply (which can include non-protein nitrogen supplements) is the first priority in optimising forage digestion (Leng, 1990; Ørskov, 1995). Protein or nitrogen supplementation can greatly enhance both the intake and digestibility of crop residues. The second major need is to increase the ratio of protein (absorbed amino acids) to energy (VFA) so that it more closely corresponds to the animal's requirements under tropical conditions (Leng et al., 1992). Ensuring that the rumen microbes have sufficient nitrogen to optimise microbial protein synthesis will help to do this.

However, it is usual to evaluate feeds using in vitro protocols (described below) which ensure that degradation takes place under conditions where there are no major deficiencies of nutrients. Therefore, there is an apparent mis-match between the conventional protocols for in vitro techniques and the desire to investigate the supplementation of nutrient-deficient feeds. Similar criticisms could also be made of some protocols used for in vivo experiments (including the nylon bag technique). It is also doubtful, given the wide variety of animal breeds, husbandry methods and environments in LDC farming systems, whether reliable predictions of animal performance are obtainable, at least in absolute terms.

4.8 The nitrogen sufficiency of feeds

Evaluation of diet components using the MP system will enable major imbalances between energy and protein supply to be identified and suggest supplements to alleviate them. This information is useful in designing animal feeding trials, but for many LDC feeding systems in itself is unlikely to be a reliable basis for advice to farmers. One way of determining if a diet has sufficient rumen degradable protein is to measure rumen nitrogen levels. Preston (1995) recommended taking a sample of rumen fluid 4 to 6 hours after feeding or following the commencement of grazing.

The method requires the use of rumen fistulated animals or taking a sample of rumen fluid by putting a tube down the animal's throat. General guidelines on the minimum ammonia nitrogen concentrations at which nitrogen supply to rumen microbes is not limiting range from 60 mg N l^{-1} (Osuji et al., 1995) to 150 to 200 mg N l^{-1} (Preston, 1995). Morrison and Mackie (1996) noted this clear lack of consensus, reporting published values between 2.5 and 18 mM (35 to 252 mg N l^{-1}).

In vitro studies have indicated minimum nitrogen requirements of 50 mg N l^{-1} for microbial growth (Satter and Slyter, 1974) and 88 to 100 mg N l^{-1} for the digestibility of low quality feeds(Oosting et al., 1989). Mehrez et al. (1977) reported that for sheep fed on whole barley the maximum rate of fermentation required a minimum rumen ammonia concentration of 235 mg l^{-1} (194 mg N l^{-1}). Erdman et al. (1986) suggested that there may be different nitrogen requirements for maximal microbial growth and fermentation of feeds. They also found that there was a relationship between the fermentability (extent of fermentation) of the feed and minimum rumen ammonium concentration required for the maximum rate of fermentation, deriving the relationship:

minimum ammonia concentration (mg l^{-1}) = 4.52 x fermentability (%) - 157.1

This indicated that feeds with a rumen dry matter degradation of 45% would required a minimum of about 50 mg l^{-1} rumen ammonia (41 mg N l^{-1}) while an 80% fermentable feed would required about 200 mg l^{-1} ammonia (165 mg N l^{-1}). The different recommended nitrogen requirements noted above may well reflect differences in the energy sources being fed (typically slowly digestible roughage and rapidly digestible by-products of sugarcane, respectively, for Osuji et al., 1995, and Preston, 1995); minimum rumen ammonia nitrogen concentrations are probably very dependent on the basal diet.

5. The in vitro techniques for estimating digestibility

5.1 Tilley and Terry two-stage method

In vitro techniques for evaluating feeds started over 100 years ago with the use of digestive enzymes to mimic physiological processes,(reviewed by Faithfull, 1984). However, it is only comparatively recently (compared to chemical analyses of feeds) that in vitro techniques have become a widely used in the evaluation of feeds for ruminants. Tilley and Terry (1963) described a two-stage method in which a feed sample (0.5 g) is digested in buffered rumen liquor taken from rumen fistulated animals for 48 hours (40 ml buffer + 10 ml strained rumen fluid), followed by pepsin digestion in an acidic solution. Fibre digestion by rumen microbes had been completed within 48 hours, but the residue contained unchanged feed protein and microbial protein. 48 h incubation in acid pepsin solution was used to digest these materials. Digestible dry matter was estimated from the weight of the residues following the two-stage digestion. The method was initially calibrated using samples of grass, clover and lucerne. The following regression equation was obtained:

In vivo digestibility = 0.99 x in vitro digestibility - 1.01

The method was standardised using two feeds of high and low digestibility. Due to variations between runs, it was recommended that similar feeds should be compared

within the same run. This method has been widely adopted due to its relative simplicity and the usefulness of the data provided.

The technique is used in the analysis of farm roughages for advisory purposes and for determining the digestibility of small samples to assist in plant breeding (McDonald et al., 1988). It has been used in various modified forms, the most common being to add a protein or nitrogen source when evaluating low-nitrogen feeds to ensure the in vitro system is not nitrogen deficient (Madsen et al., in press). There are also a range of regression equations relating in vitro and in vivo digestibility in use so that in vivo digestibilities can be predicted. For example, the expression below is used for grass products and whole-crop cereals in Denmark (Madsen et al., in press):

In vivo digestibility (%) = 4.10 + 0.959 x in vitro digestibility (%)

Variations in the regressions may relate to differences between the feeds evaluated, differences between laboratories and random differences between different studies. ADAS (1991) have suggested that one relationship could probably be used satisfactorily for fresh herbage, grass silage and grass hays. 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 apparent digestibility of mixed oak and alfalfa diets, Nastis and Malechek (1988) found the Tilley and Terry technique to be an inaccurate predictor of in vivo digestibility. It was concluded that separate regression equations are required for browses, possibly due to the influence of tannins. Hence, to obtain the most accurate predictions of in vivo digestibility, different regression equations are required for feeds with widely different compositions.

In a ring test involving three laboratories in Denmark the Tilley and Terry method appeared to be robust for a wide range of feeds (cereal grains, silages, hay and straw) over a wide range of digestibilities (from below 40% to over 90%), although a high degree of standardisation was required (Madsen et al., in press). Goldman et al. (1987) obtained a set of 482 ruminant feeds, from 23 plant species, with digestibilities from 0.25 to 0.90 determined in vivo from 21 laboratories all over the world. A sub-sample of 422 feeds was selected as being suitable and reliable (i.e. excluding doubtful data, small and highly exceptional sources), and correlations between in vitro and in vivo DMD, OMD and digestibility of organic matter in dry matter (DOMD) were investigated. The regression equations obtained were:

DMD = 0.0148 + 0.933 x IVD (R^2 = 0.922)

OMD = -0.0066 + 0.978 x IVD (R^2 = 0.907)

DOMD = 0.021 + 0.875 x IVD (R^2 = 0.931)

It was concluded that the reliability of the in vitro assay was improved when calibrated against in vivo data from several laboratories. Reproducibility of in vitro determinations was generally good, so that Goldman et al. (1987) concluded that in vitro estimates would probably yield more reliable digestibility estimates than those obtained from most individual in vivo experiments. Each digestibility trial in animals depends on many factors which are difficult, if not impossible, to repeat fully. Due to their time consuming nature and expense, it is rare that in vivo trials are repeated. It was suggested that the Tilley and Terry method would be an appropriate basis for a reliable international feed digestibility system, especially useful in countries where facilities for comprehensive in vivo studies are not available. Genizi et al. (1990) concluded that the preferred approach would be to use feed samples and a calibration

equation derived from a large data set produced by a central laboratory rather than a small set of dubious in vivo data. The use of standards for calibration appeared to be inefficient because of small and random variation of deviations from the standard in a normal run. Rather, Genizi et al. (1990) suggested that standards should be use to identify deviant runs whose data should be discarded.

The Tilley and Terry method has been the most widely used in vitro digestibility method, so approaches to standardisation and calibration are of major relevance to the newer methods such as gas production. There is a divergence of opinion reviewed above as to whether data should be standardised to reference feeds (most strongly advocated by Menke et al., 1979, for the Menke gas production method) or whether references should be used essentially to check that a particular batch of incubations has proceeded satisfactorily. A strong case for widespread collaboration to calibrate in vitro data against in vivo data has emerged. These equations may be different for different types of feeds, but there may be many types of feeds where a single equation may be appropriate. Goldman et al. (1987) found some significant species effects resulting in deviation from the general correlations given above. The extremes were -0.044 and -0.038 for oats, -0.040 and -0.021 for sudangrass, +0.033 and +0.043 for wheatgrass for DMD and OMD respectively and +0.028 for birdsfoot DMD. However, compared to the range and diversity of feeds used in tropical feeding systems, 23 species may be considered a very small range of feeds.

Nastis and Malechek (1988) found that the diets of the animals from which rumen fluid was taken affected the Tilley and Terry in vitro digestibility of the oak and alfalfa mixtures. In contrast, Coppock et al. (1988) found little substantial differences in Tilley and Terry in vitro digestibility values of grasses, shrubs and browses (trees) when inocula from indigenous (Kenyan) sheep, goats, a camel and Merino sheep were used. The different types of animals had been fed on different diets prior to the taking of rumen fluid. No significant ($P>0.05$) effects were found on grass digestibility, but browses were digested more by inoculum from indigenous sheep than from Merino sheep ($P<0.01$). Overall indigenous sheep inoculum was more active than the other three inocula. Differences were due to small, but consistent, differences between the inocula. Coppock et al. (1988) suggested that the inoculum is probably able to adapt quickly to the feed during incubation, thereby negating most differences that may occur in the microflora of the rumen fluids. It was suggested that for feed evaluation an inoculum source from laboratory animals fed a standard diet is probably preferable to trying to obtain inoculum from indigenous species consuming their usual diets.

Therefore, there are some indications that the source of rumen fluid may have some minor effects on in vitro fermentation characteristics. This appears to be particularly the case for taniniferous feeds, although even for these differences appear to be small. There is no evidence that rumen fluid donor or diet of the donor is critical to the accuracy of in vitro techniques, although standardisation of donor and diet is probably desirable.

5.2 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 pretreatment of herbage with acid pepsin then treatment with a range of cellulase preparations. Acid pepsin pretreatment 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.

The pepsin-cellulase method required separate prediction equations for grasses and legumes (Terry et al, 1978), although (with modification) cellulase techniques can be as accurate as Tilley and Terry in predicting in vivo digestibility (Dowman and Collins, 1982). The numeric values of the in vitro digestibilities are less close to the in vivo values than is achieved by the Tilley and Terry method (Omed et al., 1989), which make them less readily interpretable. Furthermore, only methods which use microbes will be useful for investigating interactions between feeds when the interactions are related to stimulation or inhibition of microbial growth. Nevertheless, NCD can be used to predict the ME of compound feeds (concentrates) with a very low residual error (reviewed by Thomas, 1990). The prediction equation derived was:

ME(MJ/kgDM) = 0.25 x Oil Content (g/kg DM)+ 0.14 x NCD

where NCD is cellulase digestible organic matter in the dry matter following neutral detergent extraction (Downman and Collins, 1982).
Madsen et al (in press) describe the following equation being used in Denmark for concentrates:

In vivo digestibility (%) = 5.38 + 0.867 x NCD (%).

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.

5.3 Rusitec

Czerkawski and Breckenridge (1977) described apparatus and a methodology for the long-term simulation of rumen fermentation. This method allows a detailed study of the long term dynamics of fermentation to be undertaken, unlike the end point methods described above or the essentially batch fermentations described below. However, it is very demanding in equipment and labour and so is generally of use in specific detailed research investigations rather than for routine feed evaluation.

5.4 Nylon bag (in sacco) method

5.4.1 Methodology

Although an essentially in vivo technique, the nylon bag method has characteristics in common with the purely laboratory in vitro methods, and is therefore included in this 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 digestibility data, or by incubating bags for different lengths of time, can give data on the rate of feed degradation (kinetic data). 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. As noted above, it has become widely used for such measurements which are used in the MP system. Similarly, the Nordic protein evaluation system (Madsen et al., in press) estimates the amino acids truly absorbed from the small intestine (AAT) and protein balance in the rumen (PBV). This also uses the degradation parameters derived from the nylon bag technique. The French PDI system was revised to similarly use the nylon bag technique (Vérité and Peyraud, 1989).

The nylon bag method can also be used to investigate the effects of changes in the rumen environment. Erdman et al. (1986) infused rumen fistulated cows with urea and measured the effects of rumen ammonia concentration on the degradation of feedstuffs incubated in the rumen in nylon bags. Fistulated animals can be fed on different diets and the effect of these diets on the degradation of a standard feed measured. Unmolassed sugar-beet pulp and dried grass have been shown to increase the rate and extent of straw degradation (Silva and Ørskov, 1988). This approach could also be used to measure the effect of protein supplements in the animal's diet on the degradation of a roughage placed in nylon bags. In this way the nylon bag method can be used to investigate interactive effects between feeds, although it does involve changes of diets of the fistulated animals which is time consuming and expensive.

5.4.2 Analysing nylon bag data

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 kinetic data (there is no theoretical reason for not using the end point assays to produce kinetic data by incubating for different times, but this approach requires labour demanding multiple incubations). This make the analysis and interpretation of the data more complex, but potentially much more useful.

Nylon bag kinetic data is usually analysed by fitting an exponential equation (Ørskov and Mcdonald, 1979):

$$Y = a + b(1-e^{-ct})$$

or a similar equation with a lag time (McDonald, 1981)

$$Y = a + b(1-e^{-c(t-L)})$$

where Y = degradability at time t

a = intercept (degradability at time 0; i.e. material washed from the bag)

b = potentially degradable fraction

c = rate constant for the degradation of b

L = lag time (from time 0 to the time when the maximum rate of degradation starts)

a + b has been termed the potential degradability as it represents all the feed which can be degraded in the rumen if incubated for a sufficiently long time.

L can be calculated using the expression:

$$L = 1/c(b/a + b - A)$$

where A is the measured amount of soluble material in the sample, b and c as defined above. Parameter B may also be used, where B is defined as the insoluble but fermentable material, calculated using the equation:

$$B = (a + b) - A$$

(Ørskov and McDonald, 1979; McDonald, 1981; Ørskov and Ryle, 1990). Sigmoidal models (a logistic and Gompertz-like model), which do not have a discrete lag time, have been suggested as being more appropriate for analysing nylon bag data (van Milgen and Baumont, 1995). However, these mathematically more complex models have not, as yet, been widely adopted.

Fitting the equation performs two functions; firstly it provides a number of interpretable parameters to describe the data and secondly it enables all the experimental data to be used across the range of incubation times to obtain these parameters (compared to taking single values at particular incubation times). These equations can be applied to dry matter and protein disappearance, or any other parameter determined by this method.

Parameters from the nylon bag technique have been correlated with in vivo digestibility, dry matter intake, digestible dry matter intake and growth rate. It is noteworthy that techniques which might initially have been regarded as useful in producing predictions of digestibility (or rumen degradability) have increasingly been explored as predictors of intake and performance as described below. Ørskov and Ryle (1990) proposed a single index figure generated from nylon bag technique parameters aimed at integrating degradation rates and extents for ranking feeds and predicting minimum feed quality for animals to meet maintenance requirements.

5.4.3 Use of nylon bag to predict in vivo parameters

There have been several investigations on the use of the nylon bag method to predict in vivo parameters such as digestibility and DMI. Nylon bag and in vivo determinations have been conducted on single feeds which usually (but not always) have been supplemented to avoid nutrient deficiencies. The literature is reviewed separately for different types of feeds, selected prediction equations are given in Table 3.

Table 3 Correlations between nylon bag dry matter disappearances and in vivo parameters

Feed	In vivo parameter	Correlation equation	Correlation coefficient etc.	Source
straw	DMI (kg/day)	= -1.56 + 0.159a + 0.0658b + 56.4c	(r = 0.88)	a
straw	Digestible DMI (kg/day)	= -2.576 + 0.0554a + 0.0640b + 37.7c	(r = 0.95)	a
straw	Growth rate (g/day)	= -1.267 + 0.0571a + 0.0126b + 17.02c	(r = 0.95)	a
hays and straws	DMI (g DM per kg $M^{0.75}$)	= 29.2 + 1.23a - 0.006ADF	(R^2 = 0.83)	b
roughages	DMD(g/kg)	= 376 + 0.41A + 0.039B + 6.44c + 2.0L	(R^2 = 0.19)	c
hay	Intake	= 13.4 + 0.58(a + b) + 203.2c	(R^2 = 0.772)	d
hay	DMD	= 10.9 + 0.71(a + b) - 82.6c	(R^2 = 0.749)	d
graminaceous hays	Intake	= -11.5 + 0.60(a + b) + 563c	(r = 0.834)	e
graminaceous hays	DMD	= 174 + 5.0(a + b) - 922c	(r = 0.775)	e
all hays	Intake	= 13.5 - 0.31(a + b) + 328c + 0.066CP	(r = 0.862)	e
all hays	DMD	= 140 + 6.43(a + b) + 175c - 0.37CP	(r = 0.775)	e
hays	DMI	= 21.3 + 0.0733a + 138c	(R^2 = 0.897)	f
tropical browses	DMI (kg/day)	= -8.286 + 0.266A + 0.102B + 17.696c	(r = 0.90)	g
tropical browses	digestible DMI (kg/day)	= -7.609 + 0.219A + 0.080B + 24.191c	(r = 0.93)	g
tropical browses	Growth rate (g/day)	= -0.649 + 0.017A + 0.006B + 3.87c	(r = 0.93)	g
forage legumes	DMD(g/kg)	= 473 - 0.032A - 0.075B + 3118c - 11.09L	(R^2 = 0.81)	

a = (Ørskov et al., 1988).
b = (Chermiti et al., 1996).
c = Nsahlai and Umunna (1996)
d = Khazaal et al. (1993)
e = Khazaal et al. (1995)
f = Carro et al. (1991)
g = Shem et al. (1995)

5.4.3.1 Straws and other poor quality roughages

For roughages (untreated and ammonia treated cereal straws) it was found that the nylon bag degradation characteristics were good predictors of growth rate and gave better measures of nutritive value than in vivo digestibility or metabolizability values (Ørskov et al., 1988). Ørskov et al. (1988) suggested that a degradability index could be used to express the nylon bag degradation characteristics as a single value using the expression below:

degradability index = (a + b) + 600c

Using ten cereal straws, Blummel and Ørskov (1993) found that the nylon bag method was able to predict dry matter intake (DMI) and digestible dry matter intake (DDMI). Nylon bag degradation characteristics also improved the prediction of intake of hays and poor quality cereal straws used to feed sheep in northern Africa (Chermiti et al., 1996). The voluntary DM intake was highly variable and the hays ingested in greater amounts than the straws although the rate and extent of the degradability of the insoluble fraction (parameters b and c of the Ørskov and McDonald, 1979, equation) were not significantly different. The material washed from the bag, the a fraction, was strongly correlated with intake (r = 0.78) and explained much of the variability between samples. In these intake trials, all feeds were supplemented with soya-bean meal and a vitamin/mineral mixture and fistulated animals used for nylon bag determinations were fed on a maintenance diet containing adequate protein, vitamins and minerals.

In contrast, Nsahlai and Umunna (1996) evaluated 26 tropical forages (legumes) and roughages and found that nylon bag degradability was generally poorly related to in vivo digestibility and DMI for roughages. The nylon bag method was better at predicting the DMI of legumes. It was noteworthy that in this study, for in vivo trials, feeds were presented unsupplemented; conventional enriched environments were used for the nylon bag technique. The roughages were probably deficient in some nutrients (almost certainly protein deficient), unlike the legumes which were probably protein sufficient, hence the poor relationships between the in vivo and in vitro parameters for roughages.

5.4.3.2 Hays

Hovell et al. (1986) described the use of the nylon bag technique to predict the intake of four hays by sheep. Intake was better related to potential degradability measured by this technique than to in vivo digestibility. In particular, hays of similar in vivo digestibility could have different intakes but were distinguished by their potential degradability. The hays were fed after the nitrogen content had been equalised at 18.5 g N per kg DM by adding an aqueous urea solution. The fistulated sheep used for nylon bag determinations were fed a good quality hay diet. It was also noted that the rumen incubation times required to match the in vivo digestibilities ranged from 23 to 67 h, hence the difficulty in relating degradabilities at any fixed time with in vivo digestibilities.

Khazaal et al. (1993), using ten hays, found that although NDF was related to intake (r = -0.68), other chemical components and the in vitro digestibility were poorly related

to animal performance (P>0.05). Nylon bag degradation gave better predictions of intake and in vivo DMD (r = 0.73 to 0.80 for incubation times between 12 and 96 h). Multiple regression using kinetic constants after fitting the McDonald (1981) exponential equation gave improved predictions of both in vivo parameters for nylon bag data. Khazaal et al. (1993) concluded that the nylon bag technique was potentially capable of predicting both intake and digestibility for hays.

More recently, Khazaal et al. (1995) used ten graminaceous hays where intake and in vivo DMD were poorly related to each other to retest the in vitro and analytical procedures. In this study, intake and in vivo apparent DMD could be predicted using NDF, ADF, ADL and CP in a multiple regression. Intake was strongly related to CP (r = 0.76). The nylon bag technique was also able to accurately predict the in vivo parameters. The addition of CP to the multiple regression improving its performance in predicting both intake and DMD. The rate constants (c) were particularly important in improving the prediction of intake, presumably because rate of degradation affects rumen fill. Apparent in vivo digestibility was strongly associated with the potential degradability of the feed. By pooling data from the two studies using hays described above, further sets of equations were obtained to predict intake and apparent DMD (see Table 3).

5.4.3.3 Browses, tree fodders

Kibon and Ørskov (1993), using five browse species fed to goats in Nigeria, found that the nylon bag degradation characteristics were correlated with various in vivo parameters. The rate constant greatly increased the correlation obtained between in sacco characteristics and in vivo parameters. Multiple correlation coefficients (R^2) of 0.88, 0.99, 0.92 and 0.99 were obtained when A + B + c were included in regression analysis against apparent DM digestibility, DMI, apparent digestible DM intake and growth rates (respectively). The inclusion of a lag phase led to some further improvement in the correlations. Shem et al. (1995) similarly correlated nylon bag degradability characteristics with in vivo parameters for 18 feeds used on smallholder dairy farms in Tanzania. For the in vivo trials each animal was supplemented with 1 kg cotton seed cake per day to ensure that there was no nitrogen deficiency in the rumen. Again, the nylon bag degradation characteristics could be correlated to DMI, apparent digestible DM intake and growth rates when the feeds were offered supplemented with cotton seed cake. One of the feeds, banana pseudostem, had a low DMI but high digestibility and rumen outflow rate. These properties were anomalous, possibly resulting from the high content of intracellular water. However, the degradation characteristics of foods, combined with their voluntary intakes, gave a generally useful description of the nutritive value of the foods.

The study of Nsahlai and Umunna (1996) on roughages and forages has been reviewed in part above. The nylon bag technique was found to give a relatively good prediction of dry matter digestibility.

5.4.4 Limitations and advantages of the nylon bag method

The nylon bag method has become widely used, however there are a number of practical limitations. A major one is the wide laboratory to laboratory variation of

nylon bag data. Osuji et al. (1993) noted that the feed sample must be able to move freely in the bag to avoid poor reproducibility between replicates. A ring test involving 23 laboratories in 17 European countries revealed appreciable variabilities in the mean protein degradabilities measured by the nylon bag technique. For example, for single samples of soybean meal and fish meal the estimated mean effective protein degradations (%) $\pm$ the standard deviations were 63 $\pm$ 11 and 23 $\pm$ 6 respectively (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 appears to be robust.

Other limitations are the need to measure or assume outflow rates, data which is time consuming to collect and generally not available for LDC 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. This is a particular problem with roughages where the microbial protein adhering to the sample can exceed the protein content of the feed itself (Preston, 1995). The reliability of the method for evaluating feeds with a large soluble component or feeds of fine particle size is doubtful as sample is 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 also be washed out of feeds and diluted to neutralise their effects (Preston, 1995). Nylon bag degradabilities also depend to some extent on the basal diet fed to the fistulated animals (reviewed by Kandylis and Nikokyris, 1991). 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. It appears that there are no generally accepted prediction equations even for single types of feeds, larger pools of data are probably required to facilitate the development of robust prediction equations. However, one more fundamental problem is that the nylon bag and in vivo studies were generally done with supplemented feeds. In the one study (Nsahlai and Umunna, 1996) where in vivo intake and digestibility were determined with unsupplemented feed, nylon bag parameters were poor predictors of in vivo parameters, particularly for roughages. There must therefore be some doubt as to how accurate predictions would be under on-farm conditions in LDCs, where supplementation is often inadequate to prevent nutrient deficiencies. Furthermore, there must also be doubts as to the usefulness of such predictions in many situations, where farmers need advice on how to achieve the feed potential of roughages rather than predictions of what that potential is.

Nevertheless the method does not require sophisticated laboratory facilities, not even a reliable supply of electricity. For this reason its use has been advocated in LDCs (Osuji et al., 1993; Preston, 1995).

6. Gas production method

6.1 General principles

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. The standard assumptions do not apply, although there are others which apply uniquely to gas production techniques.

The main products of carbohydrate fermentation by rumen microbes are volatile fatty acids (VFAs: acetic, proprionic and butyric acids), the gases carbon dioxide and methane and microbial biomass. Carbon dioxide is also released from the bicarbonate buffer usually 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 (the stoichiometry of gas production) 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.

6.2 Menke method

6.2.1 Methodology

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. 200 mg of dried feed was fermented in 150 ml glass syringes in a reduced, anaerobic nitrogen-free bicarbonate buffered medium. Two parts of medium were mixed with one part of strained, fresh rumen fluid. 30 ml of this was added to the feed in the syringe and the mixture incubated at 39°C. The syringes were calibrated to facilitate reading the volumes of gas produced. Readings were taken after 24 h incubation. The gas production from no-substrate blanks (medium with rumen fluid incubated without any feed sample) was subtracted from the test data. Reference substrates of hay meal (200 mg) and 140 mg hay meal plus 60 mg starch were used for all sets of incubations. Data was standardised by multiplying it by the sum of the average of the two references divided by standard values obtained for these reference feeds in previous experiments. New hay meal references were made every 6 to 12 months by mixing two different hay meals in different proportions so as to give a reference with the same properties as the original.

Blummel and Ørskov (1993) modified the technique to monitor gas production at regular intervals up to 72 h incubation, as well as adding trypticase as a supplementary nitrogen source. Gas production data was 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.

6.2.2 Uses of the Menke gas production method

6.2.2.1 Prediction of ME

Menke et al. (1979) found the following regression equations between gas production and digestible organic matter (DOM) and ME:

DOM (g/kg organic matter) = 13.3 x GP - 0.05 x GP^2 + 511 x CP + 76 x fat + 91.2

ME (MJ/kg DM) = 0.118 x GP + 8.72 x CP + 19.21 x fat + 3.38 x NFE + 0.691

where GP is the gas production after 24 h incubation from 200 mg feed corrected and standardised as above; NFE = nitrogen free extract. NFE, CP and fat contents were expressed as g/g DM.

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). Menke and Steingass (1988) reported that when CP, fat and NFE were included in the equations (as above), estimates were more accurate than those obtained using the Tilley and Terry method. They recommended the following equation as suitable across different groups of feedstuffs:

ME (MJ per kg DM) = 0.1181 x GP + 0.0088 x CP + 0.0247 x fat + 0.0036 NFE + 0.46 (R^2 = 0.95)

where GP = gas production after 24 h incubation, ml per 200 mg DM; CP, fat and NFE are expressed as g kg^{-1} DM.

NFE could be left out with very little reduction in the accuracy of ME estimates using the equation:

ME = 0.1457 x GP + 0.0070 x CP + 0.0224 x lipid + 1.24 (R^2 = 0.94)

For more accurate estimates, different equations for concentrates and roughages were recommended to take account of different fermentation patterns leading to different stoichiometries of gas production.

For roughages:

ME = 0.98 + 0.1049 x GP + 0.0088 x CP + 0.0268 x lipid + 0.0038 x NFE (R^2 = 0.94)

For compound feeds:

ME = -2.69 + 0.0868 x GP + 0.03132 x CP + 0.0331 x lipid + 0.0105 x NFE (R^2 = 0.90).

6.2.2.2 As an alternative to the nylon bag technique to predict in vivo parameters

There have been some limited investigations of the use of in vitro gas production (using the Menke method) to predict in vivo digestibility and DMI, comparing this to

the nylon bag technique. Selected prediction equations are given in Table 4 and described by feed type below. As with the nylon bag studies, the approach generally adopted has been to look at single feeds in supplemented rumen or in vitro environments. Gas production data was analysed by applying the Ørskov and McDonald (1979) equation.

Table 4 Correlations between gas production (Menke method) and in vivo parameters

	In vivo parameter	Correlation equation	Correlation coefficient etc.	Source
Cereal straws	DMI	1.529 + 0.455a + 0.0324b (note x)	corr. coeff. = 0.88	a
	digestible DMI	-0.933 + 0.301a + 0.0496b	corr. coeff. = 0.93	a
	growth rate (g day^{-1})	-391 + 112.5a + 6.37b	corr. coeff. = 0.95	a
	DMD(g/kg)	= 278 + 2.1b + 2167.0c	(R^2 = 0.44)	b
	Intake	= -47.7 + 4.25a + 2.12b + 444.5c	(R^2 = 0.630)	c
	DMD	= -21.7 + 4.16a + 1.52b + 268.4c	(R^2 = 0.784)	c
graminaceous hays	Intake	= 38.0 - 1.43a - 0.66b + 894c	(r = 0.894)	d
	DMD	= 99 + 5.6a + 6.2b + 3308c	(r = 0.694)	d
all hays	Intake	= -2.6 + 0.49(a + b) + 339c + 0.17CP	(r = 0.798)	d
	DMD	= 15 + 10.1(a + b) + 623c +0.51CP	(r = 0.778)	d
forage legumes	DMD(g/kg)	= 23 + 9.4b + 570.2c	(R^2 = 0.77)	b

DMI = dry matter intake
a, b, c = parameters from Ørskov and McDonald (1979) eqn.

a = Blummel and Ørskov (1993)
b = Nsahlai and Umunna (1996)
c = Khazaal et al. (1993)
d = Khazaal et al. (1995)

6.2.2.3 Straws

Using ten cereal straws, Blummel and Ørskov (1993) found that the Menke gas production and nylon bag techniques showed similar trends. Gas production at 8, 24, 48 and 72 hours of incubation were highly correlated with nylon bag degradabilities at the same times (R^2 ranging from 0.93 to 0.97). However, no significant correlation was found between the rate constants obtained by the two methods. Differences between the gas production parameters for straws were largely defined by parameter (a), the rate constant (c) making little difference in the ability of the gas production method to distinguish between the samples. Equations were derived by stepwise multiple regression analysis which correlated gas production data to DM intake,

digestible DM intake and growth rate of growing steers fed on these straws. Blummel and Ørskov (1993) suggested that while gas production may give a good estimate of rumen apparent degradability, the loss of dry matter from the residue may predict true degradability and the difference could be considered as an estimate of microbial yield. The gas production method and nylon bag method were able to predict dry matter intake (DMI) and digestible dry matter intake (DDMI) with similar precision, while the nylon bag method was slightly more accurate in predicting liveweight gains.

Nsahlai and Umunna (1996) evaluated 26 tropical forages (legumes) and roughages by gas production, the nylon bag degradability and in vivo DMI and digestibility. Gas production was the best of the techniques used for predicting roughage DMI; the nylon bag method was better at predicting the DMI of legumes. However, the ability of both techniques (and the Tilley and Terry method) to predict the in vivo parameters was generally modest or poor. As noted in Section 5.4.3.1, it was noteworthy that in this study, for in vivo trials, feeds were presented unsupplemented; conventional enriched environments were used for the in vitro and in sacco techniques.

6.2.2.4 Hays

As described in Section 5.4.3.2, Khazaal et al. (1993) used ten hays to compare gas production (Menke method), nylon bag degradability together with Tilley and Terry in vitro digestibility and chemical composition as predictors of hay apparent digestibility and voluntary intake. Nylon bag degradation gave better predictions of intake and in vivo DMD than gas production, although it was concluded that both nylon bag and gas production techniques were potentially capable of predicting both intake and digestibility for hays. The second study (Khazaal et al., 1995) used ten graminaceous hays where intake and in vivo DMD were poorly related to each other to retest the in vitro and analytical procedures. The Menke gas production method could predict intake but not DMD. The fact that protein fermentation makes little contribution to the gas production and increases buffering capacity was thought to be the reason for the relatively poor performance of the gas production method in this study. The addition of CP to the multiple regression improving its performance in predicting both intake and DMD. The rate constants (c) were particularly important in improving the prediction of intake, presumably because rate of degradation affects rate of passage. Apparent in vivo digestibility was strongly associated with the potential degradability of the feed. By pooling data from the two studies using hays described above, further sets of equations were obtained to predict intake and apparent DMD. Overall, the nylon bag method gave the most accurate predictions, followed by the gas production method.

6.2.2.5 Browses, tree fodders

The study of Nsahlai and Umunna (1996) on roughages and forages has been reviewed in part above. The nylon bag method was better than gas production at predicting the DMI of legumes. Nsahlai and Umunna (1996) were unable to explain why the gas production method was unable to predict in vivo parameters for the legumes with greater accuracy. One suggestion was that gas production may have been suppressed in some feeds, possibly by anti-nutritive factors. An alternative is that it may have been

due to relatively high protein content of forages and the low gas production derived from protein degradation.

6.3 Cornell method

A completely enclosed fermentation system in which gas pressure and gaseous products (carbon dioxide and methane) are monitored automatically has been described by Pell and Schofield (1992). The method is similar to the Theodorou method described below, except gas is not removed during fermentation but allowed to build up, potentially causing inhibition of further fermentation.

6.4 Theodorou method

6.4.1 Methodology

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. This system enables a larger volume of medium and a larger feed sample (1 g instead of 0.2 g) to be used. While this may appear trivial, the increased amount of sample is important when feed mixtures are to be used and residues are to be recovered and analysed. The system can also be automated making it particularly attractive for use in industrialised countries.

Incubations are conducted in a nitrogen-rich, bicarbonate-buffered medium and fresh rumen fluid is used as a source of inoculum. Incubations are conducted in serum bottles at 39°C. The bottles are sealed using rubber stoppers. 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. The method was proposed as a simple method of investigating fermentation (degradation) kinetics and a ranking tool for feeds on the basis of their in vitro fermentability.

Gas production data was analysed by subtracting no-substrate control data and fitting the exponential model of France et al. (1993):

$$y = A\text{-}BQ^{t}Z^{\sqrt{t}}$$

where $Q = e^{-b}$, $Z = e^{-c}$ and $B = e^{bT+c\sqrt{T}}$. y denotes cumulative gas production (ml), t is incubation time (h), A is the asymptotic value (gas production at infinite time or gas pool size), T is the lag-time (from time 0 to the time when the maximum rate of degradation starts) and b (h^{-1}) and c ($h^{-0.5}$) are rate constants. Theodorou et al. (1994) reported that in general this model gave a better description of the gas production data than the Ørskov and McDonald (1979) model as it is capable of describing sigmoidal patterns of degradation. However, other models have also been proposed, such as the modified Gompertz model of Beuvink and Kogut (1993). These models are mathematical equations which have been shown to fit the gas production data to various extents. As more complex models can potentially improve the fit using

increasing numbers of parameters, it becomes increasingly difficult to assign biological meaning to the parameters derived.

Theodorou et al. (1994) demonstrated that gas pressures were highly correlated to gas volumes, although scatter increased at pressures above 7 psi (volumes above 30 ml). Gas production kinetics were not affected by sample size between 0.2 and 2 g (using ryegrass as the feed), and the gas pool size was proportional to the amount of substrate over this range. Hence, over the sample range fermentation was not inhibited by nutrient deficiency, pH changes or the accumulation of end-products. The gas yield for ryegrass was estimated at 312±4.79 ml per g DM apparently degraded (determined by residue recovery at the end of fermentation).

6.4.2 Potential applications of Theodorou method

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 LDCs are:
- screening and ranking feeds in terms of nutritive value for ruminants (including feeds with may contain anti-nutritive factors)
- estimations of digestibility and feed intake
- investigation of interactions in feed mixtures
- as an experimental tool to investigate the fermentation of feeds and feed components
- to provide kinetic parameters for use in computer models of rumen function
- reduction or avoidance of the use of rumen fistulated animals.

The gas production technique may have the potential to replace some or all of the existing in vitro digestibility and in sacco techniques, to be used in combination with other feed evaluation techniques to assess nutritive value. In terms of the information on feeds identified in Section 1.4, gas production could contribute to obtaining some information on most of the required aspects, except data on the amino acid balance (which is not generally important to LDC ruminant production).

6.4.3 Screening and ranking feeds by gas production

The gas production method could potentially act as a type of bio-assay for investigating the effects of anti-nutritive factors on microbial microorganisms. There is, however, very little reported use of the gas production method in this context. Siaw et al. (1993), working with tree leaves, reported that the gas production method was not able to distinguish leaves which were considered by farmers to be toxic to ruminants. Khazaal et al. (1994) used browses treated with the tannin-binding agent polyvinylpolypyrrolidone (PVPP) to investigate the effects of tannins on degradation characteristics assessed by in vitro gas production (using the Menke procedure) and the nylon bag method. They found that the tannin content of the browses was more closely related to the inhibition of the gas production than the inhibition of dry matter disappearance from rumen incubated nylon bags. Khazaal et al. (1994) suggested that antinutritive factors were probably diluted in the rumen to largely negate their effects on the nylon bag technique, whereas in vitro gas production was inhibited by tannins.

Nsahlai et al. (1994) have distinguished between 23 *Sesbania* browses using in vitro gas production and chemical composition. Siaw et al. (1993) ranked leaves from 20 accessions from multi-purpose trees from 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, 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 fed on these feeds.

Thus 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, whether the in vitro ranking accurately reflects relative animal performance, or indeed whether conventional animal performance parameters always reflect the value of the feed in the context of the farming system.

6.4.4 Supplementation and interactions

The ability to use the Theodorou gas production technique to investigate interactions between feeds was seen at one of its major advantages over alternative techniques. However, the authors are unaware of any attempts to use it for this purpose outside of the activities of project A0316. Nevertheless, earlier studies have shown the potential value of this type of work. The nylon bag technique has been used to measure the fermentation characteristics of roughages in the rumens of fistulated animals fed supplemented and unsupplemented diets. Mehrez and Ørskov (1976) used the nylon bag method to test the effect of rumen ammonia concentration on the rate of dry matter disappearance. Mutsvangwa et al. (1992) used the Menke gas production method in a similar way, comparing the fermentation of a basal diet (barley) using inocula from unsupplemented and supplemented cattle. The in vitro data helped in the interpretation of a parallel animal feeding trial.

6.4.5 As an experimental tool for investigating rumen degradation

The gas production technique is particularly useful for investigating the fermentation of soluble feed components where alternative in vitro or nylon bag techniques which measure degradation of the insoluble fraction only cannot be used. This facet has been exploited by Stefanon et al. (1996), who used the gas production method to investigate the fermentation kinetics of the water-soluble and water-insoluble fractions of alfalfa and brome hay. The main differences between the feeds were in the size and fermentation kinetics of the water-soluble fractions. There were indications of positive interactive effects between the two fractions. Information of this type is of potential use in plant breeding programmes and in identifying appropriate feed combinations.

6.4.6 Avoidance of the use of rumen fistulated animals

Using in vitro gas production it may be possible to avoid (or at least minimise) the use of fistulated animals, an option clearly impossible with the nylon bag technique. Using gas production methods will already greatly reduce the use of fistulated animals compared to that required for an equivalent number of nylon bag determinations.

Current UK legislation requires scientists to seek to avoid or reduce the use of experimental animals whenever possible. El Shaer et al. (1987) first demonstrated that faeces could be used as an inoculum source in the Tilley and Terry procedure, the faecal inoculum giving values which closely correlated (r = 0.98) with the conventional rumen fluid inoculum values (although incubation time with rumen microbes was increased from 48 to 72 h for barley straw samples). A relationship: in vivo digestibility = in vitro digestibility x 1.003 was found for grass, lucerne and other feeds. Omed et al. (1989) confirmed the suitability of faecal inoculum in the Tilley and Terry method. Nsahlai and Umunna (1996) found that reconstituted faeces could successfully be used as an inoculum, replacing fresh rumen fluid, for both Tilley and Terry and gas production procedures. It has also been demonstrated that the activity of faecal microbes is reasonably robust, with faeces being successfully used as a source of micro-organisms even 5 h after being voided (Akhter et al., 1996).

Luchini et al. (1996) recommended harvesting micro-organisms from strained rumen fluid at 5,000g (30 mins at 4°C), stirring the pellet in a 50:50 (v/v) solution of glycerol-McDougall's buffer for 15 mins and then storing at -20°C. Microbes, after 6 h preincubation, were used as an inoculum source for in vitro estimation of ruminal protein degradation, giving similar results to those obtained using fresh rumen fluid. This opens up the possibilities of producing large batches of inoculum, possibly from normal slaughter, which can be stored for subsequent use thus reducing or possibly eliminating the use of rumen fistulated animals.

6.4.7 Providing kinetic parameters for computer models

The authors are not aware of any published literature on this potential use of the in vitro gas production technique. Nevertheless the potential is considerable to develop computer models which will facilitate the interpretation of the gas production data. In principle, parameters used to describe in vitro fermentation kinetics might be used to furnish parameter values for appropriately constructed rumen simulation models.

6.5 Advantages and limitations of gas production techniques

The major advantages of the technique are the abilities 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. This method of data collection greatly facilitate kinetic studies. Because of this, 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. Similarly, 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.

As noted above, potentially the technique could be used for estimating microbial protein production. The Theodorou technique in particular is potentially useful for investigating interactions between feeds due to the relatively large sample fermented

coupled with the ease of monitoring kinetic properties. Another possible advantage is that VFAs accumulating in the incubation medium can be analysed to investigate the VFA production profiles. The composition of gas evolved can also be analysed to investigate the production of methane, implicated in global warming.

The general lack of data from studies using the Theodorou gas production method means that there are no published equations which can be used for the prediction of in vivo parameters from gas production data. Nevertheless, general conclusions concerning the potential usefulness of the technique in obtaining such predictions can be made based on studies using the essentially similar Menke technique, and to some extent based on the nylon bag which investigates the same biological processes. Both techniques can give useful predictions of in vivo DMI and digestibility, predictions which may be improved by including data on composition or other indicators of nutritive value. Gas production can be useful for concentrates and roughages when fed (and assessed in vitro) under conditions when nutrients, especially nitrogen, is not limiting. As discussed elsewhere, evaluating roughages under nitrogen deficient conditions is a poorly investigated field, but one of considerable importance in LDC feeding systems.

Given that the mechanisms controlling feed intake are poorly understood, attempts to predict intake from in vitro digestibility methods must be treated with some caution. Correlation does not necessarily indicate causal relationships. Empirical correlation equations may prove to be highly misleading if applied for different feeds or under different circumstances if correlations are coincidental. Nevertheless, in the absence of alternative approaches such predictions may be of considerable practical utility.

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 DOM, but similar equations have as yet not been developed for the Theodorou method.

Variable stoichiometry of gas production would mean that there would not be a fixed relationship between gas production and feed degradation. For straws, there was a strong relationship between gas production and true organic matter fermentation (true organic matter defined as 100 - NDF of residue), so gas production did appear to be a good indicator of the degradation of straw. However, a proportion (estimated at 45% after 24 h and 30% after 48 h) of the degraded organic matter was apparently trapped in the form of microbial biomass rather than released as gas (Blummel and Ørskov, 1993). However, this property of the technique could be developed to provide an estimate of the production of microbial biomass during roughage fermentation, a parameter which is difficult to measure in vitro and in vivo by existing techniques (Blummel and Ørskov, 1993; Blummel, personal communication).

Some variability could also occur if the profile of VFA production was very variable Menke and Steingass (1988) noted that concentrates would be expected to increase propionate at the expense of acetate production, to some extent explaining the

different correlation equations obtained for predicting ME from gas production for different classes of feeds. Pell and Schofield (1993) noted that the use of mixed bicarbonate-phosphate buffers were useful in maintaining the pH, but meant that the molar yield of carbon dioxide from the reaction of VFA and bicarbonate buffer is <1. However, a strong linear correlation was found between NDF disappearances of forages and gas production. Prediction equations derived using gas production parameters may need to be adjusted to take into account the fact that little gas is derived from protein degradation. This could make gas production methods less suitable for evaluating high protein feeds in isolation. However, as such feeds are often fed as supplements in conjunction with roughages, this may not be a significant disadvantage for studying LDC feeds. It may indicate that gas production from more realistic whole diets may be preferable to studying the different components separately.

7. Conclusions

Existing in vitro techniques can be used to predict in vivo digestibility, intake and animal performance with varying degrees of success. The gas production technique shows promise as a simpler and more versatile method for predicting these parameters, and as a more general research tool, but requires further development and calibration against feeding trial data.

Feed evaluation has a history of different research groups independently developing feed evaluation systems, leading to different equations for interpreting data and different protocols for laboratory methods. A more collaborative approach to developing the gas production method would avoid unnecessary duplication and confusion.

Some of the major problems of ruminant nutrition in LDCs are nutrient imbalances, particularly shortages of dietary protein. Existing feed evaluation and in vitro techniques are not designed to assess feeds as part of protein-deficient diets. New approaches are required for the investigation of interactions between feeds to formulate more balanced diets. The Theodorou gas production technique appears to be particularly suitable for this use as feed mixtures can be fermented under controlled conditions.

There is no framework for accounting for the effects of anti-nutritive factors in ruminant feeds and more work is required to evaluate such factors.

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