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The XX International Grassland Congress took place in Ireland and the UK in June-July 2005.

The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

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Recent developments in methods to characterise the chemical and biological parameters of grass silage

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Key points

1. Chemical analysis of forages is expensive, time consuming, environmentally unfriendly and relates poorly to the feed value for production purposes.
2. *In vivo* characterisation of animal feed is not a feasible option in terms of cost and analysis time.
3. NIRS is a rapid, non destructive, environmentally friendly, multi-analytical technique which can estimate the nutritive value of the feed.
4. NIRS predictive equations developed on a master instrument can be transferred to local and international sites.
5. Future assessment of forages necessitates rapid, stable, instrumentation for 'in field' studies.

Keywords: near infrared reflectance spectroscopy, diode array, forages

Introduction

Accurate characterisation of available feeds is a key requirement for cost-effective feeding of farm livestock. It forms the basis of not only making decisions on appropriate feeding levels to meet production targets but also optimising economic combinations for differing feed sources. While traditional approaches to silage characterisation provide some insight into feed quality, they are laborious to carry out and their accuracy in estimating biological parameters is limited. Ruminant production is now facing new challenges in a difficult economic climate, with the need to predict the quality of farm output, for example milk components (fat, protein, and lactose) or meat components (fatty acids profiles) rather than just volume or weight. As a consequence there has been a need to develop more accurate, rapid and cost-effective methods to characterise grass silage.

Previous approaches

The nutritive value of grass silage has traditionally been expressed in terms of organic matter digestibility (OMD) and digestibility of crude protein (DCP). These were derived from prediction equations based on chemical analysis of the materials. Alternatively in some countries more biologically meaningful *in vitro* techniques using rumen micro-organisms or commercial cell-free enzymes (Givens *et al.*, 1995) have been adopted. Table 1 shows the relationships between laboratory measurements, which have been used to predict the OMD of silages and the actual *in vivo* data. The precision of the relationships, as shown by R^2 values of 0.34–0.74, and particularly the modified acid detergent (MAD) fibre relationship previously used in the UK, are relatively poor. Although these wet chemistry approaches were, at the time, a reasonable compromise between simplicity and accuracy of prediction, they were also labour intensive and slow to carry out.

Table 1 Correlations between *in vivo* organic matter digestibility values (mean OMD = 0.71) and a range of laboratory measurements in 122 grass silage samples

	RSD	R ²
MAD fibre	0.051	0.34
Pepsin cellulase digestibility	0.042	0.55
In vitro digestibility	0.032	0.74

(Barber *et al.*, 1990)

Silage feeding value

Silage feeding value depends on the nutritive value of the silage expressed either as the digestibility or the ME concentration and the quantity of silage the animal will consume, that is the intake potential. It must be recognised however that the intake potential is often more important than the nutritive value in determining the feeding value of a silage as it exhibits greater variation. Accurate prediction of both intake potential and ME concentration, or digestibility of a silage are both therefore essential pre-requisites to the effective rationing of dairy cows and beef cattle offered grass silage *ad libitum*. In addition to feeding value and chemical composition, it is becoming increasingly important to be able to predict the rates of digestion of the protein components in silages and the amount of fermentable metabolisable energy. These latter parameters are required in situations where rations must be formulated to produce specific animal end-products. In view of the need to develop a new approach to predicting the feeding value of grass silage a series of studies were initiated at the Agricultural Research Institute of Northern Ireland, Hillsborough (funded by the Department of Agriculture and Rural Development). The studies were designed to ensure that both chemical and near infrared reflectance spectroscopy (NIRS) methods could be explored.

Animal experimentation to provide database

At the onset of the Hillsborough research programme it was recognised that a new approach to feed evaluation could only be developed if the intake, digestibility, chemical composition and rates of digestion of a large number of silages, representing the range of types of silages across the industry, were characterised within a standard animal protocol. The study, involved selecting silages on the basis of their pH, dry matter, ammonia and metabolisable energy contents. A total of 136 silages were selected on this basis from farms across Northern Ireland. Approximately seven tonnes of each silage were brought to the Institute, mixed in a mixer wagon to achieve uniformity and stored in polythene-lined boxes until feeding one to four weeks later. Care was taken to ensure that there was no deterioration of the silages during storage and work by Pippard *et al.* (1996) showed that their chemical composition remained constant throughout the storage period. The silages were offered *ad libitum* to 192 individually fed steers, which were crosses of the continental beef breeds and had a mean initial live weight of 415 kg, in a partially balanced changeover design experiment. Detailed chemical compositions of the silages were also determined. All silages were offered to sheep to determine *in vivo* digestibility. The rates of disappearance and rumen degradabilities of the dry matter, nitrogen and fibre fractions in the 136 silages were determined using the *in sacco* method. Steen *et al.* (1998) have reported the main results of this study, particularly the prediction of silage feeding value from chemical analyses (organic matter digestibility (OMD) was predicted from chemical constituents with a coefficient of determination ($R^2 = 0.30$)).

This paper is concerned with the further development of the data from this study to explore the potential of NIRS as a means of predicting a range of feeding parameters of grass silage.

Near infrared reflectance spectroscopy

Spectroscopy means 'looking at light' and the matter to be analysed interacts with electromagnetic radiation. Near infra red light is defined as the wavelength region from 780 to 2500 nm in the electromagnetic spectrum. When a sample is scanned, light is absorbed selectively according to the specific vibration frequencies of the molecules present and gives rise to a spectrum. All organic bonds have absorption bands in the NIR region, whereas minerals may only be detected in organic complexes. In forages, NIRS is therefore detecting the bonds in the protein, oil and carbohydrate fractions. The optical data stored as the NIRS spectrum is then regressed against known parameters to produce the best correlations and the resulting equation is then used to predict the parameters in unknown samples.

NIRS offers a number of advantages over traditional chemical and *in vitro* analyses. It is a rapid, physical, non-destructive method, requiring minimal or no sample preparation and its accuracy is high. It is an environmentally friendly technique as no harmful, corrosive chemicals are used and there are no waste products to dispose. NIRS is a multi-analytical technique as many parameters can be predicted simultaneously.

NIRS was first shown to be a reliable, practicable method for predicting the chemical composition of forages by Norris *et al.* (1976) in the USA. Since then numerous workers have explored the use of NIRS for the prediction of both chemical composition and digestibility of grass silages. Attempts have also been made to use NIRS to predict the voluntary intake potential of dried, milled forages (Norris *et al.*, 1976; Shenk *et al.*, 1977; Ward *et al.*, 1982; Coelho *et al.*, 1988; Abreu *et al.*, 1991; Flinn *et al.*, 1992).

Prediction of intake and organic matter digestibility using dried samples

As all previous uses of NIRS to evaluate forages were based on scanning of dried milled samples this procedure was adopted as the first approach in the present study. Samples were air equilibrated after drying and milling and scanned at 2 nm intervals over the visible and near infrared spectral range (400-2500 nm) using a Foss NIRSystems 6500 scanning spectrophotometer. The full details of the methods and results of this work are presented by Park *et al.* (1997a).

The spectral data were subjected to a range of mathematical treatments to develop the optimum prediction methods. These methods included regression and derivatisation techniques and three scatter correction procedures. Appropriate cross validation was performed by removing one sample from the population of 136 in turn and forming a calibration on the remaining 135 samples and predicting the excluded sample. The best three mathematical treatments of the data, within each of these regression techniques, were selected on the basis of the lowest Standard Error of Calibration (SEC) and highest coefficient of determination (R^2). On the basis of the calibration statistics (SEC), the modified partial least squares (MPLS) technique achieved the best performance, having the lowest individual SEC and highest R^2 for intake (SEC 3.4 g/kg $W^{0.75}$, $R^2 = 0.90$) and organic matter digestibility (SEC 1.64 g/kg $W^{0.75}$, $R^2 = 0.94$) respectively.

Prediction of intake and organic matter digestibility using undried silage

The drying of silage is time consuming and leads to the loss of volatile acids, alcohols, esters, amines and ammonia which may influence the accuracy of predicting the components of feeding value (intake and digestibility). The development of more rapid, less expensive and potentially more accurate NIRS methodologies using fresh (undried) silage was therefore considered a desirable objective. However it was recognised that in this approach the sample preparation method could have a major influence on the accuracy of any data obtained particularly where NIRS scanning instruments only scanned small areas of sample. A programme was therefore undertaken to examine a range of sample preparation and scanning methods for undried silages using a Bran & Luebbe InfraAlyzer 500 Spectrophotometer either internally in a closed or viscous cup or externally using a probe attachment on the NIRS instrument.

In this study the 136 undried silages were scanned using eight different methods, involving various combinations of five sample preparation methods (intact with no pre-treatment; coarsely chopped; finely milled following freezing in liquid nitrogen; expressed liquor or eluent) and three scanning techniques (external probe; internal closed or internal viscous sample cup). Liquor was extracted from 100 g intact silage using a manual screw press and the eluent was decanted from 50 g intact silage immersed in 100 ml distilled water overnight. Six spectral scans ($\log 1/\text{Reflectance}$, $1/R$) were produced for each of the 136 silage samples by each of the 8 methods. The full methods and results of this study are given in Gordon *et al.* (1998) and a brief summary of the OMD data is presented in Table 2. The lowest standard error of cross validation (SECV) for the prediction of intake and digestibility was obtained by using finely milled silage presented to the NIRS instrument in the internal cup, and the highest SECV was obtained using the eluent. This work has demonstrated that in a static scanning-based system enhanced comminution has been an important factor in increasing the accuracy of the prediction of intake.

Table 2 The performance statistics for the prediction of organic matter digestibility (mean value 678 g/kg) using NIRS on undried silage samples (n=136) prepared and scanned by a range of methods

Preparation Method	Calibration and validation statistics for organic matter digestibility		
	SEC	R ²	SECV
(a) Intact tray	25	0.87	31
(b) Intact tube	23	0.89	27
(c) Coarse tube	26	0.86	29
(d) Fine tube	24	0.88	27
(e) Coarse cup	18	0.94	26
(f) Fine cup	18	0.94	26
(g) Liquor	37	0.73	46
(h) Eluent	52	0.44	56

(Gordon *et al.*, 1998)

Reeves and Blosser (1991) have indicated that enhanced comminution of fresh forages improved the accuracy of NIRS for prediction of fibre components but not crude protein.

However no similar data are available on the effects of particle size in fresh forages on the accuracy of biological parameters, such as digestibility and intake. Nevertheless it is likely that due to the linkage of digestibility and intake to the extent and nature of the fibre fraction these also will be influenced by particle size.

Calibrations based on scanning intact undried grass silage

The previous section has shown that when static scanning techniques are adopted it is preferable to finely comminute the undried material before scanning. However, this implies freezing and milling prior to scanning, both of which incur time delays and increased labour costs. Recently NIRS instruments have become available which incorporate a moving sample transport mechanism which may permit irradiation of a much larger sample surface area and minimise the effects of sample heterogeneity and so eliminate the need for sample comminution (e.g. Foss NIRSystems). Therefore, to evaluate this system NIRS calibrations for a range of chemical and biological parameters based on scanning intact undried grass silage were developed using this equipment. Two sub-samples of each silage were wrapped in non PVC cling film before being packed into a rectangular sample cell with internal dimensions of width 4.1 cm, length 17.2 cm and depth 1.4 cm. Each sample was scanned 24 times over the entire area of the sample and then automatically averaged using ISI NIR3 Version 4.00 (Infrasoft International, Port Matilda, PA, USA) software, to produce one representative spectrum, thus minimising the effects of sample heterogeneity. Scanning takes less than a minute and optical data were recorded as log 1/R at 2 nm intervals. Equations were developed to predict dry matter, pH, total nitrogen, insoluble nitrogen, neutral detergent fibre, ether extract, gross energy and organic matter digestibility (Park *et al.*, 1996). The cross validation statistics are given in Table 3 where 1-VR = the coefficient of determination showing the proportion of variation in the reference method values explained by cross validation predicted values.

Table 3 Cross-validation statistics for the equations developed on the Foss NIRSystems 6500 using 136 uncomminuted undried grass silage samples.

Parameter	Mean	SECV	1-VR
Oven dry matter (g/kg)	201	6.38	0.98
pH	4.15	0.14	0.85
Total nitrogen (g/kg fresh)	4.64	0.34	0.92
Insoluble nitrogen (g/kg fresh)	1.98	0.15	0.89
Neutral detergent fibre (g/kg fresh)	118	5.13	0.95
Ether Extract (g/kg fresh)	7.73	0.61	0.88
Gross Energy (MJ/kg fresh)	4.05	0.14	0.96
Organic matter digestibility (%)	71.0	2.60	0.87

(Park *et al.*, 1996)

While the above work was undertaken using a Foss NIRSystems 6500 instrument, another instrument, which involves a rotating (600 rpm) sample cup and also provides a similar scanning area of the sample is also available (Bran & Luebbe InfraAlyzer 500). Work undertaken by Offer *et al.* (1996) had suggested that this latter approach may be a more accurate methodology. A study was therefore undertaken to compare the accuracy of both types of scanning monochromators in the development of calibrations for seven chemical and

biological parameters of undried silage. Samples of 136 undried silages were scanned in the two instruments and calibrations were produced using the MPLS regression technique, in conjunction with either first or second order derivatisation and three scatter correction procedures. Optimum equation selection was based on the lowest SECV. The results showed no consistent differences in cross-validation statistics between the two instruments (Table 4).

Table 4 Cross-validation statistics for a range of parameters of undried silage developed on two types of NIRS instruments

Parameter	Foss NIRSystems 6500		B&L Rotating Cup	
	SECV	1-VR	SECV	1-VR
Alcohol corrected toluene dry matter (g/kg)	7.38	0.97	8.61	0.95
pH	0.14	0.85	0.16	0.83
Total nitrogen (g/kg fresh)	0.34	0.92	0.27	0.95
Neutral detergent fibre (g/kg fresh)	6.23	0.92	6.40	0.91
D-value (g/kg alcohol corrected toluene dry matter)	22.4	0.85	21.8	0.86
Voluntary Intake (g/kg $W^{0.75}$)	6.39	0.71	6.84	0.65

(Park *et al.*, 1998b)

Cloning of NIRS instruments

Whilst research at Hillsborough had shown that NIRS is an accurate, rapid method for characterising grass silage, it was important to consider the transfer of this predictive system to other NIRS instruments either within or between laboratories across the wider industry. This has been shown to be particularly difficult for heterogeneous and high moisture samples when differing types of equipment are being used. It would therefore be advantageous if calibrations developed on one NIRS instrument could be successfully transferred to another NIRS instrument, irrespective of manufacturer. Unfortunately spectral differences exist even between instruments of the same make and model (Dardenne & Biston, 1990). These differences in spectra are due to differences in the optical and electronic characteristics of the instruments and even very small differences have considerable effects on the predictions of parameters produced using the same equations.

Transfer of NIRS calibrations across instruments is commonly achieved by adjusting the equations for slope and bias. An alternative approach is to standardise the instruments so that they produce identical spectra (Shenk *et al.*, 1985). This 'cloning' approach has been shown to be successful when using homogeneous materials, such as dried milled samples (Forina *et al.*, 1995; Flinn *et al.*, 1995) or whole grain samples (Dardenne *et al.*, 1992). However difficulties arise when samples, with more than 200 g/kg moisture or high log 1/R values, are used because at high log 1/R values the reflectance data become non linear due to stray light (Shenk & Westerhaus, 1995). Grass silage is a very heterogeneous material consisting of leaves, stems, and dead material from a wide range of plant species and with a dry matter content ranging from approximately 120 to over 500 g/kg.

Two studies (Park *et al.*, 1998b and 1999) were undertaken to develop the technique of successfully transferring undried grass silage calibrations developed from one make of NIR spectrophotometer (i.e. Foss NIRSystems 6500) to another make of NIR spectrophotometer

(i.e. Bran & Luebbe InfraAlyzer 500) and also between instruments of the same type (i.e. Foss NIRSystems 6500 to Foss NIRSystems 5000).

In the first study a range of undried grass silage samples was selected to clone two different makes of scanning monochromators using the ISI (Infrasoft International) cloning software. This software produces standardisation files which when applied to spectra from the slave instrument makes them 'look like' spectra from the master instrument and so allows accurate prediction by equations developed on the master instrument. Using an independent set of silages to compare the predictions of the unstandardised and standardised slave spectra to the master spectra predictions, using the same equations, showed that the standard error of prediction (SEP) was greatly reduced and the R^2 increased after standardisation of the spectra. The standardised spectra predictions were highly correlated to the master predictions, thus proving that this method of transferring calibrations worked very successfully. In comparison the alternative and commonly used method of sloping and biasing equations for use on other instruments was examined. Again the SEP values were reduced in line with the cloning method but the resultant slope and bias values were not improved over the cloning method. In addition, the average 'H' values, a measure of the closeness to the original data set, were not reduced by sloping and biasing as this technique matches the equations and not the spectra. Calibrations for biological parameters transferred more successfully when the cloning technique was employed. This study has demonstrated that this method of cloning monochromators of two different types and without the use of sealed sample sets, has proven very successful even with forages of high moisture content.

In the second study the cloning technique of Shenk *et al.* (1985) was used to spectrally match a Foss NIRSystems 6500 and a Foss NIRSystems 5000. A range of grass silage samples were scanned through both instruments using both a coarse transport cell and a natural product cell. These are the two most commonly used cell types with this instrument for scanning fresh forages. Cloning was based on using either 30 samples or one central sample. Standardisation files were produced (using the ISI software) and applied to the spectra of the validation samples, scanned on the Foss NIRSystems 5000 (regarded as the slave instrument). These standardised spectra were predicted by the master equations developed on the Foss NIRSystems 6500 instrument and the results compared to the corresponding master validation spectra scanned in the coarse transport cell and predicted by the master equations (regarded as the reference values). The spectra of a silage sample scanned in the coarse transport cell on the master, and the slave instruments and the standardised slave spectrum are shown in Figure 1.

In all instances the cloning technique (based on either one or thirty samples) proved very successful, clearly indicating that undried grass silage calibrations can be transferred across NIRS instruments of the same type with little loss in accuracy of prediction.

Use of NIRS in the Hillsborough Feeding Information System

The NIRS prediction equations developed on the Foss NIRSystems 6500 (Park *et al.*, 1998b) for chemical composition, digestibility, intake potential and rates of digestion have subsequently been used to establish the Hillsborough Feeding Information System. This is a commercial service available from the Institute for the evaluation of grass silage and the provision of associated feeding information. The system is based around 3 main functions; sample registration, sample analysis, and sample reporting with automation being used to increase efficiency and reduce costs. Silage sample information is logged into computer software and the undried (fresh) silages are scanned via a coarse transport cell mechanism on

the NIRS instrument. This provides a NIRS spectrum which is examined through a set of calibration equations which allow the key attributes of the silage to be predicted automatically. This information is then appraised through a computer model to provide feeding strategies for dairy cows, growing cattle, suckler cows, breeding ewes and growing lambs. Wherever possible the System has been automated to keep the cost to the customer at a minimum and produce a final report within four working days for grass silage. Since its inception in 1996, the forage analysis service at Hillsborough has processed in excess of 80,000 samples of grass silage (approximately 12,000 silage samples/year at present).

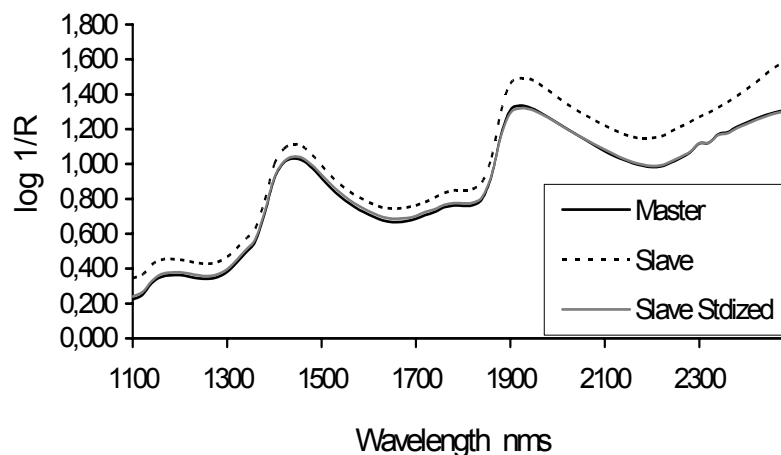


Figure 1 Comparison of the spectra of an undried grass silage sample scanned in a coarse transport cell, on the master (Foss 6500) and the slave (Foss 5000) NIRS, plus the slave spectrum standardised to the master spectrum

In January 2000 a new body known as the Forage Analytical Assurance (FAA) Group was formed. It comprises of the vast majority of silage analysis laboratories in the UK and one in the Republic of Ireland, accounting for approximately 85,000 forage samples per year. The FAA group use a range of equations developed on the Master NIRS instrument at the Agricultural Research Institute of Northern Ireland. This was accomplished by cloning thirteen instruments to the Hillsborough Master. Since it was established, the group has developed common quality control methods, with twice yearly stringent ring tests carried out between all sites plus monthly bias checks performed to maintain forage characterisation accuracy. It is hoped that these robust equations could also be expanded and new parameters developed for use internationally.

Future developments in forage characterisation

The NIRS programme of silage characterisation described here has developed an excellent advisory tool for quantifying the energy and protein components of ruminant diets – including intake, digestibility and rates of degradation etc. The key reasons for this success at the advisory level have been:

- Rapid sample turn round.
- Relatively inexpensive technique.
- Robust predictive relationships.
- Excellent repeatability.

However in the future from both an animal and an environmental perspective there is a need to provide similar accuracy in relation to some of the key cations and anions in ruminant diets, especially dairy cows. For example the relationship between dietary Na, K, Cl and S are the primary drivers of the Dietary Cation-Anion Difference (DCAD) which is important in dry cow management (control of milk fever) and lactation. Even within relatively small geographical areas (such as Northern Ireland) DCAD levels in grass silage can vary widely and this can have important implications for animal performance.

Equally phosphorus (P) levels in ensiled forages (grain and cereals) can also vary widely. With the present move towards on-farm P balances becoming a key environmental factor driving on-farm profitability it will become increasingly important to ensure that minimal levels are purchased onto the farm via concentrates. This can only be accurately achieved, and risks to animal health minimised, if P levels in individual forages are available. While it is fully recognised that NIRS may not be a good direct determinant of some of the key minerals it remains quite possible that indirectly NIRS may provide sufficiently robust relationships for the advisory situation.

To really assess the quality of forages, evaluate the nutrient status (N, P, K etc), detect early signs of disease problems and therefore improve the yield and quality of forage, we need to be able to perform 'in-field' (real time) studies with hand held or machine mounted instrumentation which can rapidly evaluate the crop. This type of evaluation is currently being enhanced by the development of new rapid scan NIR monochromator/detection systems without mechanically moving parts, such as acoustic-optic tuneable filters (AOFT), diode arrays or charge-couples devices. The advantages of new developments would be speed of measurement, mechanical robustness, temperature stability and small instrument size. In-field instruments would have to be robust in order to withstand mechanical shock as vehicles pass over rough ground. Real time data would provide objective rationale for adding fertiliser, fungicides etc and thus reduce over use of these chemicals and unnecessary expense.

Research is also progressing to determine on-line evaluation of total mixed rations in dairy feeding systems, where an NIR device is mounted directly on a feed mixer. At present problems exist in solving sample presentation in front of the scanning window of the feed mixer. When presentation and software problems are resolved, the farmer will have instant access to a complete breakdown of the concentration of all constituents in the diet enabling monitoring and adjustment of the feed for production purposes. Major minerals levels could also be monitored, especially phosphate levels which are an environmental concern in dairy rationing.

There seems to be little doubt that for reasons of speed and costs InGaAs-based diode array spectrometers are top of the instrumental shortlist. However these instruments have a reduced wavelength range and acceptable solutions for calibration transfer among these instruments still require work.

On a much larger scale, at present in America, sophisticated airborne and space-based imagers from NASA are enabling development of precision farming systems. Using hyperspectral, multispectral and infrared imaging, farmers can identify areas that are experiencing water, nitrogen or micronutrient stress and target their relief. The spectral information can be fed into a digital controller and, using the global positioning system for navigation, tractors can be guided to problem areas where they can distribute nutrients or pesticides within 19 mm of where they are needed. This technique has also allowed the farmer to reduce fertiliser

application where it is not needed, thus affording production of higher yields at a lower cost. Early identification of limiting plant nutrients could substantially benefit EU farmers who now have to comply with the limits set by the Phosphate and Nitrates Directive.

Conclusions

The Near Infrared Reflectance Spectroscopy Research Programme based at the Agricultural Research Institute of Northern Ireland has developed a rapid, cheap and effective method for predicting a wide range of chemical and biological parameters, including a prediction of intake potential of grass silage for use in dairy cow, beef cattle and sheep rationing systems. These new methods enable cost effective and accurate predictions of a range of nutritionally important parameters of grass silage and are the basis of proven commercial silage analysis systems.

Further research has demonstrated that these calibrations can be successfully transferred to other NIRS instruments of either the same or different type.

With the rapid advances in instrumentation the future looks set to enable real time assessment of forages/crops, allowing in-field decisions to be made regarding the health and nutrient status of the plant. Thus yields should improve with reduced costs due to identification of mineral/nutrient requirements of the plants. These improvements in automation and sensor technology will play a significant role for farmers seeking to compete in today's global markets plus reduce environmental pollution due to overuse of nitrates and phosphates.

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