FEED ANALYSIS 1860-1990 : HOW MUCH HAS REALLY CHANGED?

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SUMMARY

The limitations of laboratory methods used to test feeds for nutritive value are well-recognised but often ignored The traditional approach, which goes back 130 years, has been to separate feed samples into crude chemical fractions which are then used to predict animal performance, with varying degrees of success. Enzymic methods predict digestibility successfully, provided in vivo "standards" of similar type to the unknowns are run with each batch, but they are quite slow. There is still no adequate laboratory method to predict intake. Near infrared reflectance (NIR) spectroscopy is an optical technique, in which a multivariate modelling process is used to relate NIR spectral changes to chemical composition. NIR is now widely accepted as a rapid and reproducible method for the measurement of nutritive value indicators in grains, forages and mixed feeds, and is of considerable benefit to both industry and research. However, the accuracy of NIR depends heavily on the quality of reference methods used in calibration, most of which do not measure what NIR "sees". Research is now addressing the need for improved methods to isolate feed constituents which control digestion and intake, and their effect on NIR spectra. With improving techniques for spectral interpretation, NIR will also be used directly to characterise feeds in terms of animal performance.

INTRODUCTION

The analysis of animal feedstuffs for major nutrients is a subject which is usually taken for granted by agriculturalists and which generally arouses little interest among analytical chemists. In fact, feed analysis has been described by Christian (1972) as a "scientific backwater".

Because most ruminant production in Australia is from grazed pasture, the demand for feed analysis in Australia will probably never reach the level of that in the USA and Europe, where ration formulation and hand-feeding of housed animals requires much closer control of feed quality. However, Australian producers are now taking an interest in the quality of the feeds they grow, buy, sell or feed out. With continually rising costs and wildly fluctuating returns, there is a constant drive towards greater efficiency, and a realisation that feed resources must be better matched to animal requirements.

The birth of new industries, such as hay exports to Japan and the live sheep export trade has also focussed attention on objective assessment of feed quality, and there is now a move towards improving the nutritive value of Australian pastures (Hutchinson et al. 1987).

Whilst animal production studies will always remain the ultimate means of assessing feeding value, they are too time-consuming and expensive if large numbers of feeds are to be evaluated. Many laboratory techniques have been developed to attempt to simulate feed utilisation by animals, with varying degrees of success. Inevitably, a significant increase in demand for information on feed composition puts pressure on these testing procedures, some of which have changed little in 130 years.

The physical, non-destructive technique of near infrared reflectance (NIR) spectroscopy represents the most significant development in feed analysis for

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many years. Since its introduction in the 1960's for the measurement of moisture and protein in grains, the number of analytical applications of NIR in quality control has expanded dramatically, not only in agriculture but also in industry at large. The reason for this is its speed and convenience. Analyses are available in minutes, compared to days or weeks for conventional methods, and many constituents can be analysed simultaneously on intact samples, with minimal sample preparation required.

This paper reviews the benefits and limitations of conventional laboratory methods to assess the nutritive value of ruminant feeds, and the scope for NIR in Australia as a means of providing rapid and accurate feed analysis.

THE CLASSICAL APPROACH TO FEED ANALYSIS

The familiar "Weende" analytical scheme separates feeds into their "proximate" constituents of moisture, ash, fat, protein and carbohydrates. Further division of carbohydrates into soluble and structural fractions, and the degree to which each is "available" for utilisation by ruminants, has been a subject of considerable research since the attempts of the chemist Einhof in the early 19th century to measure the solubility of feeds in various solvents (Thaer 1809). However, most analytical methods developed over the years have really been variations on a theme, where a sample is treated with various chemicals and/or enzymes, and the degree of solubility taken as a measure of the value of the feed to the animal. Barnes (pers. comm.) has referred to these as the "boil and stir" techniques, and they have proved quite variable in their ability to predict animal performance, which should be the primary aim of any feed analysis. Furthermore, there is often little agreement between testing authorities on what is the "best method", with consequent confusion among both scientists and clients. Many methods are "operationally defined", with the result obtained depending on factors such as reagents and digestion time.

Although the deficiencies of the Weende system are well-recognised, they are frequently ignored. Crude protein (CP) consists of true proteins, composed of amino acids, and non-protein nitrogen. The conversion factor of 6.25 originated from early research on animal proteins which were found to contain approximately 16% nitrogen (Tkachuk 1969). This factor takes no account of non-protein nitrogen, and has been found to vary from 5.18 to 6.25 because different plant proteins contain different levels of nitrogen (Jones 1931). Although the 6.25 factor is still recommended for fibrous feeds, Tkachuk (1969) concluded that it overestimates the total CP content of cereals and 'oilseeds, and different factors have been recommended for these grains **(AOAC** 1980).

The availability of CP to the ruminant has been the subject of considerable study. Many linear regressions have been published for estimating digestible CP in forages from CP itself, and these estimates are adequate for practical purposes (Jarrige 1980, Minson 1982). Some protein can also become "unavailable" in heat-damaged hays or silages (Minson 1987) and is often measured as acid detergent insoluble nitrogen (Linn and Martin 1989). There is frequent debate about the need to measure the extent to which CP is degraded to ammonia in the rumen, and many laboratory techniques have been proposed to measure it (Minson 1987). However, Minson concluded that until there is adequate information to show which method correlates best with the level of ammonia produced in vivo, no method can be currently recommended to estimate protein degradability. Crude protein therefore, continues to reign supreme, and is wrongly considered by many farmers to be the major criterion of feed quality.

The most serious limitation of the Weende system is the assumption that crude fibre (CF) represents the indigestible portion of the feed and nitrogen-free extract (NFE) the digestible portion. The method currently used has been

virtually unchanged since 1860 (Van Soest 1966). However, CF is partly digestible and NFE contains some indigestible lignin (Van Soest and Moore 1965), which makes the division of carbohydrates in this way unrealistic, particularly for ruminants. The retention of the CF method therefore is rather surprising, but is based on the assumption that it is negatively correlated with nutritive value. However, this relationship is often inaccurate and imprecise, and a review by Barnes (1973) reported correlations between CF and herbage digestibility ranging from r=0.50 to r=-0.94.

The detergent fibre system

In the 1960's, a new approach to feed analysis was introduced, with the separation of organic matter into a readily available soluble fraction and a fibrous, partially available residue (Van Soest 1966). This was achieved by boiling feed samples for one hour in neutral and acid detergent solutions, with the residues remaining known as neutral detergent fibre (NDF) and acid detergent fibre (ADF) respectively. NDF represents the cell-wall constituents and has been verified as primarily hemicellulose, cellulose and lignin (Bailey and Ulyatt 1970). ADF represents the ligno-cellulose fraction, and the residue can be further treated with 72% sulphuric acid to isolate acid detergent lignin (ADL) (Van Soest **1963).**

NDF, ADF and ADL are extensively used for estimating forage quality (Marten 1981). Many modifications to these methods have also been proposed, mainly to reagents and digestion time. One example is modified ADF, or MAD-fibre (Clancey and Wilson 1966), which is popular in the UK. Although more meaningful than CF, all these methods are empirical and give different results. It is vital that the fibre fraction measured is always clearly specified.

In the USA, ADF is routinely used to predict digestibility and NDF to predict voluntary intake, based on a number of experiments described by Rohweder *et al.* **(1978)** and Marten (1981). Although this approach has limitations, it forms the basis of a successful hay grading system. However, Barnes (1973) concluded that correlations of NDF, ADF and ADL with digestibility or intake did not show much improvement over other chemical methods. Goering and Van Soest (1970) proposed a summative equation utilising NDF, ADF and ADL to calculate digestibility, but Minson (1971) reported that the accuracy of this equation was only slightly better than using ADL alone.

Digestibility

The digestibility of a feed is almost certainly the most useful index of nutritive value available at present (Ulyatt 1973). Digestibility is variously expressed as dry matter digestibility (DMD), organic matter digestibility (OMD) or digestible organic matter in the dry matter (DOMD) (MAFF 1984). Care is therefore needed in comparison of results.

The most accurate and successful laboratory estimate of in vivo DMD has been the two-stage in *vitro* incubation of a feed sample with rumen fluid from a fistulated sheep, followed by acidified pepsin (Tilley and Terry 1963). This procedure has been widely adopted to estimate relative DMD differences among many forage types (Marten 1981, Coleman and Windham 1989).

A more convenient alternative to the in *vitro* procedure is a two-stage incubation with pepsin and fungal cellulase (Jones and Hayward 1975). The advantages of this technique were listed by Minson (1987) and a simplified version (Clarke *et al.* 1982) has been successfully used at Hamilton for several years. However, in the case of grains and mixed feeds, starch needs to be removed prior to cellulase digestion either using amyloglucosidase (Dowman and

Collins 1982) or hydrolysis at 80°C (De Boever et al. 1986). The disadvantage of both procedures is that a batch of samples takes up to one week to process.

In order to reduce errors in predicting DMD, it is important to convert laboratory values for unknown samples to predicted in vivo DMD using a regression based on a set of "standard" feeds of known in vivo DMD and as similar as possible to the unknowns in terms of feed type and class of animal to which the results will be applied (Goto and Minson 1977, Minson 1981).

The other important use of digestibility measurements is to calculate metabolisable energy (ME), which, despite its known inadequacies (Leng 1989), has been officially adopted to express the energy content of Australian feeds (Pryor 1980). Because ME. is difficult to measure, it must be estimated from some laboratory analysis. This introduces confusion, as everyone has a "favourite" equation. There is a strong case for Australian laboratories to standardise on the equations relating either DOMD, OMD or DMD to ME, as recently recommended (SCA 1990).

Voluntary intake

Voluntary intake accounts for at least 50% of feeding value (Ulyatt 1973), but despite its importance, there is still no completely adequate laboratory technique for its estimation (Coleman and Windham 1989).

Intake is often positively related to digestibility, and satisfactory predictions of intake can be obtained from in vitro DMD for some forages (Minson 1987). However, the two properties are not always related, and the problem facing the chemist is prediction of intake of feeds with similar digestibility. NDF is commonly used to estimate intake, and Goering and Van Soest (1970) proposed a regression equation based on sheep data and NDF, but they warned of possible large errors. Rohweder et al. (1978) found correlations ranging from $r=-0.32$ to $r=-0.94$ between NDF and intake for various species.

It has been suggested that physical rather than chemical methods may hold the key to improved laboratory prediction of intake. Some workers have reported success using a grinding energy index (Chenost 1966, Minson 1981), but Foot and Reed (1981) found correlations between grinding energy and intake of mixed pasture hay to be unsatisfactory. There is scope for considerable research in other physical methods to predict intake (Minson 1987).

NEAR INFRARED REFLECTANCE (NIR) SPECTROSCOPY : A NEW APPROACH

The technique of NIR represents a radical departure from conventional analytical methods, in that the entire sample of a ground natural product is characterised in terms of its absorption properties in the near infrared region, rather than separate sub-samples being treated with various chemicals to isolate specific components. This forces the analyst to abandon his traditional narrow focus on one sample and one analyte at a time, and to take a broader view of the relationship between components within the sample and between the sample and the population from which it comes. Inevitably, this means the analyst, who may have spent much of his career dealing with traditional "wet" chemical analyses, must also come to terms with the concepts of spectroscopy (involving complex mathematical treatments of optical data), multivariate statistics, and a wide range of computer software.

Theoretical basis of NIR

The near infrared region of the electromagnetic spectrum lies between the visible and infrared regions and is usually defined by the wavelength range 700

to 3000 nanometres (nm) (Norris 1989b). However, most analytical use of NIR is between 1100 and 2500 nm. This region is characterised by absorption bands caused by stretching vibrations of hydrogen (H) bonds with either carbon (C), oxygen (0) or nitrogen (N) atoms (Kaye 1954). The absorption bands seen in the near infrared region are the weaker "overtones" (between 1000 and 1900 nm) and "combinations" (between 1900 and 2500 nm) of the much stronger fundamental absorptions which occur in the mid infrared region (Murray 1983, Williams 1987a, Barton 1989a).

Some energy is absorbed by chemical constituents present in a powdered solid, when it is irradiated with monochromatic light, and some is diffusely reflected. The difference between the energy entering the sample and the diffuse reflectance escaping is measured by the NIR instrument and is related to the concentration of the constituents (Williams 1987a). In transmission spectroscopy, the Beer-Lambert law states that absorbance (A) is directly proportional to concentration:

$$A = \log I_o/I = \log 1/T = kc\ell$$

where I_o = intensity of the incident radiation, I = intensity of the transmitted radiation, T = transmittance, c = concentration of absorbing molecules, ℓ = path length and k = a constant of proportion.

This relationship is fundamental to spectroscopy and can equally be applied to the diffuse reflectance of light-scattering materials (Birth and Hecht 1987). In the case of NIR, reflectance (R) is analogous to transmittance, so

$$A = \log 1/R = kc\ell .$$

However, path length in the reflectance mode cannot be a constant as it is in transmission measurements, so it becomes an extra unknown as well as concentration (Murray 1983, Murray and Williams 1987). This means that NIR measurements must be made at several wavelengths, with the use of complex mathematical procedures.

Hence NIR spectra are plots of log l/R versus wavelength, and a typical NIR scanning monochromator will yield 700 readings for every sample between 1100 and 2500 nm. The spectra appear as smooth, rolling lines with few well-defined features (Murray 1988), but consist of many overlapped bands, since the reflectance spectrum of an intact feed, for example, is the summation of the spectra of its major chemical components (Murray 1983, Norris 1989a). The challenge to the chemist is to extract analytically useful information on composition from the reflectance data.

It is the shape of the NIR spectral line, or the rate of change in slope with wavelength, that conveys chemical information (Murray and Williams 1987). Thus first or second derivative plots of log l/R are useful, as they can resolve overlapping peaks into component absorptions which may appear as shoulders on larger peaks, and also to a large extent remove baseline variations, i.e. spectra differences due to non-uniform particle size (Hruschka 1987, Murray 1988, Barton 1989a).

NIR calibration Procedures

Routine use of the NIR technique first requires the instrument to be calibrated against a standard reference method. Many different calibration methods have been proposed, but there are basic principles which have been shown to be vital to the success of NIR calibration.

The first major step involves the selection of a set of calibration samples from a larger population. These samples must represent all sources of variation likely to be found in future unknown samples of similar material, such as chemical, physical and botanical characteristics, including sample preservation and processing methods (Shenk et al. 1979, Abrams 1989b, Windham et al. 1989). Samples can be selected from a population in a random or structured fashion, or on the basis of spectral characteristics (Abrams 1989c).

The optimum number of samples chosen for calibration has been the subject of some debate. Windham et al. (1989) concluded that in narrow-based or "closed" populations, less than 100 samples (minimum 50) were usually adequate, whereas in broad-based or "open" populations, 150 or more samples were necessary. In a closed population, all samples (and hence all variation) are available at the time of calibration. In an open population, new samples may have to be periodically added in order that the calibration remains robust indefinitely (Barton et al. 1990). Calibration equations developed from closed populations often have better statistics but have limited value beyond those populations, compared with robust, broad-based equations from open populations (Shenk 1989).

Once the calibration set has been chosen, the samples must be analysed for the constituents of interest by conventional reference methods. Stark (1988) has described this step as the most difficult but essential in developing an NIR calibration. Poor calibration accuracy has oftenbeen incorrectly blamed on NIR instruments, when in most cases the laboratory reference method (and associated factors such as mis-numbering samples, transcription errors, etc.) was at fault (Williams 1987b). The major drawback with NIR is that wet chemistry is both the basis for developing and evaluating a calibration (Stark 1988), or both "judge and jury" (Murray 1988).

The mathematical treatment of data necessary in relating log l/R values to chemical components is a study in itself which will not be attempted in this review. The technique used at Hamilton is modified stepwise multiple linear regression (Shenk et al. 1981), which generates equations of the form:

$$Y = B_o + B_1X_1 + B_2X_2 + B_3X_3 \ldots.$$

where Y is the component of interest, B values are regression constants and X values are smoothed first or second derivative segments of log l/R. A set of six alternative mathematical treatments is evaluated for each calibration using the statistical criteria recommended by Windham et al. (1989).

After the "best" calibration equation has been selected, it must be validated with samples not included in the original calibration (Stark 1988, Windham et al. 1989). When applied to open populations, such as when feed samples are routinely tested for the agricultural community, the selected equation also needs to be monitored on a regular basis. A monitoring test, using bias and unexplained error confidence limits, has been suggested by Shenk et al. (1989). Bias is defined as the difference between reference method and NIR-determined mean values, and unexplained error is the standard error of performance of the equation on a given population, corrected for bias (SEP(C)). The performance of a calibration equation on sets of validation samples will depend on the degree to which all sources of variation in the validation samples are encompassed in the calibration set.

Errors in NIR and reference methods

Hruschka (1987) listed sampling error, reference method error and NIR method error as the three major categories of error in an NIR measurement, with

sampling error the largest component, as it is in any analysis of agricultural materials.

Sample preparation, i.e. drying (if required) and grinding can have a major impact on any system of analysis. In the case of forages, there is little agreement on which drying method to use, with freeze-drying, and oven-drying at temperatures ranging from 40°C to 100°C commonly practised (Smith 1973). Abrams (1989b) recommended that moist forage samples be dried at 60°C in a forced-air oven prior to NIR testing. Microwave drying has also been used (Dantoin 1984, Ellingboe et al. 1986, Abrams 1989b). Particle size is one of the most important factors affecting the accuracy of NIR analysis, and uniform grinding procedures are considered essential to minimise particle size differences between samples (Wetzel 1983, Williams 1987b).

Other important sample factors affecting NIR analysis can include blending of samples after grinding, sample dividing techniques, cell loading and sample storage (Williams 1987b, Abrams 1989b, Hall 1990). The important point is that sample handling methods must be consistent between calibration and unknown samples.

The crucial effect of the quality of reference method analysis on the accuracy of NIR calibrations cannot be over-emphasised. Barton (1989b) stated that "the NIR results can be no better than the data used for calibration". It follows, therefore, that the NIR calibration error for a recognised chemical entity like Kjeldahl nitrogen will be smaller than for an operationally defined property like crude fibre or an attribute like digestibility (Murray 1988).

Before assessing the statistics of an NIR calibration, the error associated with the reference method should be known. However, this is frequently ignored (Barton 1989b), and relatively few papers in the literature have quoted the standard error of their reference methods (SEL).

Another measurement of importance is the ratio of the standard error (SE) to the standard deviation (SD) of the population for a given constituent, something not often considered in traditional laboratory analysis (Murray1988). Standard error should be much lower than SD; Murray (1983) stated that the SE/SD ratio should preferably be below 0.3. If SE/SD = 1, analysis is pointless as it is no better than using the mean value of the original data.

Despite the errors which can affect NIR analysis, it has proved highly successful. NIR has been shown to be superior to chemical methods in terms of precision and repeatability (Shenk et al. 1981, Murray 1987b, Barton 1989b), and its dependence on calibration data has actually resulted in improvement in the quality of chemical analysis through closer examination and testing of methods needed to produce the data (Norris 1988).

Protein measurement of oat grain using NIR

Although the most widely used grain for feeding livestock in Victoria is oats, its protein content is commonly low, which can be a problem especially if fed during droughts or dry seasons when quality and quantity of pasture is also low. Protein content of oats across Victoria can vary from 4 to 16% (Foot and Flinn, unpublished data), and demand for rapid testing has increased markedly, with many farmers now wanting to know the protein content of oats for sale before they buy. NIR offers the means to meet this demand. The greatest impact of NIR has been in the grain industry, particularly wheat, where the segregation of wheat on the basis of protein content has transformedbulk wheat handling and marketing procedures. There have been fewer reports of NIR measurement of

protein in oats. Williams (1975) reported a standard error of 0.47% for protein in ground whole oats, which is somewhat higher than that routinely found with wheat (0.2 to 0.3%). This is due to the problem of obtaining homogeneous ground samples of whole oats due to their high husk and oil content relative to wheat or barley.

In a study at Hamilton (Flinn 1990), 118 oats samples, covering a range of 5.2 to 14.8% CP (nitrogen x 5.83), were selected on the basis of their NIR spectra from a population of 856 samples tested from 1987 to 1990, embracing many varieties and growing environments. The statistics of the "best" calibrationequationobtained (R^2=0.94, standarderrorofcalibration (SEC)=0.45) compared favourably with those of Williams (1975), and when tested on another subset of 154 samples from the same population, SEP(C) was 0.44 and bias was -0.17. Results were not as good when sample preparation methods differed between calibration and target populations.

NIR analysis of forages

The application of NIR to the measurement of forage quality was first reported by Norris et al. (1976). In what has come to be regarded as a classic paper, the authors found standard errors of 0.95, 3.1, 2.5, 2.1 and 3.5% respectively, for CP, NDF, ADF, lignin and in vitro DMD measured in a diverse set of forage samples. This paper stimulated wide interest and a great many investigations soon began across the world to extend and refine the use of NIR in forage analysis.

The successful use of NIR to measure major organic components of forages has been demonstrated by many workers. Depending on the instrument and calibration procedure employed and the forage species investigated, standard errors of performance have been found to range from 0.32 to 1.15% for CP, 1.00 to 2.46% for ADF, 1.24 to 3.50% for NDF, 0.30 to 1.13% for lignin, and 1.47 to 4.10% for in vitro DMD or OMD (Barton and Burdick 1979, Burdick et al. 1981, Shenk et al. 1981, Marten et al. 1983, Marten et al. 1984, Brown and Moore 1987, Flinn and Murray 1987, Smith and Flinn 1990). The Association of Official Analytical Chemists has now accepted NIR as an official method for the determination of CP and ADF in forages (Barton and Windham 1988).

Forage analysis using NIR has not been confined to traditional protein and fibre fractions. Parnell and White (1983) measured water soluble carbohydrates in perennial ryegrass with standard errors of performance ranging from 0.92% to 1.6%. Similar satisfactory standard errors for total non-structural carbohydrates in lucerne roots were found by Brink and Marten (1986) and tropical grasses (Brown et al. 1987). Determination of more precisely defined cell-wall carbohydrates (glucose, xylose, arabinose and galactose) in lucerne (Albrecht et al. 1987) and sub-tropical grasses (De Ruiter et al. 1988) using NIR was reported as either accurate and precise or promising for ranking samples in a breeding program.

Mineral analysis of forages by NIR appears unlikely, as minerals do not absorb in the near infrared region. However, Shenk et al. (1979) suggested the possibility due to close associations between minerals and organic compounds. They reported standard errors of performance of 0.14, 0.04 and 0.41% respectively for calcium (Ca), phosphorus (P) and potassium (K) in hay samples. Valdes et al. (1985a) found comparable errors for Ca, P and K in hay, haylage and corn silage samples, but they reported low r^2 values (possibly due to the narrow range of chemical values) and high variability in the calibrations. In a study involving a wide range of minerals in various forages, Clark et al. (1987b) concluded that accurate NIR analysis was limited to Ca, P, K and magnesium (Mg), which were found to have coefficients of variation (CV) ranging

from 10 to 20%. The CV values for other minerals generally exceeded 20%. They recommended caution when using NIR-determined mineral values. In a further study involving several trace elements, Clark et al. (1989) found that only aluminium and silicon could be determined by NIR in the forages studied. At Hamilton, we have found that Mg could be measured in perennial ryegrass samples (mean 0.25%, range 0.15-0.37%, SD 0.06%) with a standard error of 0.04% and a CV of 16% (Smith et al. 1991). We concluded that NIR may be a useful tool for preliminary screening of ryegrass lines for Mg content, but that other techniques should be employed if a higher level of accuracy was required.

There have also been suggestions that NIR may have a role in screening various forage species for anti-quality factors. These are normally present in small quantities but may be detectable if they affect NIR spectra. The first report of this nature was by Clark et al. (1987a), who used NIR to measure total alkaloid concentration (TAC) in tall larkspur and velvet lupine samples. They found r^2 values to exceed 0.90 and SEC values of 0.10% for larkspur (range 0.26-1.72%) and 0.04 for lupine (range 0.09-0.60%). Spectral comparisons of plant material and alkaloids showed strong relationships between wavelengths selected in the equations and the alkaloid peaks. They suggested that NIR could have great potential for rapid screening of forage species for toxicity, particularly in breeding programs, but that in the case of TAC, NIR was limited by the accuracy of chemical methods. In another investigation, Windham et al. (1988) successfully used NIR to determine tannin concentration in sericea lespedeza, and found accuracy to be similar to the reference method.

NIR monitoring of pelleted mixed diets for live sheep exports

There is less information available on NIR analysis of mixed feeds than for grains and forages. Abrams (1989d) found poor results in preliminary attempts to evaluate mixed feeds, which he attributed to their great heterogeneity. Murray and Hall (1983) examined 24 compound feeds containing up to 35 ingredients and attempted NIR calibration against 15 chemical or biochemical properties. Conclusions were limited due to the small number of samples, but promising predictions were obtained for CF, NDF, CP, fat, modified ADF, lignin, starch, in vitro OMD, DM, in vivo ME and gross energy. Poultry and pig feeds have also been analysed successfully by NIR for DM, CP, CF, fat, Ca and P (Charles and Shenk 1986, Valdes et al. 1985b, Pazourek and Cerny 1988). All these feeds could be generally described as concentrate rations, containing only small amounts of roughage.

NIR has been used successfully at Hamilton to determine CP, predicted in vivo DMD and ADF in pelleted complete diets for live sheep exports, with standard errors of 0.7, 2.4 and 1.3% respectively. Ash determination was less accurate, with a large SE/SD ratio and low r^2, but was adequate to screen out high-ash samples (>13%), which is the industry requirement. However, when a calibration equation derived from Australian pellets was tested on 22 Scottish diets, error and bias levels were unsatisfactory, due to the latter samples being totally different in composition to the calibration set (Flinn 1990).

Interpretation of NIR spectra

Many of the early NIR papers tended to treat the technique as a "black box", with good calibration statistics considered sufficient to justify its adoption. This is hardly surprising, as the major progress in NIR during the 1960's and 1970's was led, not by spectroscopists or chemists, but engineers and agriculturalists (Davies 1987). The technique was demonstrated to work effectively, and was used merely as a rapid analytical tool to produce the figures required.

However, it is now considered essential to try to understand how NIR works, and to ensure that the wavelengths chosen for a particular equation make chemical sense. A calibration justified on the basis of spectra is more likely to be robust during routine use (Hruschka 1987). Murray (1986) recommended that the final step in the calibration process should be an attempt to explain the NIR equation in terms of the wavelengths chosen. In a comprehensive examination of the NIR spectra of families of organic compounds, Murray (1987a) showed that compounds having common functional groups shared spectral features relating to the presence of those specific groups, mainly -CH, -NH and -OH. He concluded that if the weaknesses of traditional chemical analysis were to be avoided, a clearer understanding of the spectra was vital.

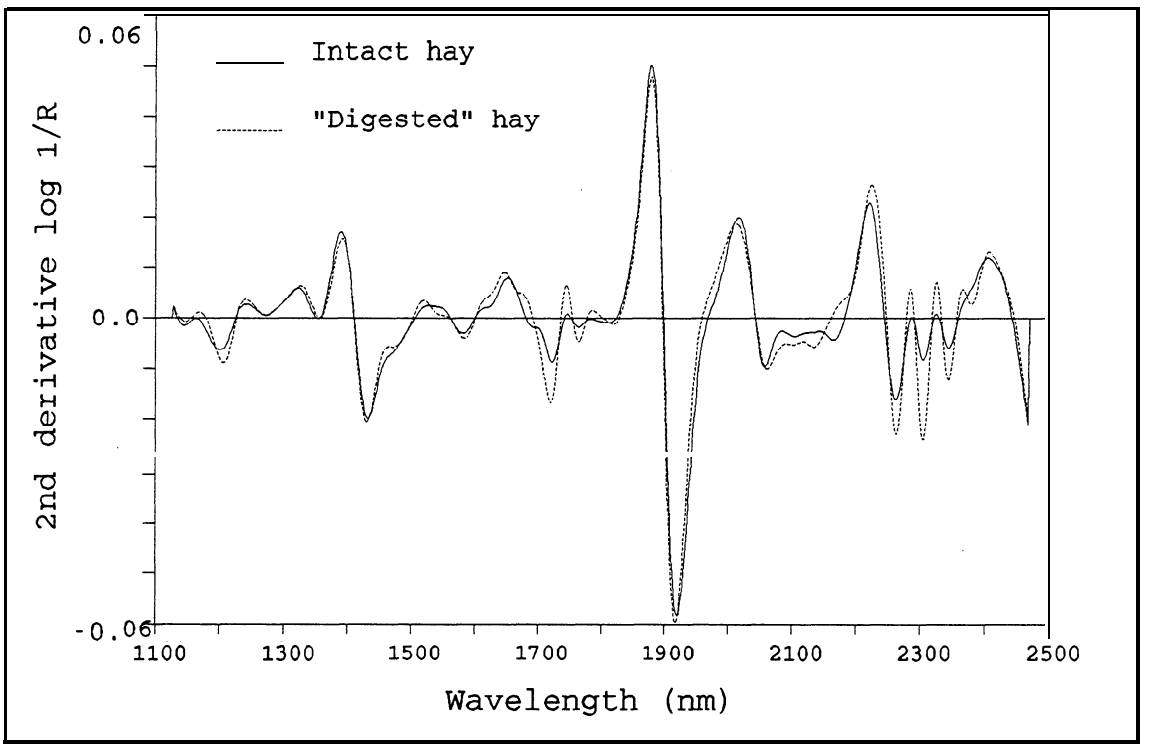

Fig. 1. Effect of pepsin-cellulase digestion on the second derivative NIR spectrum of a lucerne hay sample. Deeper troughs near 1700 nm and 2300 nm for the "digested" hay indicate higher levels of lignin, and lignin and cellulose respectively

Interpretation of NIR spectra is a subject still in its infancy, but some spectral features are relatively clear. Norris et al. (1976) found that low-protein forages exhibited a broad absorption band near 2100 nm, whereas high-protein samples produced almost a straight line in the same region. Barton et al. (1986) studied changes to the spectra of untreated and ammoniated barley straw during ruminant digestion, and found that when difference spectra were used, absorbance at 2260 nm (where cellulose and lignin are known to absorb) in ammoniated samples disappeared faster than in untreated samples, indicating that the "fibre" had been made more available. Murray (1990) pointed out 'that calibration equations for forage digestibility frequently selected 2266 nm and 1666 nm as important wavelengths. This was explained by the 2266 nm band being an absorption due to both cellulose and lignin, whereas the 1666 nm band was a weak aromatic -CH first overtone band unique to lignin. The effect of pepsin-cellulase digestion on the second derivative spectra of a lucerne hay sample is shown in Fig. 1. The troughs are the absorption bands, and the clear

differences between the spectra near 1700 and 2300 nm reflect higher levels of indigestible cellulose and lignin in the residue following treatment with pepsin and cellulase.

Direct NIR calibration of animal performance - dream or reality?

The main problem with NIR determination of nutritive value is that the technique is generally calibrated on empirical factors which in turn attempt to predict animal performance. The possibility of directly relating NIR spectra to animal response measurements without recourse to chemical analysis is an attractive possibility. This was first attempted by Norris et al. (1976), who, as well as deriving calibrations for laboratory measurements, also estimated in vivo DMD and voluntary dry matter intake (DMI) with standard errors of 5.1% and 7.9 g/kg metabolic body weight ($W^{0.75}$)/day for a range of forages fed to sheep. When evaluating grazed forage via oesophageally fistulated cattle, Ward et al. (1982) reported an SEC of 9.6 g/kg $W^{0.75}$/day for organic matter intake using NIR. Eckman et al. (1983) compared NIR with CP, NDF and IVDMD in predicting DMI, digestible energy and digestible energy intake by sheep fed pure and mixed forage-based diets. They concluded that NIR had the potential to predict animal response as accurately as could laboratory analyses. Barber et al. (1990) compared NIR with a number of laboratory methods for the determination of OMD in 72 grass silages and found NIR to be superior, with a standard error of performance of 2.6%. Standard errors for the other techniques varied from 3.6 to 5.3%.

A major problem when using animal measurements for NIR calibration is variability between animals, which results in lower coefficients of determination andhigher standard errors than for laboratory procedures (Windham and Coleman 1989). This variability was found to account for one half of the variation in NIR determination of DMI and digestible energy intake of cattle (Redshaw et al. 1986). Variability in calibration data for DMD was about threefold less than that for intake.

NIR equations have also been developed to predict intake and diet quality of grazing cattle, by using the technique as a "faecal index" (Coleman et al. 1989). Despite some limitations, this procedure may have considerable potential. One problem is the difficulty in obtaining a sufficient number of accurate sets of intake data, both for calibration and validation.

Work in progress at Hamilton, in conjunction with CSIRO Plant Industry, Canberra, will attempt to relate NIR spectra to grazing intake of sheep measured in different seasons on a variety of pastures, using the "alkane" technique. This involves the measurement of the faecal levels of n-alkanes (with odd-numbered carbon chain length) from plant cuticular waxes, together with those of synthetic alkanes (even-numbered chain lengths) used as faecal markers (Dove et al. 1989).

Another application showing promise is NIR measurement of apparent metabolisable energy (AME) in feed ingredients for broiler and adult poultry. In a preliminary assessment, 115 samples of various feed ingredients having AME data were scanned by NIR at Hamilton, with best results being obtained with cereal grains (R^2 0.85, SEC 0.37) (Johnson et al. 1991).

CONCLUSION

After 130 years, conventional methods of feed analysis are widely discredited but still widely used. NIR has been described as the most exciting technique to hit the agricultural and feed industries since the introduction of the Kjeldahl test, and can rapidly test the quality of agricultural (and other)

products on a scale not previously imagined, with proven benefits to industry and research. However, the problem remains that "we are using 19th century chemistry to calibrate 20th century technology" (Murray 1988). It would be a pity if the role of NIR merely served to perpetuate the old methods, thus allowing the mistakes of the last 130 years to be made faster and more efficiently! No-one is suggesting that "wet chemistry" is dead; on the contrary, NIR has focussed attention on the limitations of crude chemical fractionations, and the need to develop methods to isolate plant components which better relate to NIR spectra, as well as "tuning up" the precision of existing procedures. As spectral interpretation techniques improve, future progress may depend on the direct use of NIR spectra alone to characterise agricultural materials, without recourse to intermediate chemical procedures.

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