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What are feeds worth?: A critical evaluation of selected nutritive value methods

A.T. Adesogan
Department of Animal Sciences
University of Florida, Gainesville

Introduction

Feeding can account for up to 60% of the costs of livestock production and even under the intensive concentrate feeding systems of ruminant animal production in the USA, forages continue to represent the single most important feed resource (Jung and Allen, 1995). Yet forage quality varies with species, variety, physiological maturity, regrowth, season, time of harvest, cutting height, fertilization and other factors. Feed analysis is therefore important for nutritionally characterizing forages and highlighting the supplemental nutrients needed, such that rations can be effectively formulated to optimize animal production. Feed analysis is also valuable for quality assurance in feed manufacturing and for identifying the presence and concentrations of undesirable substances in feeds, which adversely affect animal health and productivity. Feed analysis is therefore indispensable for efficient resource use and profitability in livestock production.

Although livestock performance is the best index of feed quality, the public dislikes animal experimentation and it is too costly, labor intensive and protracted to be routinely practicable. Therefore animal performance is generally estimated from less animal-based techniques that measure related parameters such as feed composition, digestibility, degradation, fermentation and passage. This review appraises some of such analytical methods in terms of factors like their accuracy, biological relevance, cost, reproducibility, appropriateness for routine use and ability to handle large numbers of samples. A basic understanding of the techniques is assumed and the reader is referred to Givens *et al.* (2000) for their descriptions. Traditional wet chemistry techniques for estimating chemical components are intentionally omitted due to their diversity and the abundance of reviews on such subjects in the literature.

In vitro digestibility methods

The rumen fluid-pepsin method of (Tilley and Terry, 1963) is one of the most useful methods for predicting digestibility *in vivo* (Clancy and Wilson, 1966; De Boever *et al.*, 1988). Unlike other techniques, which only attempt to simulate ruminal digestion, the technique and its' variants also mimic gastric digestion and therefore accurately predict the *in vivo* digestibility of many forages (Tilley and Terry, 1963; De Boever *et al*., 1988).

The main drawback of the technique is its' reliance on rumen fluid which is typically sourced from fistulated animals. It is becoming increasingly difficult to obtain the licenses required to surgically prepare such animals and they are expensive to keep and must be concealed from the public in some countries. The results from the technique are also affected by variability in the quality of the rumen fluid which can be due to processing, host animal diet and species, time of collection and the extent to which anaerobic conditions and optimal pH and temperature are maintained (Tilley and Terry, 1963; Clancy and Wilson, 1966). Most of these problems can be prevented by including standards in experiments (Tilley and Terry, 1963) but more complicated shortcomings include the disregard of post-gastric digestion, outflow of digesta and the digestion of pepsin-insoluble nitrogenous compounds. Some of these factors have led to differences between *in vitro* and *in vivo* digestion residues and cause poor predictions of *in vivo* digestibility. For instance malliard products in silages are digested *in vitro* but not *in vivo* and underestimation of metabolic fecal N in *in vitro* residues impairs the prediction of N digestibility (Ibbotson *et al.*, 1982).

Several workers have found that though accurate for fresh grasses, the rumen fluid-pepsin technique gives less accurate predictions of digestibility in silages and straw (Klopfenstein *et al*., 1972; Adesogan *et al.,* 1998b; Givens *et al*., 1995). To better account for post-ruminal digestion, Van Soest *et al.* (1966) introduced a technique, which measures true digestibility *in vitro* by replacing the acid-pepsin step of the Tilley and Terry (1963) technique with a neutral detergent digestion step. Although the resulting technique is shorter and often more accurate, it requires using rumen fluid and hence retains the attendant problems.

Akhter *et al.* (1996) developed a method that replaces the rumen fluid in the Tilley and Terry (1963) procedure with fecal inoculum. Relatively good predictions of *in vivo* digestibility are obtainable. However this technique will probably be limited to centers that can't obtain rumen fluid as it gives low digestibility values and combines the protracted, labor-intensive aspects of the rumen fluid-pepsin technique with feces collection and activity problems.

Several cellulose-based techniques have been used with some success, to estimate forage digestibility. Compared to rumen fluid-based methods, such methods are generally simpler, less time consuming, more convenient and reproducible and don't require fistulated animals. The main problem with such techniques is the variability in the activity of the enzyme preparations due to the batch and source of the enzyme. Such differences can account for up to 15 digestibility units (g/kg dry matter (DM) (De Boever *et al*., 1988). This problem can be avoided by using standards or by regressing cellulose digestibility on mass of cellulose used as a substrate (De Boever *et al*., 1988).

Nevertheless, enzyme-based predictions of *in vivo* digestibility and energy value also vary with forage species, population and season of harvest (Barber *et*

al., 1989; Givens *et al.*, 1995) such that predictive relationships developed have limited application. Furthermore, procedural differences have limited the widespread use of the techniques. Depending on the laboratory, cellulase solubilisation is either preceeded with either pepsin or neutral detergent treatment or without treatment. Further variations involve including amylase and or gamannase pretreatments for starch and oil rich feeds respectively. While some results indicate that the pepsin-cellulase procedure is more accurate others favor the neutral detergent cellulase procedure. Nevertheless, the pepsin cellulase method is easier to manipulate, prone to less errors and requires fewer hours to complete even though the neutral detergent technique requires fewer days (Dowman and Collins, 1982). While such methods don't require fistulated animals, their use continues to be limited by variability in enzyme activity and because they inadequately represent the array of enzymes employed during *in vivo* digestion.

The ANKOM Daisy incubator was recently introduced in order to simplify the estimation of digestibility *in vitro.* The method entails digesting several forage samples in bags within glass jars, which are themselves rotated in an insulated chamber. The technique significantly reduces the labor input associated with *in vitro* digestibility estimation because it obviates the need for filtration and allows batch inoculation of several samples with the rumen fluid – buffer mixture. Several authors have shown that the technique gives relatively accurate predictions of *in vitro* apparent and true digestibility (Julier *et al.,* 1999; Vogel *et al.,* 1999; Wilman and Adesogan, 2000) and it has the potential to be used to estimate the degradation rates of feeds. However the digestibility results obtained can be affected by sample size and processing method, the proximity of the incubation jars to the heat source and the extent to which individual bags are submerged throughout the incubation. Adesogan A. T. (unpublished) also observed that predictions of *in vivo* digestibility from the technique were more accurate when the forages were incubated in non-standard bags. However, when such non-standard bags are used, the results obtained will depend on the pore size, seal treatment and weave type. The potential for loss of soluble or fine particulate, undigested substrate also limits the feed types and sample-processing methods can be used. In addition, associative effects between samples incubated in the same vessel can also influence results. Some of these factors may have contributed to slightly less accurate predictions of *in vivo* digestibility from the technique in relation to those from traditional rumen fluid–pepsin techniques (Wilman and Adesogan,1999). In spite of these factors, the ANKOM technique represents a faster, more convenient way to determine the *in vitro* digestibility of feeds.

In situ degradability methods

The dacron bag technique (Orskov *et al.*, 1980) for measuring the *in situ* rumen degradability of feeds has received widespread attention partly because it

can be readily used in developing countries since it is not reliant on a steady electricity supply, and more importantly because it is one of the few techniques that describes the kinetics of feed degradation in the rumen. The technique has also provided relatively good predictions of forage intake and digestibility (Orskov, 2000) and has greatly improved the understanding of nitrogen (N) supply to ruminants and their microbes. It now forms the basis of describing N requirements of ruminants in the feeding systems of several countries. Yet the technique is plagued by low reproducibility and repeatability (Noziere and Michalet-Doreau, 2000) and it is notoriously difficult to standardize despite repeated attempts (Madsen and Hvelplund, 1994). Several excellent reviews on the technique (Huntington, 1995; Nocek, 1985; Noziere and Michalet-Doreau, 2000; Orskov, 2000) indicate that the results obtained vary with sample preparation method, washing and drying procedure, extent and nature of particulate losses, incubation site and sequence, host animal species and diet, bag size, weave type and pore size, and removal of microbial contamination. The effect of some of these factors on degradability are shown in Table 1. These factors have hampered the comparison of results from different experiments.

Some of the problems of the technique stem from the methods currently used to characterize incubated substrates. Noziere and Michalet-Doreau (2000) suggested that sample particle sizes should be stated instead of their grinding screen size because ground particles contain an array of particle sizes that differ in chemical composition and rate and extent of degradability. In addition, the technique may not adequately account for effects of supplementation or antinutritive factors in feeds and it is not appropriate for characterizing soluble and small particulate feeds or single-celled proteins (Orskov *et al.,* 2000; Noziere and Michalet-Doreau, 2000). Although there is widespread use of first order exponential models for characterizing degradability profiles, most of such models erroneously assume that a discrete lag phase occurs before the onset of degradation (Sauvant, 1997) and poorly describe the N degradability profiles of feeds high in soluble N (Givens, 1994). There has also been relatively little validation of the *in situ* degradability measurements with *in vivo* data, such that it is difficult to accept or refute the accuracy of the protein fractions derived from the technique (Beever, 2000). Attempts to characterize the degradability of starch and NDF with the technique have yielded variable and sometimes conflicting results (Beever, 2000).

The shortcomings of the *in situ* degradability technique highlighted above reflect the need for caution in interpreting the results. However, the technique has advanced our knowledge of protein metabolism in ruminants significantly. In the absence of a valid alternative, it will continue to be a valuable tool for assessing the kinetic parameters of feed degradation.

Table 1. Factors affecting the accuracy of *in situ* rumen degradability techniques.

Factor	Effect	Reference
Oven drying	Reduces N degradability and solubility.	(Lopez, *et al.,*1995)
Freeze drying	Enhances particulate losses but is better than other drying methods for silages.	(Lopez, *et al.,*1995; Vik-Mo, 1989)
Grinding / pre-wetting samples	Underestimates the lag phase and overestimates degradation rates due to increased microbial colonization.	Noziere and Michalet-Doreau (2000)
Particle size	The lag phase is prolonged with larger particles.	Emmanuele and Staples, (1988)
Washing procedure	Machine washing overestimates solubles and particulate losses but is less subjective than hand washing.	Cockburn, *et al.* (1993)
Particulate losses	Overestimates rumen solubles and the extent of degradation but can underestimate degradation rates if the particles lost would have degraded rapidly.	Emmanuele and Staples, (1988)
Incubation sequence	Reverse sequence incubation can reduce degradation rates due to interruptions and differences in rumen environment of samples incubated for different periods.	Nocek (1985)
Incubation site	Substrate incubation in the dorsal rumen sac underestimates degradability due to lower colonization rates than those in the ventral sac.	Stewart (1979)
Bag pore size	If < 15µm can reduce degradation by restricting microbial colonization and diversity and trapping fermentation gases. If > 40 µm, causes losses of insoluble / undegradable particles.	Huntington and Givens (1995)
Bag weave type	Unlike multifilamentous cloth, the pores of monofilamentous cloth are prone to stress-induced distortion that can enhance particulate losses.	Marinucci *et al.* (1992)
Microbial contamination of residues	Underestimates N degradation in low N feeds. Removal methods can be expensive, laborious or inaccurate.	Olubobokun *et al.* (1990)

In vitro gas production methods

The *in vitro* gas production technique also generates kinetic data but rather than measuring the disappearance of dietary components, it measures the appearance of fermentation gases notably CO_2, CH_4, H_2. Compared to the *in situ* degradability technique, gas production methods are less animal dependent, more appropriate for characterizing soluble or small particulate feeds and they can be automated thus reducing the labor input. They can also be used to generate information on rates and extents of digestion, proportions of volatile fermentation products and microbial protein production. However automated gas production methods are expensive and may or may not handle large numbers of samples. While manual methods are cheap, they are labor intensive, restricted in capacity and they often generate inadequate kinetic data for precise descriptions of fermentation rates. The results generated from both types of equipment are dependent on several procedural details and they are often misunderstood. Table 2 shows the effect of several factors on gas production. In addition to these factors, the results obtained vary with the type of system used whether closed or opened and the source, activity and consistency of the rumen fluid used (Schofield, 2000).

Table 2. Factors affecting the accuracy of *in vitro* fermentation gas production techniques.

Factor	Effect	Reference
Sample form	Wilting increases fermentation rate and freeze-drying and milling increases gas production relative to chopped /unchopped fresh forage.	Sanderson *et al.* (1997)
Oven-drying samples	Eliminates volatile constituents from fermented substrates thus reducing the indirect gas produced from their reaction with the buffer.	Deaville and Givens (1998)
Buffer composition	High phosphate buffers reduce gas production by utilizing protons that would have been used for CO_2 production.	Schofield (2000)
RF inoculum to buffer ratio	When greater than 1:2, blanks no longer truly represent the contribution of the inoculum to gas production.	Cone *et al.* (1997)
Size of liquid-gas interface	Determines the potential for gas supersaturation and solubilisation, which reduces, gas production.	Theodorou *et al.* (1998)
Prevailing pH and temperature	Decreases gas production if below optima for cellulolytic bacteria growth.	Russell and Dombrowski (1980)
Atmospheric pressure	Determines actual gas volumes. Yet it is often omitted such that it is difficult to compare results from different labs.	Williams, (2000)
Stirring	Reduces CO_2 supersaturation which causes erroneous volume / pressure readings.	Pell and Schofield (1993)

Several models have been proposed for describing kinetic gas production data. Such models vary in complexity from single pool models digesting at a variable fractional rate (France *et al.,* 1993) models to empirical multipool models (Groot *et al.,* 1996). Many of such models contain parameters that have little biological relevance and perhaps more importantly, many are often used with insufficient attention to their appropriateness for describing the fermentation profile of the feed being studied. Yet several reports have emphasized the inadequacy of some models for describing the fermentation of certain feeds (Beuvink and Kogut, 1993; Adesogan *et al.,* 1998; Dhanoa *et al.,* 2000).

The results of gas production experiments are often misinterpreted and used to draw inappropriate deductions. Gas production is often assumed to be directly proportional to substrate digestion and hence nutritive value. This is inaccurate because gas production is dependent on substrate composition, microbial population and hexose utilization for microbial yield. Several authors have shown that less gas is produced from feeds high in propionate precursors relative to that in feeds high in acetate and butyrate precursors (Beuvink and Spoelstra, 1992; Beever and Mould, 2000; Williams, 2000). Others have shown that the ammonia in high protein feeds can decrease gas production by reaction with volatile fatty acids (Schofield, 2000). In spite of its' importance, very few reports have quantified the extent of hexose utilization for microbial biomass production during gas production experiments. All of these factors determine the quantity of gas produced during substrate fermentation. Consequently Beever and Mould (2000) stated that *in vitro* gas production values alone provide little direct information, apart from estimating fermentation rates. Therefore gas production data should be supplemented with measurements of substrate disappearance, volatile fatty acid profiles and microbial yield in order to give comprehensive nutritional information on the feed tested (Schofield, 2000). However, the additional labor and cost implications will continue to limit and perhaps prevent this suggestion from being implemented.

Near infrared reflectance spectroscopy

Near infrared reflectance spectroscopy (NIRS) is a physical analytical method, which is based on the absorbance of light at wavelength regions that relate to chemical components within feeds. Compared to other methods, NIRS is unique because it is non-destructive, it requires no reagents and is therefore non-polluting and it characterizes the entire forage rather than specific components of interest (Deaville and Flynn, 200). The technique is also suited for large numbers of samples and the procedure involved is cheap after the initial capital outlay for the equipment. NIRS has been successfully used to predict a wide range of forage quality parameters. In several instances, it gives superior predictions of nutritive value than chemical analysis or bioassays (Barber *et al.,* 1990; Adesogan *et al.*, 1998). However, it must remembered that NIRS is not a 'stand alone' technique. Rather the NIRS equipment has to be calibrated with

plant characteristics determined by traditional wet chemistry. Therefore the validity of forage composition data obtained using NIRS will never be better than the databases used to establish the calibrations (Beever and Mould, 2000). The technique therefore requires large, frequently updated data sets, which must be similar in nature and variability to the samples that are to be tested. The attendant costs have limited the use of the technique as have the potential for transferring errors from the original wet chemistry technique to the NIRS prediction. It is therefore crucial to know the error associated with the reference method before assessing the accuracy of the NIRS calibration (Beever and Mould, 2000).

The adoption of NIRS has also been limited by its' dependence on a knowledge of the appropriate wavelengths for the entity being analyzed. This has given the technique the infamous 'black box' reputation. The problem is compounded by the complex algorithms required to develop the calibrations, none of which is consistently best (Deaville and Flynn, 2000) . Multiple stepwise regression is probably the simplest and most familiar of the regression techniques used but it allows intercorrelation between spectral data (Reeves III, 2000). Principal components analysis and Partial least squares analysis avoid the latter problem but are more complicated even though Partial least squares analysis is often preferred because it uses reference method data as well as spectral data to derive the predictions (Deaville and Flynn, 2000).

Reeves III (2000) noted that NIRS estimates of forage quality are also affected by particle size, temperature, sample homogeneity, packing density and type of feed or spectrometer and suggested solutions to such problems. Other common determinants of calibration accuracy including residual moisture in samples, light scatter and path length variation can be accounted for using the repeatability files, standard normal variate-detrending procedure, multiplicative scatter correction and derivatisation (Deaville and Flynn, 2000). Nevertheless, the number of factors affecting NIRS estimates highlights the important of validating the calibration relationships with large independent data sets. Although the requirement for large validation populations can be reduced by internal cross validation, the need to ensure that similar variability exists between the calibration and validation population remains. Several reports have ignored this by using equations derived for specific forages to predict nutritional parameters in other forages. Yet most of the accurate NIRS predictions tend to be species-specific, such that inaccurate estimations of nutritive value are obtained when they are used for other forages. Such misuse of the technology is quite common as is the use of NIRS to predict nutritional value indices, which themselves have not been validated against *in vivo* measurements; Consequently, while NIRS has much to offer, it should be used only where there is established confidence in the data (Beever and Mould, 2000).

General conclusions and future recommendations

This review has highlighted some of the shortcomings of a few methods of feed evaluation. In each case, the extent to which the problems are corrected should depend on an unbiased cost benefit analysis in relation to the objectives of the experiment. However there are some general problems, which apply to most methods of feed evaluation that require more urgent attention.

The first of these is the widespread use of measures of nutritive value, which have not been validated against *in vivo* data. This can take the form of extrapolating equations developed for one class of feeds to another or using lab. derived measurements as references for other *in vitro* data, without ensuring that the former accurately predicts *in vivo* measurements. As mentioned previously, livestock performance is the best index of feed quality and techniques should only be classed as reference methods where a good relationship with animal performance has been shown.

Secondly, too many methods are currently available for measuring the same nutritional parameter, such that the results obtained vary with the procedure used substantially. For instance the starch and crude protein contents of low DM corn silage sent to up to nine laboratories in Europe and the USA using different analytical techniques, ranged from 165-172 g/kg DM and 57-119g/kg DM respectively (Beever and Mould, 2000). Such variation is clearly untenable. A related problem is the use of antiquated predictive relationships, which have been shown to be inaccurate. For instance, modified acid detergent fiber is currently used in some UK laboratories for predicting the metabolisable energy value of grass silages. Yet the error associated with the prediction is much higher (standard error of prediction = 1.30; r^2 = 0.14; Givens, 1989) than that recommended as the maximum permissible (0.5MJ/kg corrected DM) to prevent inaccurate metabolisable energy predictions and attendant wastes in feed outlay or lost production (Offer, 1993). These problems reflect the need for accreditation agencies that will ensure nation-wide standardization of analytical methods and the maintenance of quality assurance standards.

Thirdly, rather than continuing with the current focus on measuring nutritionally related plant characteristics, future feed evaluation methods should determine the concentrations of specific nutrients in feeds and their rates of release to facilitate the prediction of animal response. In addition to refining current methods for the estimation of intake, microbial protein production and outflow rates, future analytical methods should aim to accurately determine diet selection, supplementation effects and associative effects of different ingredients fed together. Non-conventional factors such as the environmental impact of feeds and their potential for transferring bioactive compounds to humans should also be the focus of future evaluation methods (Givens *et al.,* 2000).

Literature Cited

Adesogan, A.T., Owen, E. and Givens, D.I. 1998a. A comparison of the suitability of different models for describing the gas production kinetics of whole crop wheat. In: *In vitro* techniques for measuring nutrient supply to ruminants, British Society of Animal Science Occassional Publication No. 22, Reading, pp 215-216.

Adesogan, A.T., Givens, D.I. and Owen, E. 1998b. Prediction of the *in vivo* digestibility of whole crop wheat from *in vitro* digestibility, chemical composition, *in situ* rumen degradability, *in vitro* gas production and near infrared reflectance spectroscopy. *Animal Feed Science and Technology*, **74**: 59-272.

Akhter, S., Owen, E. and Hossain, M.M. 1996. Effluent from RUSITEC inoculated with rumen liquor or cow faeces as sources of micro-organisms for in vitro digestion of forages. *Asian-Australasian Journal of Animal Sciences*, **9**: 375-379.

Barber, G.D., Givens, D.I., Kridis, M.S., Offer, N.W. and Murray, I. 1990. Prediction of the organic matter digestibility of grass silage. *Animal Feed Science and Technology*, **28**: 115-128.

Beever, D.E. and Mould, F.L. 2000. Forage evaluation for efficient ruminant livestock production. In: D.I. Givens, E. Owen, R.F.E. Axford and H.M. Omed (Editors), Forage Evaluation in Ruminant Nutrition. CABI Publishing, Wallingford, UK, pp. 15-42.

Beuvink, J.M.W. and Kogut, J. 1993. Modelling gas production kinetics of grass silages incubated with buffered rumen fluid. *Journal of Animal Science*, **71**: 1041-1046.

Beuvink, J.M.W. and Spoelstra, S.F. 1992. Interactions between substrate, fermentation end products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen microorganisms *in vitro*. *Applied Microbiology and Biotechnology*, **37**: 505-509.

Clancy, M.J. and Wilson, R.K. 1966. Development and application of a new chemical method for predicting the digestibility and intake of herbage samples. Proceedings of the Xth International Grassland Congress, Helsinki, pp. 445-453.

Cockburn, J.E., Dhanoa, M.S., France, J. and Lopez, S. 1993. Overestimation of solubility when using dacron bag methodology. Proceedings of the 1993 Annual Meeting of the British Society of Animal Science, Scarborough, p 188.

Cone, J.W., Van Gelder, A.H. and Driehuis, F. 1997. Description of gas production profiles with a three-phasic model. *Animal Feed Science and technology*, **66**: 31-45.

De Boever, J.L., Cottyn, B.G., Andries, J.I., Buysse, F.X. and Vanacker, J.M. 1988. The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. *Animal Feed Science and Technology*, **19**: 247-260.

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: D.I. Givens, E. Owen, R.F.E. Axford and H.M. Omed (Editors), Forage Evaluation in Ruminant Nutrition. CAB International, Wallingford, pp. 301-320.

Deaville, E.R. and Givens, D.I. 1998. Investigation of direct gas production from silage fermentation acids. Proceedings of the British Society of Animal Science Annual Meeting, Scarborough, p 64.

Dhanoa, 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 gas production profiles observed in vitro: comparison of models. *British Journal of Nutrition*, **83**: 131-142.

Dowman, M.G. and Collins, F.C. 1982. The use of enzymes to predict the digestibility of animal feeds. *Journal of the Science of Food and Agriculture*, **33**: 689-696.

Emmanuele, S.M. and Staples, C.R. 1988. Effect of forage particle size on *in situ* digestion kinetics. *Journal of Dairy Science*, **71**: 1947-1954.

France, J., Dhanoa, M.S., Theodorou, M.K., Lister, S.J., Davies, D.R. and Isac, D. 1993. A model to interpret gas accumulation profiles associated with *in vitro* degradation of ruminant feeds. *Journal of Theoretical Biology*, **163**: 99-111.

Givens, D.I., 1994. Assessment of forage quality with particular reference to protein. Proceedings of the conference on 'Metabolisable protein and forage evaluation'. Society of Chemical Industry, London.

Givens, D.I., Cottyn, B.G., Dewey, P.J.S. and Steg, A., 1995. A comparison of the neutral detergent-cellulase method with other laboratory methods for predicting the digestibility *in vivo* of maize silages from three European countries. *Animal Feed Science and Technology*, **54**: 55-64.

Givens, D.I., Owen, E. and Adesogan, A.T. 2000. Current procedures, future requirements and the need for standardisation. In: D.I. Givens, E. Owen, R.F.E. Axford and H.M. Omed (Editors), Forage Evaluation in Ruminant Nutrition. CABI Publishing, Wallingford, UK, pp. 449-474.

Huntington, J.A. and Givens, D.I. 1995. The *in situ* technique for studying the rumen degradation of feeds: A review of the procedure. *Nutrition Abstracts and Reviews (Series B)*, **65**: 65-93.

Ibbotson, C.F., Phillips, M.C., Turner, P.J. and Delaney, M. 1982. The alkali treatment of whole crop cereals. Part **I** - Farm scale feasibility trial with barley. *Experimental Husbandry*, **38**: 154-162.

Julier, B., Lila, M., Furstoss, V., Travers, V. and Huyghe, C. 1999. Measurement of cell-wall digestibility in lucerne using the filter bag technique. Animal Feed Science and Technology, 79: 239-245.

Jung, H.G. and Allen, M.S. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. *Journal of Animal Science*, **73**: 2774-2790.

Klopfenstein, T.J., Krause, V.E., Jones, M.J. and Woods, W. 1972. Chemical treatment of low quality forages. *Journal of Animal Science*, **35**: 418-422.

Lopez, S., Hovell, F.D.D., Manyuchi, B. and Smart, I. 1995. Comparison of sample preparation methods for the determination of the rumen degradation characteristics of fresh and ensiled forages by the nylon bag technique. *Animal Science*, **60**: 439-450.

Madsen and Hvelplund. 1994. Prediction of *in situ* protein degradability in the rumen: Results of a European ring test. *Livestock Production Science*, **39**: 201-212.

Nocek, J.E. 1985. Evaluation of specific variables affecting *in situ* estimates of ruminal dry matter and protein digestion. *Journal of Animal Science*, **60**: 1347-1358.

Noziere, P. and Michalet-Doreau, B. 2000. *In sacco* methods. In: J.P.F. D'Mello (Editors), Farm animal metabolism and nutrition. CAB International, Wallingford, pp. 233-254.

Offer, N. 1993. Background to the new in vivo ME silage prediction equation. Proceedings of the 1993 Society of Feed Technologists Conference, Reading.

Olubobokun, J.A., Craig, W.M. and Pond, K.R. 1990. Effects of mastication and microbial contamination on ruminal *in situ* forage disappearance. *Journal of Animal Science*, **68**: 3371.

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

Orskov, E.R., Hovell, F.D.D. and Mould, F. 1980. The use of the nylon bag technique for the evaluation of feedstuffs. *Tropical Animal Production*, **5**: 195-213.

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

Reeves III, J.B. 2000. Use of near infrared reflectance spectroscopy. In: J.P.F. D'Mello (Editors), Farm animal metabolism and nutrition. CAB International, Wallingford, pp. 185-207.

Russell, J.B. and Dombrowski, D.B. 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. *Applied and Environmental Microbiology*, **39**: 604-610.

Rymer, C. 2000. The measurement of forage digestibility *in vivo*. In: D.I. Givens, E. Owen, R.F.E. Axford and H.M. Omed (Editors), Forage Evaluation in Ruminant Nutrition. CABI Publishing, Wallingford, UK, pp. 113-134.

Sanderson, R., Lister, S., A, S. and Dhanoa, M. 1997. Effect of particle size on *in vitro* fermentation of silages differing in dry matter content. Proceedings of British Society of Animal Science Annual Meeting, Scarborough, p 197.

Sauvant, D. 1997. Rumen mathematical modeling. In: P.N. Hobson and C.S. Stewart (Editors), The rumen microbial ecosystem. Blackie Academic & Professional, London, pp. 685-708.

Schofield, P. 2000. Gas production methods. In: J.P.F. D'Mello (Editors), Farm animal metabolism and nutrition. CAB International, Wallingford, pp. 209-232.

Stewart, C.S. 1979. Problems in the assessment of fibre digestion in the rumen. In: E. Grossbard (Editor), Straw decay and its effect on disposal and utilisation. John Wiley and Sons, UK, pp. 315-319.

Terry, R.A., Mondell, D.C. and Osbourn, D.F. 1978. Comparison of two *in vitro* procedures using rumen liquor-pepsin or pepsin-cellulase for prediction of forage digestibility. *Journal of the British Grassland Society*, **33**: 13-18.

Theodorou, M.K., Lowman, R.S., Davies, Z.S., Cuddeford, D. and Owen, E. 1998. Principles of techniques that rely on gas measurement in ruminant nutrition. In: *In vitro* techniques for measuring nutrient supply to ruminants, British Society of Animal Science Occassional Publication No. 22, Reading; pp 55-63.

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

Van Soest, P.J., Wine, R.H. and Moore, L.A. 1966. Estimation of the true digestibility of forages by the in vitro digestion of cell walls. Proceedings of , The Xth International Grassland Congress, Helsinki. Finish Grassland Association., pp 438-441.

Vik-Mo, L. 1989. Degradability of forages *in sacco.* I. Grass crops and silages after oven and freeze drying. *Acta Agriculturae Scandinavica*, **39**: 43-52.

Vogel, K.P., Pedersen, J.F., Masterson, S.D. and Toy, J.J. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Science, 39: 276-279.

Williams, B.A. 2000. Cummulative gas-production techniques for forage evaluation. In: D.I. Givens, E. Owen, R.F.E. Axford and H.M. Omed (Editors), Forage Evaluation in Ruminant Nutrition. CAB International, Wallingford, pp. 189-213.

Wilman, D. and Adesogan, A. 2000. A comparison of filter bag methods with conventional tube methods of determining the in vitro digestibility of forages. *Animal Feed Science and Technology*, **84**: 33-47.