

RESEARCH ON FORAGE QUALITY: PROGRESS AND PRIORITIES

J.A. Milne

Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen, UK

Abstract

A broad definition of forage quality is used and the purposes for which research on forage quality is undertaken currently are outlined. These range from research that provides simple measurements that describes the forage quality of a commercial product to research on forage quality to improve understanding of digestion processes. Much of the research in the last decade has related to improving predictive measurements. The different degrees of progress that have been made in the development and use of measurements to further our understanding of forage quality are described and related to their fitness of purpose. It is concluded that progress in the last decade has mainly been made in the development of the use of near infrared reflectance spectroscopy to reduce the need and time required for other types of analysis. Other areas of progress have been in the development of techniques to reflect a greater understanding of the nutrition of ruminants, particularly which factors are important in determining nutrient supply. Examples of these are developments in *in vitro* gas production and *in situ* methods. These have been valuable but they are unlikely to be adopted on a large-scale for forage evaluation. For a research purpose the major advance has been in the development of improved marker techniques to allow the measurement of intake, digestibility and diet selection of grazing animals.

Priorities for research in the future depend on the identification of future needs and the opportunities for developing solutions. It is likely that research on processes in relation to forage quality will be a priority for intensively managed grassland systems. The pressure in parts of Europe and North America will be for the de-intensification of much of the grassland to meet sustainability, nature conservation and landscape objectives and in these circumstances the definition of forage quality changes and this requires new approaches to the study of forage quality. In many other parts of the world there will be a continuing need to develop simple methods for describing forage quality which do not rely on sophisticated equipment but utilise the experience and knowledge of local grassland managers. It is argued that the diversity of research on forage quality will continue to expand. It will be important to identify needs precisely so that appropriate innovative solutions can be developed.

Introduction

The term forage in English and its equivalents in other languages provide no uniform definition. For the purposes of this paper the definition proposed by the Forage and Grazing Terminology Committee (1991) is used, namely “edible parts of plants, other than separated grain, that can provide feed for grazing animals, or that can be harvested for feeding”. Such a definition covers a wide range of types of feed and includes grazed and conserved herbage but also straws and crop residues, forage crops and browse. It is not possible to include only grazed and conserved herbage in considering forage quality because straws and crop residues, forage crops and browse are important components of grassland-based systems throughout the world. In the future this is likely to continue to be the case. Whilst extensive grassland will still continue to be the most important form of management in the world, intensive grassland will continue to

evolve with improved forage quality and yield of other forage crops making a greater contribution to such systems. Moreover, treatment and breeding of cereal and protein crop straws will increase their feeding value such that they will possibly contribute more to nutrient supply from grassland systems in the future. The same argument can be applied to browse species. In a temperate European and North American context management strategies are likely to evolve not only in the context of animal production but also in meeting sustainability, nature conservation and landscape objectives. It is argued that a broad definition of forage is essential to capture the importance of forage quality in grassland systems.

Within grassland systems research on forage quality has focused in the last decade on the measurement of forage quality and revolved around the need to describe the attributes of forages, measured chemically or biologically, to meet the objectives of the system. These objectives used to be defined solely in terms of animal outputs and the system boundary was conventionally set as the farm. However, because of the vertical and horizontal integration of grassland systems, the boundaries have widened. For example, feed compounders are now among the widest users of measurements of forage quality in designing rations. Moreover, nature conservationists now require to know the quality of hays, for example, in designing systems in which hay is cut at particular times in the season to meet the requirements of nesting birds. It is no longer sufficient to consider forage quality purely in terms of efficiency of forage utilisation and animal output although these remain important objectives. It can be concluded that in the last decade the demands placed on measuring forage quality have expanded as the diversity of grassland systems has increased.

Another demand placed on the measurement of forage quality has arisen as a result of increases in understanding of the processes of digestion and metabolism in ruminants in the previous decade. This is particularly in the case in relation to protein digestion. Furthermore the prediction of forage intake has been recognised as a continuing weakness in the prediction of animal performance and the demands of appropriate measurements of forage quality to be used in mechanistic modelling of, for example dairy cow systems, has increased (France *et al.*, 1999). Another strand of development in the field of forage quality has been in the use of indigenous knowledge as an adjunct to conventional approaches to improve predictions of system performance (Thappa *et al.*, 1997). The use of a variety of inputs into an integrated approach to the prediction of forage quality to predict system performance is a new demand on the measurement of forage quality.

These trends in the scope of measurements required to describe forage quality have accelerated the amount of research on the development of techniques for the measurement of intake, digestibility and diet composition using marker techniques, and the refinement of *in vitro* and *in situ* methods in the past decade. Whilst this has been at the expense of research on chemical analytical techniques, the application of Near infrared reflectance spectroscopy has highlighted the inadequacy of many conventional laboratory reference methods and this will stimulate more research in this area in the future. The progress in these approaches will be reviewed below and this will be followed by a discussion of the priorities for these approaches and new approaches in the future.

As the above discussion indicates, the focus in the last decade has been on finding simple means of predicting elements of forage quality rather than on increasing understanding of forage quality itself. The amount of funding in the developed world for research on ruminant nutrition, and hence on research that pertains to increasing an understanding of forage quality, has declined considerably. The ability to influence the chemical composition of plants, and hence forage quality, through the study of molecular genetics is still in its infancy and this may provide a new

stimulus for more fundamental research on forage quality in the future. This will be discussed further when future priorities are considered at the end of this paper.

Methods of estimating intake, digestibility and diet composition in grazing animals

Markers have been the most widely used method of estimating daily intake and digestibility of forage by grazing animals on swards containing few forage species because of their simplicity despite the need to make several key assumptions. The advent of the use of n-alkanes, found in the cuticular wax of all plants, as markers (Mayes *et al.*, 1986) removed some of these assumptions and the technique has become recognised as the method of choice. The technique relies on the recovery rates of adjacent dosed even-chain and naturally occurring odd-chain alkanes for measuring forage intake and a known assumed recovery rate of C₃₆-alkane to estimate digestibility. Table 1 shows the good agreement between estimates of intake, estimated using the alkane technique, and actual intakes of forages by sheep and cattle. In ten validation studies reported by Dove and Mayes (1996) the largest discrepancy between known and estimated intake amounted to 2.6%. The intake of forage can also be determined when supplements are fed (Dove *et al.*, 1995). The use of slow-release capsules in the rumen increases the application of the technique to more extensive situations (Mayes and Dove, 2000).

Before the development of the alkane marker technique, the measurement of the diet composition relied upon the use of oesophageal-fistulated animals. This technique had a number of difficulties, not least being the use and maintenance of surgically prepared animals. Exploitation of the fact that different plant species have different patterns of alkanes has allowed the estimation of the diet of herbivores grazing a small number of species from the composition of alkanes in the faeces. Simultaneous equations or least-squared optimisation methods, after correction for the incomplete recovery of different alkanes in the faeces, can be used to estimate diet composition (Dove and Mayes, 1996). Validation studies, in which known plant mixtures have been fed to herbivores, have shown that the species composition of two-, three- and four-component mixtures can be estimated satisfactorily (Dove and Mayes, 1991; Dove, 1992).

A challenge for the future is the development of the technique to measure a greater number of species in the diet. The use of cluster analysis, canonical variate analysis and principal components analysis to group sets of species on the basis of their alkane composition is one approach and this has been used successfully in a few studies (Dove *et al.*, 1999; Bulgalho, 2000). The latter author used the approach to discriminate between browse and herbage species in a Mediterranean environment (see Figure 1) and this approach has the potential to be extended more widely. It has also been suggested that cuticular wax markers other than alkanes could be used as a means of discriminating between species (Mayes and Dove, 2000). The same authors suggested that near infrared reflectance spectrometry could potentially be used to predict diet composition in the future.

The use of markers, excreted in urine, as a means of estimating diet composition has been demonstrated by Martin *et al.* (1978). There is renewed interest in combining faecal and urinary markers, not only to estimate intake and diet composition but to predict microbial protein supply to the animal's tissues (Mayes *et al.* 1995; Mayes and Dove, 2000). The principal difficulties are in the measurement of urine volume, the preservation of urine and the identification of unique markers.

The significance of estimating diet composition is not only because of the prediction of the nutritive value of the diet but also because it can lead to the prediction of the long-term consequences for the nutrition of herbivores through an understanding of the vegetation dynamics

of the pasture (Milne, 1991). This is important in assessing the sustainability of extensively managed grasslands. Such knowledge is also important in managing grasslands to achieve specific nature conservation objectives. This aspect of forage quality will become a priority for the future in order that ecological sustainability and sustainable development objectives can be achieved.

***In situ* methods**

The lack of suitable chemical analyses to estimate nitrogen digestion in the rumen and the production of microbial protein led to the development of biological methods of predicting the degradability of nitrogen. These methods have also stimulated the revisiting of *in situ* techniques to measure the degradation of dry matter and fibre as predictors of intake and digestibility. Estimation of *in vivo* degradability of forage protein requires the differentiation of duodenal protein flow into that of undegraded forage protein, microbial protein and endogenous protein. Endogenous protein is difficult to measure and the techniques of measurement of all the components are prone to error and are expensive to make. Moreover, forages are seldom fed as sole feeds. For these reasons it is difficult to compare *in vivo* with *in situ* estimates of protein degradability and there has to be doubt on the validity of the *in situ* method. Because the demand for a method in the last decade was so strong, the normal rigorous approach to validation was not taken.

The current state of *in situ* degradability methods in the rumen have been reviewed recently and readers are referred to these reviews for a detailed description of the methods (Hvelplund and Weisbjerg, 2000; Orskov, 2000; Noziere and Michalet-Doreau, 2000). Essentially the method describes the rate of disappearance of dry matter, fibre or nitrogen of forage from nylon or Dacron bags suspended in the rumen. The rate and extent of disappearance are measured and the results subjected to first-order kinetics (Orskov and McDonald, 1979) such that, in the case of protein, the soluble fraction, degradability and rate of degradation are described.

The major factors influencing variation in the results that can be obtained for protein degradability are the choice of basal diet, bag characteristics, sample preparation, replication, incubation conditions, washing technique and microbial correction (Broderick and Cochran, 2000). Whilst standardisation of the technique can reduce the variation, intra- and inter-laboratory variability have been found to be high (Michalet-Doreau and Ould-Bah, 1992; Madsen and Hvelplund, 1994). Other difficulties with the technique are the potentially variable nature of the degradability of the soluble protein fraction, the physical separation of the contents of the bag from the ruminal digesta that can lead to an underestimation of degradation, and inappropriateness of the technique with forages containing anti-nutritional factors. It must be concluded that the technique is unlikely to be developed much further and other methods of measuring protein degradability require to be sought if an universal method is going to be developed.

The *in situ* technique has also been used to estimate the dry matter and fibre degradability in a similar manner to that for protein and has been used to predict intake and digestibility of a range of tropical forages and feeds (Shem *et al.*, 1995). This work requires to be extended to a wider range of forages to explore the potential of the approach further.

The requirement to use surgically prepared animals means that the method will not be available in all laboratories and the variability of the results obtained makes it difficult to see the method developing to have universal applicability. However, as Orskov (2000) has argued, the

simplicity of the measurements makes it suitable for use in many situations, particularly in evaluating the effect on the rumen environment of different feeding regimes and combinations of forages, and using standard local forages in the bags. Gas production methods *in vitro* have the potential to provide similar answers in relation to evaluating forage quality as *in situ* methods (Cone *et al.*, 1998) without the need for surgery as rumen fluid can be obtained by stomach pump. This method is discussed in the section below.

***In vitro* methods**

The gas production system is an alternative analytical technique to the conventional *in vitro* technique pioneered by Tilley and Terry (1963). Instead of measuring the dissolution of insoluble plant components, as in the method of Tilley and Terry (1963), gas production is measured. This has two main advantages, namely that, using electronic pressure sensors, measurements can be recorded by computer and the rich detail of the digestion of soluble feed components can be studied in the same manner as that of insoluble components in the *in sacco* method. Although it is not a new technique, it has come to prominence in the past decade.

Schofield (2000) has divided the alternative methods currently being used into three groups: the syringe method, the closed automatic method and the open automated method. The first method is non-automated, insensitive and subject to error from sticking plungers. The second method (Pell and Schofield, 1993) is the simplest and easiest to maintain automated method but is not readily adapted to sample sizes greater than 250 mg. The third method (Cone *et al.*, 1996) can deal with larger sample sizes but for the method to be sensitive the excess pressure to trigger valve opening must be small.

The common procedure in gas systems is to run in tandem a “blank” container and a sample container and subtract at each measurement point the gas produced in the “blank” container from that produced by the sample. The “blank” contains rumen fluid in the same proportions to that in the sample. Standards are included in each run. For a kinetic analysis of forage digestion, electronic pressure sensor measurements are recorded using a computer-based data collector. Fuller details of the approach are given in Schofield (2000) and Williams (2000). The choice of equation to fit the cumulative gas production data has to take into both mathematical and biological considerations and is a matter of much debate. A comparison of the principal models and their application is described by Dhanoa *et al.* (2000).

The gas production technique measures fermentation in the rumen, in other words the interaction between a substrate and microbes. It has been used to evaluate grass, silage and hay quality (Stefanon *et al.*, 1996), the efficacy of different browse species as forages (Newbold *et al.*, 1997) and different chemical and physical treatments of crop residues (Castro *et al.*, 1994).

Cumulative gas curves also have the potential to be used in nutritional models, such as the Cornell model, that require rate and pool size information for different carbohydrate fractions. A combination of chemical extraction and gas production methods has been proposed to achieve this (Schofield *et al.*, 1995). Moreover, attempts have been made to predict protein degradation and microbial production rate from gas production (Cone and Van Gelder, 1999). The use of the technique to predict the voluntary intake of roughages has also been proposed (Blummel *et al.*, 1997).

There is a danger that attempts may be made to use the technique beyond its potential. However, the simplicity of the method and the major gap that still exists in the ability to predict degradability of constituents of forages as inputs to nutritional models makes the use of the technique to determine fermentation kinetics attractive. It is less easy to predict how useful the

technique will be for predicting voluntary intake other than as one of several variables in a prediction equation.

Chemical analyses

In a similar manner to *in situ* and *in vitro* methods, chemical characterisation cannot give a direct estimate of forage quality but relies on statistical associations. Because of the complexity of ruminant digestion, single measurements of chemical characterisation have a relatively low ability to predict forage quality. In the last decade most progress has been made in the automation of techniques and minor adjustments to them rather than in the development of techniques for the measurement of new chemical entities. Looking to the future, the use of modelling to predict forage quality will increase and these models are likely to continue to rely upon chemical characterisation, particularly on soluble fibre and starch analyses. Automation and refinement of techniques will increase speed, reliability and, as a consequence, reduce the cost of analyses. Moreover, arguments for maintaining animals for routine determinations of forage quality will become increasingly difficult to make. However, the major driver for improved methods will be the need to develop better methods to use as reference methods in the use of near infrared reflectance spectroscopy to estimate forage quality. As Murray (1988) pithily stated, “we are using 19th century chemistry to calibrate 20th century technology”

Near infrared reflectance spectroscopy

Near infrared reflectance spectroscopy is based on the absorption of C-H, N-H and O-H groups in organic compounds. Calibration is required to relate the near infrared optical measurements, made predominantly using diffuse reflectance, to the constituent or property of the forage required to determine forage quality. Depending upon the type of sample, instrument and constituent or property being estimated, a few to several hundred wavelengths may be used. Because calibration is a complicated process, chemometrics are a key element of the successful use of near infrared reflectance spectroscopy. Since the constituent or property of the forage is both being determined and a part of the matrix being measured, and because each sample will consist of different relative amounts of constituents or properties of the sample, it is important that appropriate samples be used for the calibration. For most of the applications of the technique, analyses of open populations of samples will be undertaken. In other words, the calibration has to be continually updated to take into account of new types of samples or types of samples no longer produced. This is particularly the case if the technique is used for a research application, where accuracy is paramount, rather than a routine analysis of a sample for a standard commercial application, where speed may be important. It is not possible to deal in depth with the important issues involved in chemometrics but the key issues of data exploration, regression analysis, calibration validation and transfer are dealt with fully in Shenk and Westerhaus (1994).

Following on from the classic work of Norris *et al.* (1976), the use of the technique has been extended in the past decade from estimating the chemical composition of dry milled samples to the analysis of fresh unmilled samples. The technique has also been successfully used to predict the *in vivo* digestibility of grass and legume hays and silages (Robert *et al.*, 1986; Gordon *et al.*, 1998) and to predict the voluntary intake of grass herbage and silages (see Table 2) and do as well as other predictors (for example, Park *et al.*, 1997). The considerable interest that exists in predicting the soluble and degradable fractions of protein and structural

carbohydrate of forages in estimating forage quality, and the disadvantages of using *in situ* and *in vitro* methods, has aroused much interest in the extent to which these components could be estimated using near infrared reflectance spectroscopy. Although there are some studies which suggest that this may be possible (Waters and Givens; Herrero *et al.*, 1996), the jury is still out on the use of near infrared reflectance spectroscopy for this application. However, if such measures of forage quality are to become used in a widespread manner, then they will probably be required to be predicted using near infrared reflectance spectroscopy.

One limitation to the wider use of near infrared reflectance spectroscopy is the cost of the equipment. Only if there is a major reduction in its cost will the technique have world-wide applicability.

Future priorities

Current research on forage quality, as was argued in the Introduction, has to meet an increasing number of challenges as the diversity of objectives for grassland systems increases. This is particularly the case in the developed world. The range of systems is likely to increase as the need to meet environmental as well as production objectives becomes part of the expanded number of objectives that will require to be met. Within intensive grassland systems, the current and indeed future environmental focus is and will be on reducing losses of nitrogen from such systems. In a systems context, these can be alleviated by reducing the amount of nitrogenous fertiliser used and by manipulating the overall composition of the diet. One option is to change herbage quality by altering the ratio of nitrogen to readily available carbohydrate in the herbage through plant breeding (Miller *et al.*, 1999). Such opportunities for the manipulation of forage quality will increase in the future as genome analysis, sequencing, genomics and proteomics allow more rapid progress in conventional breeding programmes through the use of quantitative trait loci analysis and molecular markers. The challenge is for clear messages to be given to plant breeders what traits in forage quality they should focus on, now that plant breeders have a new range of tools to use. Conversely the greater facility with which plant breeders can produce varieties with different chemical composition will allow advances to be made in our understanding of forage quality.

The application of new breeding technologies is likely to lead to the production of new cultivars tailored specifically to meet system objectives and, to justify their development, requiring greater precision in the management of the system (Pollock, 2000). The list of attributes of forage quality that could be used in this manner is long, ranging from altering the balance of constituents of forages, decreasing the solubility of proteins, increasing the degradability of fibre and optimising the presence of secondary plant compounds. The emphasis on grasses may well decline as the potential of other forages to contribute to meeting objectives of systems is realised. The greater precision of management will require the continued use of improved *in vitro*, *in situ* and near infrared reflectance spectroscopy techniques to predict forage quality and the output of animal products. The output of these products whether as meat, milk or fibre, will need to be predicted more specifically in terms of "quality". To a greater extent in the future, outputs of excreta and the location of their deposition in grazing situations will also need to be predicted.

From the review of the state of development of techniques given above, there must be concern that our current understanding of forage quality and our ability to predict forage quality is inadequate to meet the objectives of intensive management systems for developed countries in the future. Emphasis on research requires to be placed on increasing understanding of the

processes in the efficient digestion of nutrients and in nutrient intake. For example, associative effects in the rumen when different feeds are fed and passage rate in influencing intake are key processes that influence forage quality with major implications for intensive systems. In grazing systems there will be greater need to estimate the intake and excretion of nutrients. This will lead to the wider use of faecal and urinary markers and the use of techniques, such as geographical positioning systems (GPS), to describe the location of the animal and when the animal occupied that location. In this way clear guidance can be given to plant breeders and designers of systems not only as to how forage quality will influence animal outputs but nutrient deposition as it may influence pollution of water courses. This greater understanding may lead to the bypassing of the current *in vitro* and *in situ* techniques and to the wider use of near infrared reflectance spectroscopy to estimate forage quality.

This is not to imply that the feed compounders will not still seek to use cheap and rapid methods of assessing forage quality. Regulation by government and their agencies, and indeed the farming industry and consumers themselves, to ensure a knowledge of the source of feeds and their quality will become more important. Quality will not only be determined by its value as a feed but also in terms of animal and human health, for example, as mycotoxins, their value as substitutes for veterinary drugs and as sources of n-3 fatty acids. Research will be required to identify such potential uses but also, in the context of this paper, in developing cheap and rapid methods for their assessment. Techniques, such as gas or high performance liquid chromatography coupled to mass spectroscopy, will have an important role to play.

In developed countries there will be greater emphasis on more extensively managed grassland providing environmental “goods”. These will be provided predominantly by grazing animals where the agricultural output may be secondary to the environmental “goods” produced. The environmental “goods” will be in the form of more species-rich pastures which are more structurally diverse and which fit into more designed landscapes. The management input will be less and the animals will be able to exercise a maximum choice in their diet. In terms of forage quality, the challenge will be to determine the composition of the diet in complex situations and where the animals graze. On the one hand, the implications for vegetation dynamics can be assessed and, on the other, the animal production and welfare consequences can be predicted. As Mayes and Dove (2000) have pointed out, there is considerable potential to use a wider range of faecal and urinary markers to predict diet composition. The development of controlled-release devices to administer markers over a sustained period to wild and semi-domesticated animals offers the opportunity to estimate intake and faecal output of animals from the collection of faecal samples from pasture through an unique combination of administered markers. The combination of different measurement techniques, for example marker techniques and GPS, will provide the opportunity to describe forage quality in a manner that meets the objectives of the system. The further development of current methods of evaluating forage quality and the development of GPS-derived methods should be able to provide the necessary inputs to the management and monitoring of such systems.

In less developed countries many of the same issues will begin to come into play in the need to produce animal products at world-market prices in the context of sustainable development within an ecologically sustainable framework. However, the background knowledge of many of the forages used is still being collected and there will continue to be a need to use current chemical, *in situ* and *in vivo* methods to develop an appropriate database of forage quality applicable to the particular circumstances. Indeed the opportunity to use grazed forage, cereal by-products and straws, and browse as part of a multi-purpose system affords the possibility of providing the most-efficient system in many non-temperate countries. Cost-

effective and simple-to-apply techniques, such as *in situ* and *in vitro* methods, will continue to provide opportunities for increasing understanding and prediction of forage quality in these circumstances. Indeed, the opportunities for increasing productivity from forages through a greater understanding of the treatment and processing of straws and crop residues and the breeding of forages of appropriate quality are considerable.

In some circumstances, particularly with browse plants, conventional chemical and biological methods do not provide adequate measures of forage quality. Although our understanding will increase using these techniques in the future, the greater use of simple measurements of forage quality, such as leaf-to-stem ratio, colour and smell (Thorne, 1998), and local knowledge, can fill a current information gap for the future.

This is an example of where lateral thinking can lead to significant advances in our knowledge and the use of that knowledge in predicting forage quality. With the wide range of grassland systems that will develop in the future to meet multiple objectives, the challenge for research on forage quality will be to develop new and innovative approaches to meet the diversity of objectives for grassland systems in the future.

References

- Blummel, M., Steingab H. and Becker K.** (1997). The relationship between *in vitro* gas production, *in vitro* microbial mass yield and ¹⁵N incorporation and its implications for the prediction of for the prediction of voluntary intake of roughages. *British Journal of Nutrition*, **77**: 911-921.
- Broderick, G.A. and Cochran R.C.** (2000). *In vitro* and *in situ* methods for estimating digestibility with reference to protein degradability. In: Theodorou, M.K. and France, J. (eds) *Feeding systems and feed evaluation models*. pp. 53-85. Wallingford: CAB International.
- Bugalho, M.** (1999). *The foraging behaviour of red deer (Cervus elaphus) in a Mediterranean environment*. PhD Thesis, University of Aberdeen.
- Castro, F.B., Hotten P.M. and Orskov E.R.** (1994). Inhibition of rumen microbes by compounds formed in the steam treatment of wheat straw. *Bioresource Technology*, **50**: 25-30.
- Cone, J.W. and Van Gelder A.H.** (1999). Estimation of efficiency of microbial growth in rumen fluid with the gas production technique. *Proceedings of the British Society of Animal Science Annual Conference, 1999*, p. 39.
- Cone, J.W., Van Gelder A.H. and Valk H.** (1998). Prediction on nylon bag degradation characteristics of grass samples with the gas production technique. *Journal of the Science of Food and Agriculture*, **77**: 421-426.
- Cone, J.W., Van Gelder A.H., Visscher G.J.W. and Oudshoorn L.** (1996). Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time-related gas production apparatus. *Animal Feed Science and Technology*, **61**: 113-126.
- Deaville, E.R. and Flinn P.C.** (2000). Near infrared (NIR) spectroscopy: an alternative approach for the estimation of forage quality and voluntary intake. In: Givens, D.I., Owen, E., Axford, R.F.E. and Omed, H.M. (eds) *Forage evaluation in ruminant nutrition*. pp. 301-320. Wallingford: CAB International.
- Dhanao, M.S., Lopez S., Dijkstra J., Davies D.R., Sanderson R., Williams B.A., Sileshi Z. and France J.** (2000). Estimating the extent of degradation of ruminant feeds from a description of their grass production profiles observed *in vitro*: comparison of models. *British Journal of Nutrition*, **83**: 131-142.

- Dove, H.** (1992). Using the n-alkanes of plant cuticular wax to estimate the species composition of herbage mixtures. *Australian Journal of Agricultural Research*, **43**: 1711-1724.
- Dove, H. and Mayes R.M.** (1991). The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: a review. *Australian Journal of Agricultural Research*, **42**: 913-952.
- Dove, H. and Mayes R.W.** (1996). Plant wax components: a new approach to estimating intake and diet composition in herbivores. *Journal of Nutrition*, **120**: 13-26.
- Dove, H., Mayes R.W. and Freer M.** (1995). Using cuticular wax alkanes to estimate herbage intake in animals fed supplements. *Annales Zootechnie*, **44**: 237.
- Flinn, P.C., Windham W.R. and Dove H.** (1992). Pasture intake by grazed sheep estimated using natural and dosed n-alkanes – a place for NIR? In: Hildrum, K.L., Isaksson, T., Naes, T. and Tandberg, A. (eds) *Near Infrared Spectroscopy*. pp. 175-178. Chichester: Ellis Horwood.
- Forage And Grazing Terminology Committee** (1991). *Terminology for Grazing Lands and Grazing Animals*. Blacksburg, Virginia: Pocahontas Press.
- France, J., Theodorou M.K., Lowman R.S. and Beaver D.E.** (1999). Feed evaluation for animal production. In: Theodorou, M.K. and France, J. (eds) *Feeding systems and feed evaluation models*. pp. 1-10. Wallingford: CAB International.
- Gordon, F.J., Cooper K.M., Park R.S. and Steen R.W.J.** (1998). The prediction of intake potential and organic matter digestibility of grass silage by near infrared spectroscopy of undried samples. *Animal Feed Science Technology*, **70**: 339-351.
- Herrero, M., Murray I., Fawcett R.H. and Dent J.B.** (1996). Prediction of the *in vitro* gas production and chemical composition of kikuyu grass by near infrared reflectance spectroscopy. *Animal Feed Science and Technology*, **60**: 51-57.
- Hvelplund, F. and Weisbjerg M.R.** (2000). *In situ* techniques for the estimation of protein degradability and post-rumen availability. In: Givens, D.I., Owen, E., Axford, R.F.E. and Omed, H.D. (eds) *Forage Evaluation in Ruminant Nutrition*. pp. 233-258. Wallingford: CAB International.
- Madsen, J. and Hvelplund T.** (1994). Prediction of *in situ* protein degradability in the rumen. *Livestock Production Science*, **39**: 201-212.
- Martin, A.K., Milne J.A. and Moberly P.** (1982). The origin of urinary aromatic compounds excreted by ruminants. 4. The potential use of urine aromatic and phenol outputs as a measure of voluntary food intake. *British Journal of Nutrition*, **49**: 87-99.
- Mayes, R.W. and Dove H.** (2000). Measurement of dietary nutrient intake in free-ranging mammalian herbivores. *Nutrition Research Reviews*, **13**: 107-138.
- Mayes, R.M., Lamb C.S. and Colgrove P.M.** (1986). The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. *Journal of Agricultural Science*, **107**: 161-170.
- Mayes, R.W., Dove H., Chen X.B. and Guada J.A.** (1995). Advances in the use of faecal and urinary markers for measuring diet composition, herbage intake and nutrient utilisation. In: Journet, M., Grenet, E., Farce, M-H., Theriez, M. and Demarquilly, C. (eds) *Recent developments in the nutrition of herbivores*. pp. 400-406. Paris: INRA Editions.
- Michalet-Doreau, B. and Ould-Bah M.Y.** (1992). *In vitro* and *in sacco* methods for the estimation of dietary nitrogen degradability in the rumen; a review. *Animal Feed Science and Technology*, **40**: 57-86.
- Miller, L.A., Theodorou M.K., Macrae J.C., Evans R.T., Adesogan A.T., Humphreys M.O., Scollan N.D. and Moorby J.M.** (1999). Milk production and N partitioning responses in dairy

cows offered perennial ryegrass selected for high water-soluble carbohydrate concentrations. *IXth International Symposium on Ruminant Physiology, Pretoria, South Africa* (in press).

Milne, J.A. (1991). Diet selection by grazing ruminants. *Proceedings of the Nutrition Society*, **50**: 77-85.

Murray, I. (1988). Aspects of the interpretation of NIR spectra. In: Creaser, C.S. and Davies, A.M.C. (eds) *Analytical applications of Spectroscopy*. pp. 9-20. London: Royal Society of Chemistry.

Newbold, C.J., El Hassen S.M., Wang J., Ortega M.E. and Wallace R.J. (1997). Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. *British Journal of Nutrition*, **78**: 237-249.

Norris, K.H., Barnes R.F., Moore J.E. and Shenk J.S. (1976). Predicting forage quality by infrared reflectance spectroscopy. *Journal of Animal Science*, **43**: 889-897.

Noziere, P. and Michalet-Doreau B. (2000). *In sacco* methods. In: D'Mello, J.P.D. (ed.) *Farm Animal Metabolism and Nutrition*. pp. 233-253. Wallingford: CAB International.

Orskov, E.R. (2000). The *in situ* technique for the estimation of forage degradability in ruminants. In: Givens, D.I., Owen, E., Axford, R.F.E. and Omed, H.D. (eds) *Forage Evaluation in Ruminant Nutrition*. pp. 175-188. Wallingford: CAB International.

Orskov, E.R. and McDonald I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science, Cambridge*, **92**: 499-503.

Park, R.S., Gordon F.J., Agnew R.E., Barnes R.J. and Steen R.W.J. (1997). The use of near infrared reflectance spectroscopy on dried samples to predict biological parameters of grass silage. *Animal Feed Science and Technology*, **74**: 2664-2673.

Pell, A.N. and Schofield P. (1993). Computerised monitoring of gas production to measure forage digestion *in vitro*. *Journal of Dairy Science*, **76**: 1063-1073.

Pollock, C.J. (2000). Farming for the future: biotechnology and engineering in perfect harmony? *Journal of Agricultural Engineering Research*, **70**: 219-225.

Robert, P., Bertrand D. and Demarquilly C. (1986). Prediction of forage digestibility by principal component analysis of near infrared reflectance spectra. *Animal Feed Science and Technology*, **16**: 215-224.

Schofield, P. (2000). Gas production methods. In: D'Mello, J.P.D. (ed.) *Farm Animal Metabolism and Nutrition*. pp. 209-232. Wallingford: CAB International.

Schofield, P. and Pell A.N. (1995). Measurement and kinetic analysis of the neutral-detergent-soluble carbohydrate fraction of legumes and grasses. *Journal of Animal Science*, **73**: 3455-3463.

Schenk, J.S. And Westerhaus M.O. (1994). The application of near infrared reflectance spectroscopy (NIRS) to forage analysis. In: Fahey, G.C., Collins, M., Mertens, D.R., Moser, L.E. (eds) *Forage quality, evaluation and utilisation*. pp. 406-449. Wisconsin, U.S.A.: American Society of Agronomy.

Stefanon, B., Pell A.N. and Schofield P. (1996). Effect of maturity on digestion kinetics of water-soluble and water-insoluble fractions of alfalfa and brome hay. *Journal of Animal Science*, **74**: 1104-1115.

Thapa, B., Walker D.H. and Sinclair F.L. (1997). Indigenous knowledge of the feeding value of tree fodder. *Animal Feed Science and Technology*, **67**: 97-114.

Thorne, P.J. (1998). *DRASIC: a dairy rationing system for the tropics. Evaluation version for Windows 3.1*. Natural Resources Institute, Chatham, U.K., 40 pp.

Tilley, J.M.A. and Terry R.A (1963). A two-stage technique for the *in vitro* digestion of forage crops. *Journal of the British Grassland Society*, **18**: 104-111.

Ward, R.G., Smith G.S., Wallace J.D., Urquhart N.S. and Shenk J.S. (1982). Estimates of intake and quality of grazed range forage by near infrared reflectance spectroscopy. *Journal of Animal Science*, **54**: 399-402.

Waters, C.J. and Givens D.I. (1992). Nitrogen degradability of fresh forage: effect of maturity and growth type, and prediction from chemical composition and by near infrared reflectance spectroscopy. *Animal Feed Science and Technology*, **38**: 335-349.

Williams, B.A. (2000). Cumulative gas-production techniques for forage evaluation. In: Givens, D.I., Owen, E., Axford, R.F.E. and Omed, H.D. (eds) *Forage Evaluation in Ruminant Nutrition*. pp. 189-231. Wallingford: CAB International.

Table 1 - Results of indoor studies in which DM intakes, estimated using C₃₂ and C₃₃ alkanes, were compared with actual intakes in sheep and cattle (adapted from Mayes *et al* (1995)).

Animals and diets	Alkane dosing method*	Mean true DM intake	Mean bias of estimate
Sheep			
Fresh perennial ryegrass	Shredded paper	579 g/d	0 g/d
Freeze-stored perennial ryegrass	CRD	913.5 g/d	-0.2 g/d
Fresh herbage	Gelatin Capsule	778 g/d	20 g/d
Cattle			
Fresh grass	Shredded paper	4.00 kg/d	-0.07 kg/d
Fresh perennial ryegrass	Shredded paper	13.70 kg/d	0.05 kg/d
Fresh perennial ryegrass	Shredded paper	12.39 kg/d	0.35 kg/d
Fresh perennial ryegrass	Gelatin Capsule	12.64 kg/d	-0.96 kg/d

* Dosing methods:

Shredded paper - Pellet of shredded paper, impregnated with C₃₂ alkane.

CRD - Intraruminal controlled release device, containing C₃₂ alkane.

Capsule - Gelatin capsule, containing cellulose powder coated with C₃₂ alkane.

Table 2 - Prediction of voluntary dry-matter intake of forages by NIR spectroscopy (from Deaville and Flinn (2000)).

Forage type	Number of measurements	of	Intake (g kg ⁻¹ LW ^{0.75} day ⁻¹)		NIR prediction		Reference
			Species	Range	R ²	SE _p	
Mixed	76		Sheep	39.7-114.3	0.62	7.8	Norris <i>et al</i> (1976)
Grazed pasture	21		Cattle	52.6-112.3	0.72	9.6	Ward <i>et al</i> (1982)
Grazed pasture	80		Sheep	430-1458*	0.80	140	Flinn <i>et al</i> (1992)

* Organic-matter intake animal⁻¹ day⁻¹; LW, live-weight.
SE_p Standard error of prediction.

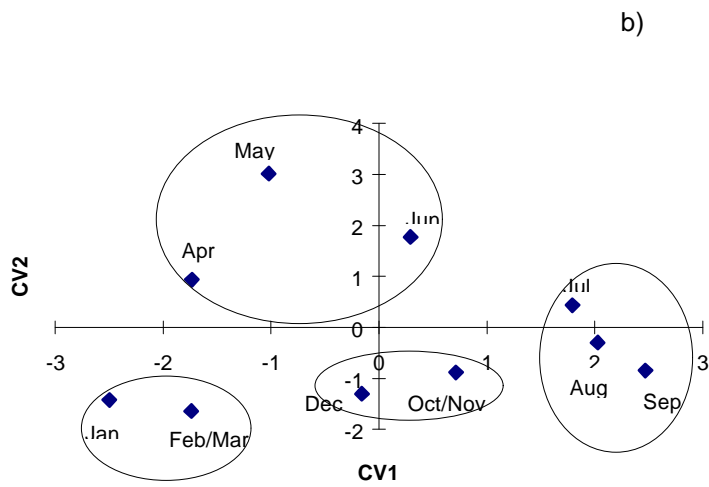
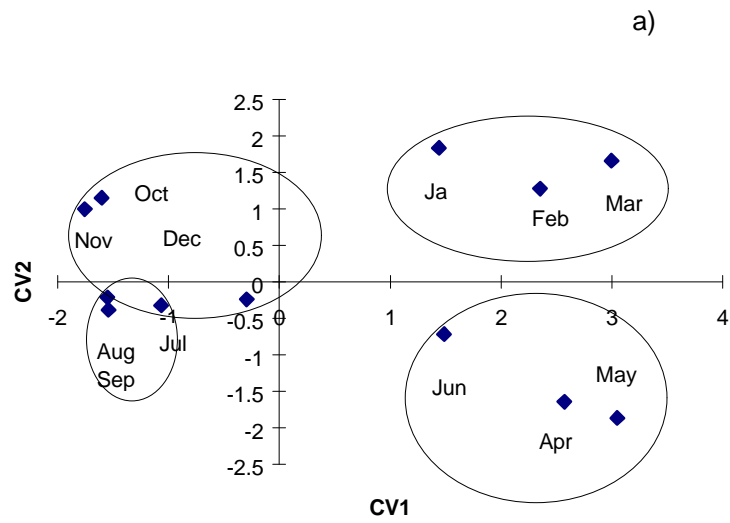


Figure 1 - Canonical variate means of the concentrations of odd-chain alkanes of samples of browse species and herbage species in a “montado” system in Portugal at different dates in 1997.