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Application of Near Infrared Reflectance Spectroscopy (NIRS) for forage evaluation

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1. Introduction

The attractivity of the NIRS technique for the analysis of forage quality parameters stems from a number of factors. First and foremost are the superior speed of analysis and the resulting lower laboratory running costs as compared to conventional wet chemical methods. Ease of sample preparation and the physical nature of the measurements are the reasons for the complete absence of health hazards in its use so that even inexperienced laboratory personnel can be entrusted with the routine analytical work. Furthermore, a number of methodological studies indicate the superiority of NIRS over several conventional methods of feedstuff fractionation in terms of analytical precision and reproducibility. Lastly, it is noteworthy that one single spectroscopic analysis allows the simultaneous derivation of several diverse constituents such as fibre, protein, fat and others. Taken together, NIRS offers the potential of a low cost but reliable analytical tool for a comprehensive feedstuff evaluation.

With such large benefits to be expected, NIRS deserves the full attention of forage quality analysts and the occasion of this symposium is therefore taken to present an overview of the physical and statistical basis of NIRS, to name factors influencing its analytical reliability, to present the accuracy and precision of NIRS for predicting forage digestibility and finally to formulate a proposal for future NIRS method development specifically for the analysis of fresh forages.

2. Physical basis of NIRS

In the past, one of the more destructive arguments against the analytical use of NIRS has been the supposed "black box" nature of the technique. Very often, this repeated criticism has been based on a lack of understanding of the physical and statistical principles which form its methodological basis.

For animal nutritionists controlled feeding experiments and balance studies represent the only acceptable method for obtaining "true" data on such a complex characteristic as the digestibility of a feedstuff. However, it is also accepted that approximations of these "true" digestibility data may be obtained by a variety of methods for the controlled fractionation of feeds into digestible and undigestible components. With regard to the use of NIRS for feedstuff analysis, the basic hypothesis is that light absorbance patterns in the near infrared are equally suitable and legitimate predictors for various complex feedstuff characteristics as for instance crude fibre, ADF or the digestibility in vitro. After all, the primary spectroscopic measurements in the NIR-range are taken amidst other analytically highly useful regions of the electromagnetic spectrum (Fig. 1). This is true for the fingerprint region of the far infrared and for the medium infrared as well as for the ultraviolet and visible part of the spectrum which all form the basis of an immense number of spectrophotometric methods. Obviously, the peculiarities of the measurements in this range have to be taken into account. They concern the physical form of the sample and the physical basis of the signals during the measurements.

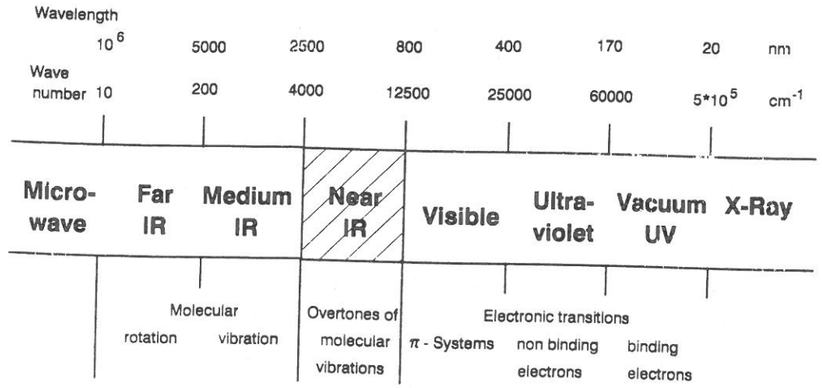

Fig. 1: Spectral ranges of electromagnetic radiation

Necessitated by their opacity in the near infrared, samples made up from fine to coarse particles are usually measured in diffuse reflectance rather than in transmission mode. Although quantitative relationships between absorption and concentration are complicated under these conditions, the work of physical chemists like the recently deceased Professor Gustav Kortüm (1969) has shown that upon suitable transformation, diffuse reflectance values are linearily related to concentration when the scattering coefficient of the samples is held constant. This has the practical advantage that NIRS measurements on dried and finely ground forages can be easily carried out and used for quantitation.

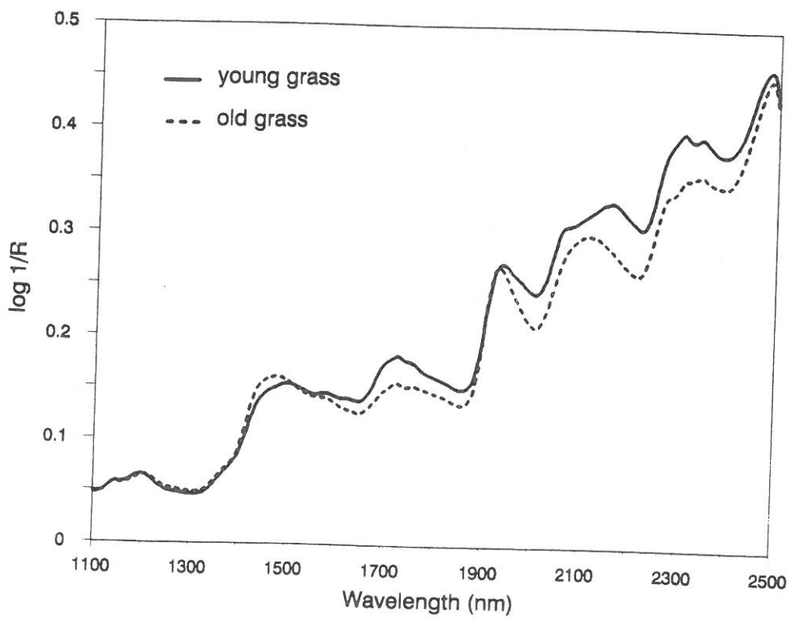

Fig. 2: NIR spectra of young and old grass in primary growth.

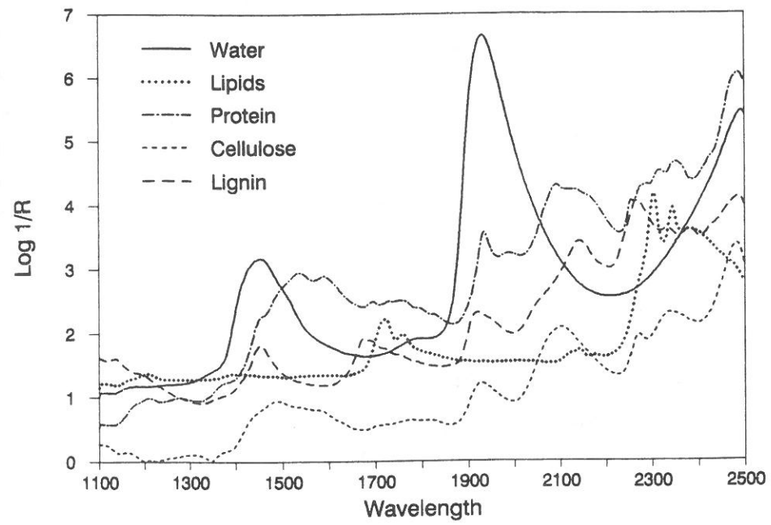

Fig. 3: NIR spectra of pure compounds

Viewed superficially, NIR spectra of two forage grasses, of which one is high and the other low in digestibility, show relatively little differentiation and few characteristic features (Fig 2). However, both these spectra may be considered as linear combinations of the pure component spectra in these samples like those shown in Fig.3. However, in practice whole forage spectra are far too complex to be reconstructed from pure component spectra. This is due to physico-chemical interactions between different molecular species which even causes water bound in grass hay to differ spectrally from water bound in wheat or barley grains.

Irrespective of whether complex biological samples or pure components are considered, their spectra are made up of convolutions of single absorption bands which primarily can be assigned to the OH-, CH- and NH-groupings of pure compounds or their mixtures. Their position is highly predictable from spectrochemical theory in pure substances but less so in multicomponent samples due to the immense interactions and overlap of the single bands.

Particularily these last mentioned interactions between different molecular species on the one hand and different absorbance bands on the other hand are the reason why the analytical utilization of the NIRS region can only be realised by empirical means.

3. Statistical basis of NIRS

The first scientist to realise that statistical modelling provides the essential methodological backbone of NIRS was Karl Norris of the USDA Instrumentation Research Laboratory at Beltsville (see NORRIS 1982). He divided the development of NIRS methods into two steps, namely calibration and validation (Fig.4). During calibration many specially selected samples of a given product are measured by NIRS to provide optical data and by a particular conventional method, e.g. Kjeldahl nitrogen to provide the so called reference data on the calibration samples. By means of linear regression, suitable NIRS calibration equations are then derived.

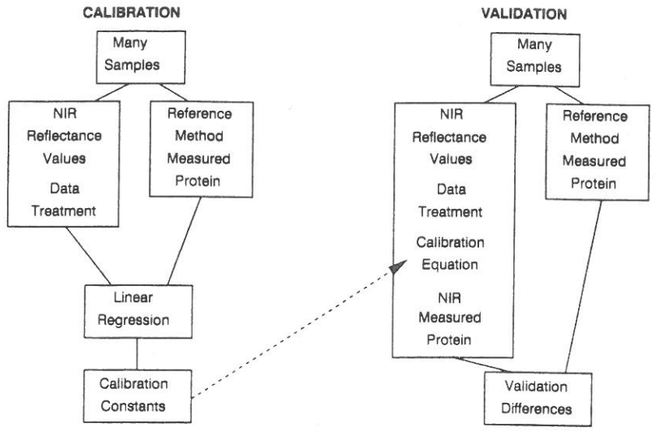

Fig. 4: Procedure for empirical NIRS method development (HRUSCHKA 1987)

	Crude Protein		Crude Fibre	
	WL(nm)	b[1]	WL(nm)	b[1]
0	-	26.2	-	22.5
1	1272	-523.0	1128	-598.2
2	1806	1391.4	1732	1379.9
3	2142	-804.1	1810	-2825.4
4	2256	161.9	2308	-1446.9
DT[2]	1/20/5/1		1/10/5/1	
RSQ[3]	0.96		0.96	
SEC[4]	1.28		0.95	

[1] Regression coefficients b_0- b_4 [2] Spectral data treatment

[3] Coefficient of determination [4] Standard error of calibration

Tab. 1: NIRS calibration equations for crude protein and crude fibre in primary growths of permanent grassland on PSCO monochromator system 6250

As shown in Tab. 1, NIRS equations are usually characterised by specific wavelengths for given constituents after the spectral data have been subjected to a certain mathematical transformation. The goodness of fit obtained in calibration is also given in terms of the coefficient of determination and the standard error of calibration.

The NIRS equations are then tested in validation with yet another set of samples (Fig.4). Based on the previously derived NIRS equations, reference data are predicted and also measured by the reference method itself. The comparison between both data sets then represents the validation test of the equation. Its result allows to decide whether a given NIRS equation is satisfactory in terms of accuracy and precision.

4. Determining factors for analytical reliability

4.1 Reliability of reference method

One of the most evident prerequisites to be fulfilled for successful NIRS method development is the condition that the reference method itself must be analytically reliable. Apparently, the determination of the nitrogen content by means of the Kjeldahl method and the subsequent derivation of the crude protein content by N x 6.25 would have to be considered as one of the most classical and reliable methods for determining a nutrient in conventional feedstuff analysis. Yet recent collaborative experimentation between the University of Halle-Wittenberg in East-Germany and this Institute

demonstrated conclusively that during routine laboratory work substantial random and systematic errors may occur even in the Kjeldahl method.

The NIRS equation shown in Tab. 1 for crude protein, originally developed for samples from mixed permanent grassland swards in North-Germany (PAUL, BORSTEL and SOMMER unpublished) was used for predicting crude protein contents in samples from experimental grass seed multiplication plots in East-Germany. When the NIRS predicted data were plotted against the data obtained in the Kjeldahl reference laboratory, not only a systematic deviation between the samples from the two harvest years 1989 and 1990 was observed but also a number of outliers in the 1990 data (Fig. 5). Extensive reanalysis of the Kjeldahl laboratory reference data uncovered the errors in these data. After a corresponding correction, the goodness of fit and the standard error of prediction (SEP(C)) were clearly improved in the 1990 data and also the differential systematic deviation (Bias) between NIRS and laboratory data in the two harvest years was reduced (Fig. 6).

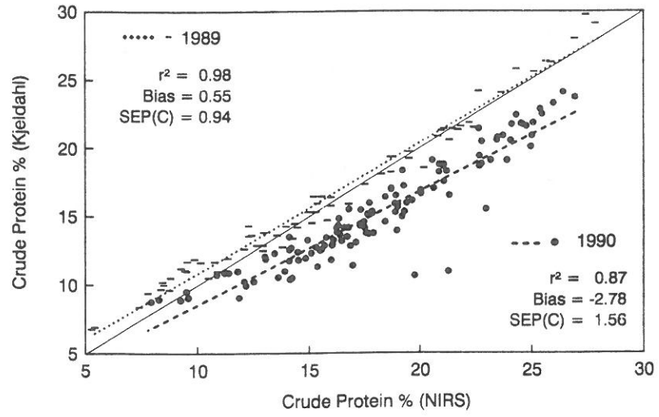

Fig. 5: Comparison of crude protein determined after Kjeldahl and predicted by NIRS before renalysis of Kjeldahl reference data

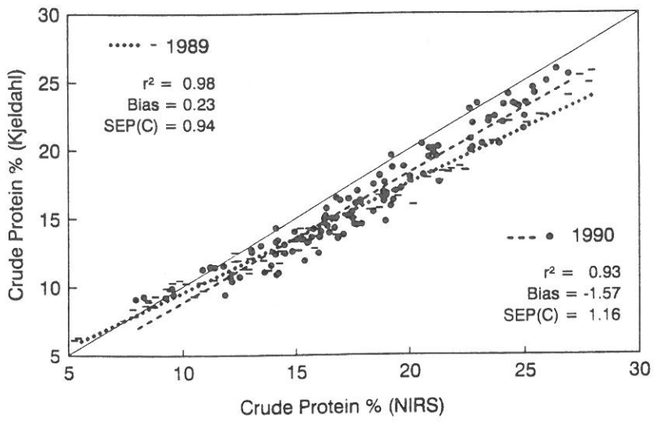

Fig. 6: Comparison of crude protein determined after Kjeldahl and predicted by NIRS after reanalysis and correction of Kjeldahl reference data

In this way NIRS had improved the quality of the conventional laboratory data as well as the consistency in the results of the underlying field experimentation (PAUL and SCHÖBERLEIN 1991). This shows that it is wrong to attribute a possible lack of fit between NIRS and the conventional laboratory method always to the NIRS method. The conventional laboratory method itself is subject to a number of errors and needs to be properly quality controlled to serve as a satisfactory basis for NIRS calibration and validation.

4.2 Adequacy of the calibration sample set

The empirical nature of NIRS method development requires a strict comparability between the samples used for calibration and those unknown samples to be analysed or predicted by means of the newly developed NIRS method. An optimal set of calibration samples would have to built up in such a way that any unknown sample would be represented in the set by chemical and spectroscopical counterparts.

The importance of adequate calibration samples can best be demonstrated by a project conducted to provide NIRS equations for rapid quality determinations in forage maize breeding and variety testing. It entailed the stepwise build up of three calibration sets: starting with an initial base of calibration samples from the harvest of 1986 from German origin (set 1, n=112), later supplemented by European samples from the harvest of 1987 (set 2, n=98) and finally combined with samples from the 1988 harvest (set 3, n=147). Based on these three consecutive sets of calibration samples three NIRS equations were derived for crude protein as well as for digestibility of the organic matter (in vitro). Obviously, the chance of representing the necessary spectrochemical variation in the third generation equation was higher than in the first generation equation. The analytical reliability of these equations was tested on altogether 478 samples which were statistically independent of the calibration samples and had originated from three different trials conducted in 1987 and 1988. The overall results show that the error of the first generation equations in these samples was far too high to be acceptable (Tab. 2).

Validation Set	n	Parameter/Equation					
		Crude Protein			IVDOM		
		CP1	CP2	CP3	IV1	IV2	IV3
KWSH87	180	1.39	0.57	0.41	5.45	2.11	1.85
BSA88	268	1.09	0.49	0.27	5.15	1.77	1.47
FAP88	30	0.52	0.90	0.69	7.10	0.96	1.28
Σ	478	1.19	0.56	0.37	4.97	2.20	1.64

Tab. 2: Performance (SEP) of NIRS equations from three successive calibration cycles in forage maize in independent validation (after MAINKA 1990).

However, for crude protein as well as for in vitro digestibility, the accuracy of the third generation equation was much improved and had in fact reached the level observed in interlaboratory trials of the respective reference methods (see MAINKA 1990). In conclusion, these results demonstrate the necessity to optimise NIRS equations by means of optimising calibration sample sets.

4.3 NIRS system management and quality control

The application of any NIRS equation - even if it has been developed in a process of thorough optimisation as described above - needs to be checked for potential misuse and thus quality controlled like any conventional laboratory method.

One case in point arose at this Institute in a collaborative project with the Chamber of Agriculture of Hannover. The project aimed at supporting a scheme for advising farmers on optimal cutting dates in the primary growth of North German grassland. For the screening of crude fibre contents in samples harvested between the end of April and the beginning of May an old NIRS tilting filter system of the type Neotec 51A had been overhauled and calibrated with the same calibration samples as were used for establishing the NIRS monochromator equation given in Table 1. When the actual samples of the new harvest year 1990 became available, the accuracy of the crude fibre predictions was first checked in comparison with the conventional Weende crude fibre method. The results pointed to a systematic error of about 2% crude fibre of the equation (Fig. 7). It was assumed that an unknown hardware problem was the cause of this bias and that it could be corrected for by adjusting the equation intercept. After the corresponding bias adjustment of the equation all subsequent samples were predicted with highly acceptable accuracy (Fig. 8; Unpublished results from PAUL, BORSTEL and SOMMER).

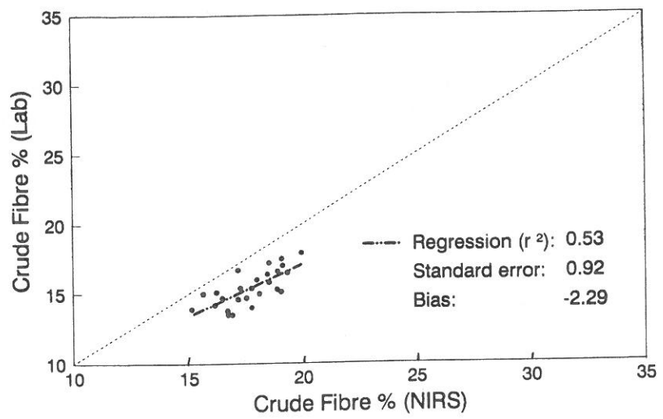

Fig. 7: NIRS prediction of crude fibre in first sampling of primary grassland growth before initial bias correction on Neotec 51A tilting filter system

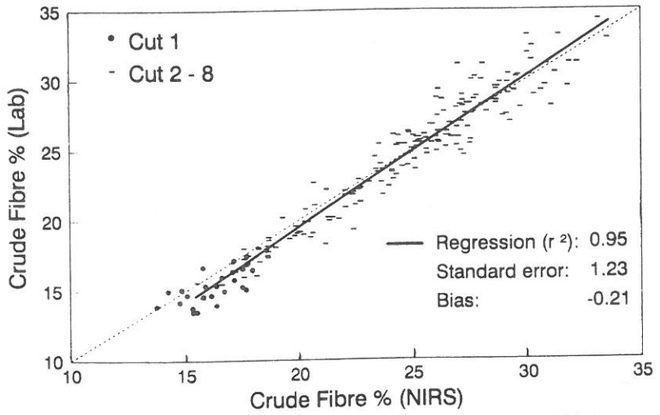

Fig. 8: NIRS prediction of crude fibre in primary grassland growth after initial bias correction on Neotec 51A tilting filter system

In conclusion, this example showed the need for monitoring the analytical reliability particularily of old NIRS instruments which has to be acknowledged like with any other conventional analytical method. Furthermore, some kind of NIRS system management needs to be implemented in order to support calibration updates, equation transfers and the calibration of newly developed instruments.

5. NIRS prediction of animal performance

In many centers of NIRS research and development for forage quality evaluation attention has shifted from merely predicting constituents like crude fibre, ADF or modified ADF which are in themselves only predictors for digestibility. Now the concept has gained acceptance that NIRS absorption patterns should be directly related to the digestibility of feeds and forages.

Correspondingly, a set of oven dried grass samples which in parallel as fresh grass samples had been subjected to FAL feeding trials for in vivo digestibility was used for NIRS calibration. As regression alternatives, stepwise multiple linear regression based on selected wavelengths (SMLR), principal component regression (PCR) and partial least square regression (PLSR) were tested in the calibration procedure. For validation, comparable samples with known in vivo digestibility values had been kindly supplied by the Institute of Forage Production at Paulinenaue / East-Germany and by the LÖLF Department of Grassland and Forage Research at Kleve-Kellen / West-Germany. The goodness of fit between NIRS predicted and actual in vivo digestibility data was high in consideration of the fact that animal feeding trials are generally known to give results with a lower reproducibility than laboratory methods (Fig. 9). PLSR proved to be the best regression method and achieved a bias corrected standard error of prediction (SEP(C)) of 2.5% DOM in the samples from Kleve-Kellen and of 3.6% DOM in the samples from Paulinenaue (PAUL, unpublished). A systematic deviation between the two data sets of about 3% DOM was there and was possibly due to a difference in feeding trial regimes between the two feed evaluation units.

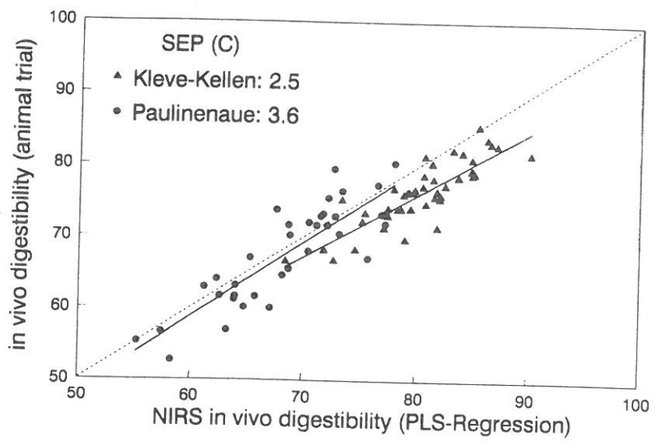

Fig. 9: Accuracy and precision of in vivo DOM prediction in grass samples from Paulinenaue and Kleve-Kellen by FAL NIRS equation established with patrial least squares regression

The results permit the conclusion that NIRS absorption patterns are indeed potentially powerful predictors for such a complex characteristic as the digestibility of a feed or forage.

6. The challenge for future NIRS research and development

Successful NIRS applications have considerably improved the turnout of forage quality analyses in forage plant breeding and grassland advisory work by substantially reducing analysis costs. A further reduction in costs seems feasible if forage samples would not have to be dried and ground prior to analysis. As an additional benefit, artefacts caused by drying and grinding treatments could be prevented if fresh forages high in moisture could be analysed directly by NIRS.

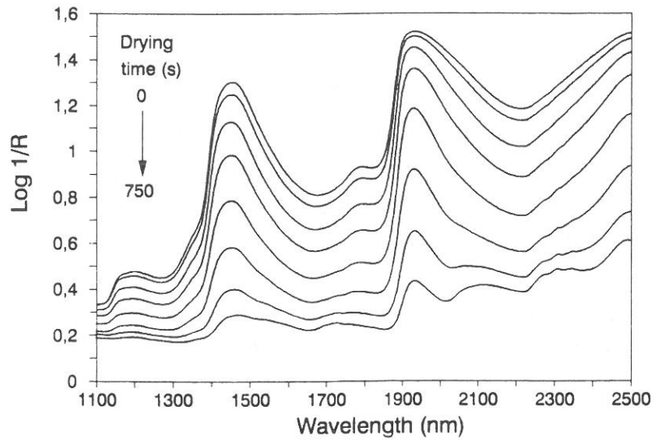

Fig. 10: NIR spectra of grass silage during sequential drying

The change in the NIRS absorption characteristics during the gradual drying of a silage (Fig. 10) indicates why previous NIRS methods for forages mainly relied on diffuse reflectance measurements on dried, ground material. The absorptivity of moist material is extremely high and increases further with increasing wavelength. This is why the absorption patterns even of pure fermentation products such as short chain fatty acids and ethanol have to be analysed by NIRS transmission in 0.5mm pathlengths. If dilute solutions are analysed in transmission or moist materials in diffuse reflectance their absorption characteristics are mainly determined by the absorption characteristics of water. As a consequence, the contribution of the organic compounds in the sample to its NIR spectrum is much less if fresh rather than dry samples are analysed. The only way in which this effect may be lessened is by ensuring higher energy throughputs through the sample during the actual measurement. This may be achieved by more powerful light sources and detectors, by analysis in the NIR region below 1300nm where the absorptivity of water is greatly reduced or by a combination of both.

Range	Absorption bands		Absorptivity	Pathlength	Dispersion-element	Light fibre absorptivity	Calibration approach
	Type	Resolution					
1300 nm-2500 nm	Combination-bands, Primary Overtones	Satisfactory	High	Low	Gratings Interference-filters	High	Wavelength selection
800 nm-1300 nm	Secondary and tertiary Overtones	Relatively low	Low	High	Photodiodes, Pulsed Laser diodes	Low	Factor analysis "full spectrum"

Tab. 3: Factors determining methods for fresh grass analysis in alterative spectral ranges in the near infrared region

The theoretical and practical consequences of this approach would be manifold (Tab. 3).

- the absorption bands below 1300nm are secondary and tertiary overtones of highly overlapping nature
- the absorptivity in this region is relatively low so that high pathlengths for thick forage samples could be measured in transmission or diffuse reflectance
- in this spectral region photodiodes and pulsed laser diodes are available so that robust instruments without mechanically moving gratings are feasible
- light fibres are particularily well suited for this spectral range which should allow repeated measurements to be taken outside a measuring cabinet like for instance on the cutting face of a silo
- the low resolution of absorption bands below 1300nm appears to require full spectrum calibration methods like PCR or PLSR rather than SMLR on the basis of selected wavelengths

It is to be expected that the future realisation and embodiment of these ideas and suggestions into suitable sensor technology will boost forage quality analysis by allowing the forage to be analysed in its native state in situ close to the points where it is produced and consumed.

7. Summary

With reference to applications in forage analysis, the spectroscopic and statistical basis of near infrared diffuse reflectance spectroscopy (NIRS) is explained. The factors judged to be essential for the analytical reliability of NIRS methods such as the reliability of the reference method, the adaequacy of the calibration sample set and NIRS system management are illustrated by results obtained in research work at the Institute of Grassland and Forage Research at FAL. It is pointed out that NIRS succeeds in predicting the digestibility as studied in animal performance trials with acceptable accuracy and precision. The main challenge for future NIRS methodological work on forages is seen as the development of applications on fresh forages by adapting the technology to achieve higher pathlengths for in situ measurements outside the measuring cabinet by means of light fibre optics.

Acknowledgments

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MODELLING THE PROCESS OF FORAGE CONSERVATION

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Abstract

Two main types of models which have been used in the study of forage conservation are described, models of individual physical processes, and 'Operational Research' models in which economics are considered. A wide range of physical processes which have been modelled are listed. Models of two of these processes, field drying of swaths and air infiltration to silos, are described in more detail. An Operational Research model, which links models of individual processes to consider the economics of a whole conservation system, is also described. A few results for models of individual processes are presented, together with some of the more far-reaching results obtained when physical models are linked into a whole system model. In particular, the substantial economic benefits from wilting silage to about 30% dry matter content are highlighted. Opportunities for further modelling work are described.

1. INTRODUCTION

For conservation of farm produced forage for winter feeding of ruminant livestock, farmers have a wide range of crops, processes, conservation systems and ancillary products to choose from. Also, new processes and technologies are continually being pioneered, both by companies with a vested interest in selling their products to farmers, and by research scientists. It is the daunting task of independent researchers to provide a sound research basis to farmers and their advisers, about the appropriateness of each system or process in a particular situation, taking account of location, climate (both average and extremes), farm size, animal enterprise, etc. For this purpose, modelling can be a valuable research activity to complement experimental research programmes.

In this paper, a range of models which have been used or could be used for the study of forage conservation systems and processes, are discussed. Models fall into two broad categories: models of physical processes and models with an economic stand-point ('Operational Research' models).

Models of forage conservation systems and processes can be used for a range of purposes, including:

1) to improve our understanding of physical processes, and hence to pin-point possible improvements;
2) to study the effects of alternative systems or system parameters, often in a much more cost effective way (in terms of researchers' time and resources) than by carrying out