



# Near Infrared Reflectance Spectroscopy: Applications in Deer Nutrition

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by G. McL. Dryden

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# Foreword

The Australian deer farming industry is well supported with information on the nutrient requirements of farmed deer, but it is difficult to continuously monitor the animals' nutritional environment, so as to optimise the performance of deer herds. Farmers need timely information on the nutritional status of their animals and the nutritive value of pastures and supplementary feeds if they are to apply successfully this existing nutritional information.

This report reviews the use of near infrared reflectance (NIR) spectroscopy to monitor the nutritive value of animal foods and the nutritional status of grazing animals. NIR has been used over the last forty years to analyse rapidly animal concentrate and forage foods. Food nutrient content can be predicted accurately with NIR spectroscopy. NIR methods have been shown to predict *in vivo* digestibility at least as well as conventional "wet chemistry" methods, and much more rapidly. NIR technology has been applied to the routine monitoring (through analysis of faecal samples) of the nutritional status of cattle, deer and other grazing animals, and appears to have potential for identifying pregnancy, gender and animal species.

NIR spectral information must be calibrated against a reference data set of adequate size and range if robust NIR calibrations are to be obtained. Once this has been done, the evidence from the application of NIR technology to faecal profiling of cattle (i.e. the continuous monitoring of grazing animals' nutritional status) which is presently available in the USA and which is being developed in northern Australia, suggests that a similar technology could be developed to monitor the nutritional status of deer herds and predict the performance of farmed deer.

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This report, a new addition to RIRDC's diverse range of over 900 research publications, forms part of our Deer R&D program, which aims to foster an Australian deer industry as a profitable and efficient mainstream agricultural enterprise.

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# Executive Summary

Near infrared reflectance (NIR) spectroscopy has been used over the last forty years to analyse accurately protein, fibre, and other organic components in animal foods. NIR spectroscopy is a rapid, non-destructive, and non-polluting technology.

NIR information can not be used to determine analyte concentrations directly because of the way in which near infrared radiation passes into, through, and is reflected from, the sample. We have to predict the concentrations of the constituent we wish to measure from relationships which have been developed between reflectance and reference data, i.e. we have to use prediction equations. Robust prediction equations are based on calibration data sets which encompass the range of sample characteristics which we expect to encounter when the equation is used. It is also important to apply appropriate mathematical techniques (e.g. smoothing and derivatisation) to the NIR data, and to make sure that the samples which we analyse are uniform in particle size and water content.

“Universal” equations have been developed to predict the nutrient composition of a wide range of foods of that type. There are several examples of European universal equations for grains and forages, and an equation for Australian mixed temperate pasture. It may be necessary to calculate “local corrections” before universal equations are used in any new context.

When properly calibrated, NIR spectroscopy predicts protein contents with great accuracy. We can predict other constituents less precisely, although with precisions which are similar to those of conventional laboratory determinations. NIR spectroscopy is used successfully with both concentrate and forage foods. NIR information is obtained from the interactions of near infrared radiation with chemical bonds between non-mineral elements and so does not always accurately predict food mineral contents. NIR methods predict *in vitro* digestibility accurately and precisely, and can predict *in vivo* digestibility at least as well as conventional “wet chemistry” methods such as *in vitro* digestion or the pepsin-cellulase method, and much more rapidly. The DM intake of animals can also be predicted, although with less precision than chemical composition or digestibility.

Faecal indices, i.e. the concentrations of certain constituents in faeces, have been used to monitor the nutritional status of grazing animals, including wild deer. Faecal indices determined by wet chemistry have given mixed success, but substantially better results have been obtained with NIR spectroscopy. NIR spectroscopy may measure characteristics of faeces which integrate several different aspects of faecal chemistry, while wet chemical analyses focus on single entities.

NIR technology has been used to routinely monitor (through analysis of faecal samples) the nutritional status of cattle, and appears to have potential for identifying tick infestation, pregnancy, gender and animal species. Nutritional status data obtained by NIR analysis of grazing cattle faeces is used as an input to the NUTBAL Pro expert system for North American ranchers. The combination of NIR analysis and nutritional profiling with the NUTBAL Pro program has improved yearly economic returns to American cattle ranchers by up to USD26.50 per cow mated. These results, the preliminary evidence from similar attempts in northern Australia, and preliminary results of a NIR-based nutritional profiling

program for deer in Texas, suggest that a similar technology could be developed to monitor the nutritional status of deer herds and predict the performance of farmed deer.



# 1. Introduction

Near infrared reflectance (NIR) spectroscopy is a long-established, and now mature, technology. Norris and his colleagues developed the first application of NIR spectroscopy to measure water in grains and seeds (Norris and Hart 1965, cited in Givens, *et al* 1997). The technology uses simple sample preparation methods (drying and grinding), is very rapid (once the sample has been prepared, measurements are made in seconds), and inexpensive. As noted by Mark, *et al* (2002) it avoids the problems of organic and other chemical waste disposal, and there are few if any hazards associated with the technique because it uses no toxic or corrosive reagents.

NIR spectroscopy has been used in a remarkably wide range of analytical situations. NIR methods were first developed for the rapid analysis of water, oil, and protein in grains and seeds. More recently, NIR spectroscopy has been used to identify waxy wheat (Delwiche and Graybosch 2002), authenticate the origin of meat (Fumiere, *et al* 2000), assess chemical pulping traits in wood (Greaves, *et al* 1996), assess the sugar content of fruit (Walsh, *et al* 2000), and predict the nutrients in *Eucalyptus* leaves and the feeding rate of greater gliders and ringtail possums (McIlwee, *et al* 2001). The potentials for NIR techniques in ecology and agriculture have been reviewed by Foley, *et al* (1998) and Givens and Deauville (1999). NIR methods are becoming widely used in animal science to predict the chemical composition of forages and other foods, food digestibility, and animal responses to foods including food intake and growth (e.g. Coates 2000; Stuth and Tolleson 2000), and to identify animal species, gender, and pregnancy (Tolleson and Stuth 2002).

## 2. Elements of near infrared reflectance spectroscopy

The theory of NIR spectroscopy has been described by several authors, e.g. Hruschka (1987) and Givens, *et al* (1997). A short review of the basic elements of NIR spectroscopy is given in this section.

### 2.1. Measurement of the absorbance of radiation by a sample.

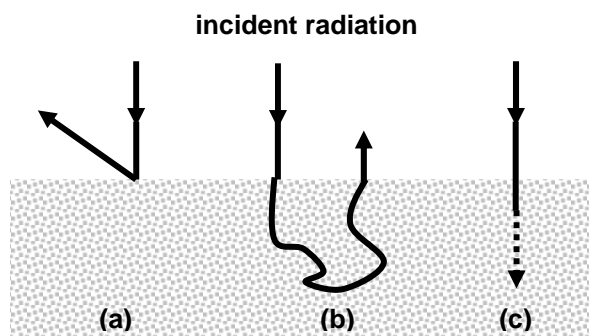
The Beer-Lambert law describes the relationship between the concentration of a solute and the amount of light absorbed by the solution:

$$C_x = A_x / e.l$$

where:  $C_x$  = concentration of the test solute  
 $A_x$  = absorbance of the test solution  
 $e$  = molar absorptivity of the test solute  
 $l$  = path length travelled by the light through the solution

The important feature of this relationship is that it allows the measurement of  $C_x$  directly from  $A_x$ .

When infrared radiation is incident on a solid sample, some of it is reflected (specular reflectance) from the surface of the sample. Another proportion of the radiation enters the sample (by about 2 mm, Hruschka 1987) and may be absorbed within it. Radiation which is not absorbed may be transmitted through the sample or reflected from it (diffuse reflectance, Fig. 1).



**Fig. 1. Diagrammatic representation of specular (a) and diffuse (b) reflectances, and absorption (c) of near infrared radiation from a sample (from Givens, *et al* 1997).**

While the Beer-Lambert law generally describes the relationship between radiation diffusely reflected from a solid sample and characteristics of that sample, the path length of diffusely reflected radiation can not be predicted because it is scattered by random reflections, refractions and diffractions within the sample. The variations within NIR diffuse reflectance spectra are mainly a result of (1) non-specific scatter of radiation, (2) variable path length, and (3) the chemical composition of the sample (Barnes, *et al* 1989).

As a result, the relationship between reflectance and analyte content can not be described by any mathematical relationship (Givens, *et al* 1997). Thus while the characteristics of near infrared radiation reflected from a sample can be used to predict certain sample characteristics, each application of this type must be obtained by calibration. This introduces a number of complications such as the choice of wavelength, mathematical treatment of the reflectance data, methods of sample preparation and the effects of instrumentation differences.

The amount of radiation reflected from the sample is quantified as the reflectance (R) of the sample. The value is usually expressed as  $\log(1/R)$ , which gives higher values at higher levels of absorbance (i.e. lower reflectance). There is an almost linear relationship between  $\log(1/R)$  and the concentration of an absorbing component (Hruschka 1987). The  $\log(1/R)$  curve is comparable to an absorption curve with peak values occurring at wavelengths which correspond to absorption bands in the sample (Norris, *et al* 1976).

## 2.2. Choice of wavelengths.

The near infrared spectrum is between 730 and 2600 nm. Reflectance spectroscopy uses wavelengths between 1000 and 2600 nm (Hruschka 1987), although the extremes of this range are not often used (Williams 1987 and Table 1). This region of the infrared spectrum gives results which have a signal : noise ration of about 10,000 : 1 and are attenuated enough so that samples do not have to be diluted and possible non-linearity caused by strong absorbances is less likely (Givens, *et al* 1997).

**Table 1. Wavelengths commonly used in the application of NIR spectroscopy to animal nutrition.**

<b>Sample type / analyte</b>	<b>Wavelength (nm)</b>	<b>Author</b>
Grass / ME content	1658 - 1668	see references in Givens, <i>et al</i> (1997)
Forages / protein	2100 - 2164	see references in Williams (1987)
Forage / fibre, IVDMD <sup>a,b</sup>	1555 - 1674, 2294	see references in Williams (1987)
Grass / fibre <sup>b</sup>	1416 - 2400	Garcia-Cuidad, <i>et al</i> (1993)
Grass / protein	1536 - 2236	Garcia-Cuidad, <i>et al</i> (1993)
Grass / cellulose	1320 - 2044	Garcia-Cuidad, <i>et al</i> (1993)
Whole-plant maize / IVDMD	1722 - 2336	Valdes, <i>et al</i> (1987)
Whole-plant maize / protein	1680 - 2336	Valdes, <i>et al</i> (1987)

<sup>a</sup> acid detergent fibre, neutral detergent fibre

<sup>b</sup> *in vitro* dry matter digestibility

**Table 2. Near infrared wavelengths and their association with chemical structures (from Barnes 1988; Osborne and Fearn 1986; Smith and Kelman 1997).**

<b>Wavelength (nm)</b>	<b>Chemical entity</b>
1143	aromatic compounds, lignin
1496, 1668, 1976	amide bonds
1660 - 1670, 1720 - 1730, 2100 - 2200	condensed tannins
1772	ester bonds
1930	water
1960, 2180	protein
2140, 2180	peptide bonds
2088, 2410 - 2460	cellulose
2380	hemicellulose
2461	starch

Reflectance in the near infrared spectrum represents the chemical structure of the sample. In particular it indicates the presence of chemical bonds and functional groups (e.g. C-H, O-H, N-H; Table 2). Peaks in the  $\log(1/R)$  spectrum represent the harmonics, overtones and combinations which arise from the primary absorption in the mid infrared spectrum (Coleman and Murray 1993). Purnomoadi, *et al* (1996) have discussed the relationships between wavelengths and the constituents of OM. However, although the reflectance of near infrared radiation is related to the sample's organic chemistry there are no necessary chemical or physical relationships between the analyte under consideration and the wavelength(s) which may be selected to predict it. Examples of this can be seen in Table 1 and Villalobos, *et al* (1991) and Purnomoadi, *et al* (1996).

There are instances of different authors working in different laboratories and with different samples selecting the same wavelength for a particular analyte (e.g. Valdes, *et al* 1987). Nevertheless, in every particular situation the optimal wavelength combinations may change between laboratories and sample types, and even between years when similar samples are analysed (e.g. Valdes, *et al* 1990). An important consideration is that the chosen set of wavelengths should optimise the contrast between the benefits of using wavelengths at which absorption by the analyte under consideration is maximised v. the benefits of minimising interference at the chosen wavelengths by other sample constituents.

### **2.3. Mathematical treatment of the reflectance data.**

NIR spectral variations related to different analytes are small (Hruschka 1987; Barnes 1988) and reflectance spectra are characterised by noise (random errors caused by instrument function), the effects of sample preparation (especially water content and particle size), and overlaps between the reflectance peaks of different constituents. There is usually a baseline variation such that  $\log(1/R)$  values are greater at wavelengths approaching 2500 nm, and this effect can be curvilinear with densely-packed samples (Barnes, *et al* 1989). Further, reflectances at different wavelengths may be highly correlated (Barnes, *et al* 1989). This collinearity is acceptable when it occurs at chemically-related wavelengths, but should be corrected for if it occurs at unrelated wavelengths.

Noise (i.e. random variation in the signal caused by equipment or other variations) can be reduced by smoothing. In moving average smoothing each reflectance value is replaced by the mean of a predetermined number of values on each side of it. Data can also be smoothed by averaging multiple (40 to 120) readings made at each wavelength (Williams and Cordeiro 1985; Williams 1987). The Savitsky-Golay and Fourier transform methods of smoothing are described by Hruschka (1987). Baker, *et al* (1994) used the “noise file” approach recommended by Westerhaus (1991) to control random error over the course of several months work. The “noise file” was developed by repeatedly re-sampling a standard sample which had been exposed to the changing environmental conditions in the laboratory. “Scatter” in log(1/R) data can also be corrected with the Geladi, *et al* (1985) multiple scatter correction.

Variations in sample water content are important because water absorbs near infrared radiation strongly (e.g. Fig. 1 in Baker, *et al* 1994). Additionally, variations in sample particle size and temperature influence the scattering of radiation as it passes through the sample (Givens, *et al* 1997). Large particles do not scatter infrared radiation as much as small particles (Hruschka 1987). More radiation is absorbed, giving higher log(1/R) values, and this effect is greater at those wavelengths which are absorbed more strongly. Robert, *et al* (1986) suggested that correction for variations in particle size are particularly important when principal components analysis is used, and devised a regression approach to correct for this. The correction involves calculating the mean values for log(1/R) at each wavelength in the calibration data set, then regressing individual log(1/R) values for each sample against these “reference” values, and using the deviations from the predicted value in the principal components analysis.

Effects of different particle sizes and water contents between samples on the log(1/R) values can also be controlled by the “standard normal variate” method of Barnes, *et al* (1985) such that:

$$SNV_i = (Y_i - \bar{Y}) / [\sum(Y_i - \bar{Y})^2 / (N - 1)]^{0.5}$$

where: SNV<sub>i</sub> = standard normal variate for the value of log(1/R) at the i<sup>th</sup> wavelength  
Y<sub>i</sub> = value of log(1/R) at the i<sup>th</sup> wavelength  
 $\bar{Y}$  = mean of all Y  
N = number of log(1/R) values

Overlapping of the raw reflectance spectra (log(1/R)) of different analytes can be addressed by calculating derivatives, i.e. by subtracting from the value for log(1/R) obtained at a particular wavelength the values obtained at second (and third, in the case of the second derivative) wavelengths usually 20 nm distant (Williams and Cordeiro (1985):

$$A' = (2 \times A) - B - C$$

$$\text{or } A' = A - (2 \times B) + C$$

where: A' = second derivative of A  
A, B, C = log(1/R) obtained at wavelengths a, b and c

Hruschka (1987) has given a detailed description of the effects of derivatisation. The effect of using a second derivative to resolve overlapping peaks is illustrated in Fig. 2. Choice of derivative may depend on the analyte and matrix under consideration. Norris, *et al* (1976)

developed good prediction equations (coefficient of determination,  $R^2 = 0.85$  to  $0.99$ ) for protein, fibre, digestibility and intake from second derivative data. Shenk, *et al* (1981) recommended  $\log(1/R)$  for protein and *in vitro* digestibility, and second derivatives for fibre and minerals. Shenk and Westerhaus (1991b) later concluded that first derivatives gave better results for protein and second derivatives for acid detergent fibre (ADF) when hays, haylages and grains were examined. Marten, *et al* (1984) recommended second derivatives for all forage constituents. Garcia-Cuidad, *et al* (1993) compared  $\log(1/R)$ , and the first and second derivatives of these, in the development of equations to predict protein, fibre, lignin and cellulose of grasses. They reported generally similar  $R^2$  but concluded that the second derivative usually gave better fits. Equations developed by Brown, *et al* (1990) to predict protein and neutral detergent fibre (NDF) contents and *in vitro* OM digestibility (IVOMD) of grass hays used the first and second derivatives in almost every case. However, the preferred mathematical treatment varied between hays for each analyte.

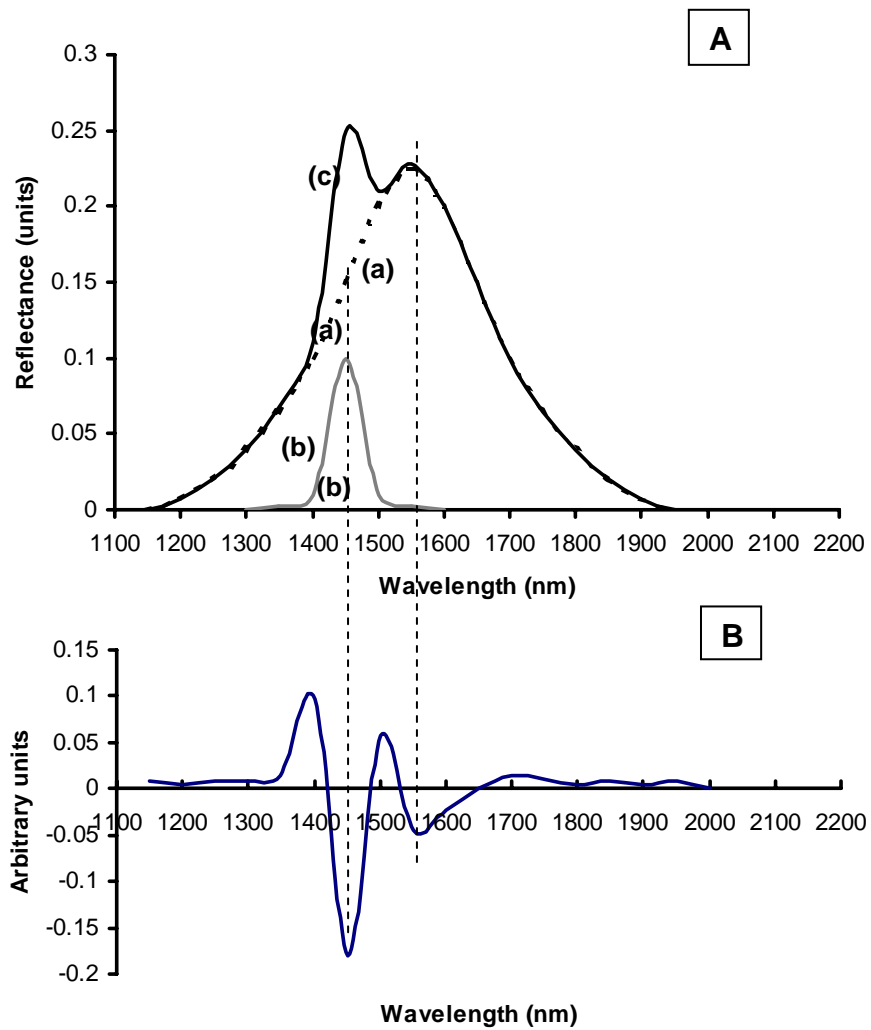
Collinearity is also reduced by the SNV correction. Table 3 reports data from Barnes, *et al* (1985). These show that the SNV correction removes collinearity between chemically unrelated wavelengths while retaining it between related wavelengths. The second derivative also reduced collinearity but to a less extent. Other advantages of the SNV correction in relation to principal component analysis are described by Barnes, *et al* (1985).

Barnes, *et al* (1985) suggested a method of using a second-degree polynomial to remove the tendency for the  $\log(1/R)$  baseline to increase over the range of wavelengths used in food evaluation. De-trending is now commonly used. Shenk and Westerhaus (1991b) reported that de-trending reduced the standard error of performance (SEP) for predictions of protein and ADF in forages and grains in more than half the cases.

**Table 3. Collinearity between reflectances at chemically-related and unrelated wavelengths before and after the SNV correction.**

<b>Mathematical treatment</b>	<b>Straw <math>R^2</math></b>	<b>Hay <math>R^2</math></b>
<i>Chemically unrelated wavelengths (1700 and 2100 nm):</i>		
Log(1/R)	0.937	0.965
D2 log(1/R) <sup>1</sup>	0.371	0.059
SNV	0.010	0.029
<i>Chemically related wavelengths (1420 and 1932 nm):</i>		
Log(1/R)	0.945	0.890
D2 log(1/R)	0.759	0.593
SNV	0.878	0.568

<sup>1</sup> D2 = second derivative



**Fig. 2. (A) Reflectance spectra showing (a) and (b) overlapping spectra of two different analytes, and (c) the combined spectrum. (B) Second derivative of  $\log(1/R)$  showing separation of peaks (after Hruschka 1987).**

## 3. Calibration and Validation

### 3.1. Characteristics of the calibration data set.

Equations are developed from a calibration data set, i.e. values for the analyte under consideration which have been generated by some reference method. Calibration data sets should be obtained from material which encompasses all of the chemical and spectral variation (Williams 1987) and the physico-chemical characteristics that are likely to be found in the population to be analysed using the calibrations (Williams and Cordiero 1985). This avoids any need to extrapolate beyond the boundaries of the calibration data. Calibration sets should have a wide range and even distribution in composition (Valdes, *et al* 1990).

Construction of a calibration set involves the balancing the cost of obtaining a widely representative data base, against the desirability of having the calibration set contain representatives of all the samples that are likely to be analysed by the prediction equation. Several authors have commented that the removal of outliers from the calibration set improves (as would be expected) the goodness of fit of the resulting calibration equation. Shenk and Westerhaus (1991a) recommended that the calibration set should exclude samples with extreme (i.e. outliers) or very similar, spectra (they used Mahalanobis distances of  $>3.0$  and  $<0.6$  to define the samples included in the set). They suggested that this would help to reduce the cost of obtaining reference values. On the other hand, Lyons and Stuth (1992) suggest that outliers which are identified when the equations are used should be referred back to the calibration set to identify those sample types that should be better represented in the calibration. Smith, *et al* (1997) comment as follows: “We conclude that outlier samples should not be removed during the calibration process unless the sample was identified as an outlier through the detection of some extraneous factor .... These outlier samples have been shown to provide essential information when the regression was used to analyse other similar samples.”

The optimum size for calibration data sets has not been resolved. Williams (1987) suggests 35 to 40 for “simple” calibrations, while J. Stuth (personal communication) recommends no less than 100 in the development of equations to predict animal performance from faecal data. Several hundreds of samples are generally used in forage calibration sets (Aastveit and Marum (1993), and they recommended 50 samples for principal component regression analysis. Hruschka (1987) recommends, on the basis of experience, that at least 10 samples are required for each different constant and for each varied parameter in the regression equation. Examples of calibration data sets are given in Table 4.



**Table 4. Calibration data sets used in the application of NIR spectroscopy to animal nutrition.**

<b>Purpose</b>	<b>n</b>	<b>Characteristics</b>	<b>Authors</b>
IVDMD of lucerne	166	Different cultivation methods and harvest dates.	Bertrand, <i>et al</i> (1987)
Protein and IVOMD of grass hay	35 of each sp.	Samples collected over 6 years, 4 grass species, maturities, fertilisation, hay-making procedures, storage conditions.	Brown, <i>et al</i> (1990)
Dietary quality of whole-plant maize forage	40 to 60	3 years, 6 locations, various hybrids.	Valdes, <i>et al</i> (1990)
Protein, ADF in hay	650	Samples from North America, Japan, Europe, pure and mixed grasses and legumes, various sample drying methods; samples with extreme spectra were removed.	Shenk and Westerhaus (1991b)
Chemical composition of maize grain	262	Samples of shelled, ear, grain + cob, high moisture, various sample drying methods; samples selected to remove those with extreme spectra.	Shenk and Westerhaus (1991b)
Nutritive quality of grasses	237	Samples collected over 4 years, from up to 5 sites, at up to three maturities, considerable variation in species composition.	Garcia-Cuidad, <i>et al</i> (1993)
Diet quality from faecal profiling	148	76 diets constructed from more than 50 forage species.	Showers (1997)

### 3.2. Derivation of calibration equations.

Least squares multiple linear regression analysis has been commonly used to develop prediction (i.e. calibration) equations. The selection of wavelengths into an equation can be done by several methods – stepwise inclusion is frequently used. In this process, the wavelength which is most highly correlated with the analyte concentration is identified, then tested with each other individual wavelength to find the best 2-term equation. This process is repeated using the 2-term equation together with each other individual wavelength, then the best 3-term equation, etc., until some predetermined statistical standard has been reached (Bertrand, *et al* 1987). Other methods of selecting independent variables (i.e. wavelengths) in a multiple regression equation (e.g. stepwise inclusion, backward elimination, etc.) are discussed in Statistical Analysis Systems (1988). Irrespective of the method used, there is no guarantee that the best equation is finally obtained. Testing all possible combinations by the combination regression method (Bertrand, *et al* 1987) may identify the best prediction equation. This takes considerable computing time and does not protect against overfitting, collinearity or selecting wavelengths which are excessively correlated with the analyte concentration.

In most cases, more than one wavelength is used to predict analyte concentrations. For example, protein, NDF and IVOMD in subtropical forages were predicted with 1 to 6 wavelengths (Brown and Moore 1987) and 3 to 7 (Brown, *et al* 1990) with  $R^2 = 0.67$  to  $0.98$ .

Shenk, *et al* (1985) used between 5 and 9 wavelengths to predict protein, fibre, lignin and *in vitro* DM digestibility (IVDMD) in a variety of grasses and legumes with  $R^2$  between 0.95 and >0.99. Valdes, *et al* (1990) achieved  $R^2$  of not less than 0.97 in predictions of ADF, protein and IVDMD in whole-plant maize by equations which included between 5 and 10 wavelengths. In this study, different numbers of wavelengths were selected to analyse these constituents in plants harvested in different years.

“Overfitting” involves using a large number of wavelengths giving a highly accurate equation as judged by statistics such as the  $R^2$ , the standard error of cross validation (SECV), or the regression F value. However, these equations may not accurately predict analyte concentrations when applied to data other than those used to derive the equation. The problem of overfitting is related to the high correlation between absorbances at different wavelengths (Bertrand, *et al* 1987) and to the equation recognising features of the calibration data set which are not representative of the data which will be used in predictions (Hruschka 1987). Overfitting is recognised (Hruschka 1987) by standard errors of calibration (SEC) being much less than the standard error of the reference (laboratory) determinations (SEL), large differences between reference values and NIR spectroscopy values, and a SEP greater than twice the SEC. Biston, *et al* (1989) suggested that over-fitting can be avoided by choosing equations which give the least SEP, rather than the lowest SEC.

Standard stepwise methods of developing a multiple regression equation ultimately select only some of the available wavelengths, and thus ignore much potentially useful information. Statistical treatments which use the whole of the available spectral information (so-called “full spectrum” methods) are available. These include principal component analysis (Robert, *et al* 1986; Berglund, *et al* 1990), principal component regression analysis (Cowe and McNicol 1985; Bertrand, *et al* 1987; Aastveit and Marum 1993), and partial least squares analysis (Manne 1987; Berglund, *et al* 1990; Shenk and Westerhaus 1991a). According to Givens, *et al* (1997) the variables developed from full spectrum methods are independent of each other, which addresses the problem that absorbances at nearby wavelengths are related. Haaland and Thomas (1988) have discussed the advantages and disadvantages of these methods v. the classical and inverse least squares approaches. Shenk and Westerhaus (1991a) and Smith and Kelman (1997) reported greater precision from equations derived by partial least squares than stepwise multiple regression methods, although Smith, *et al* (1998) found that both methods gave equations of similar accuracy when applied to the chemical composition of perennial ryegrass.

### **3.3. Statistical assessments of the quality of NIR calibrations and predictions.**

Several statistics are used to describe the quality of calibration and prediction equations. These are listed, with methods of calculation, by Williams (1987) and are summarised in Table 5.

**Table 5. Statistics to describe the quality of NIR spectroscopy calibration and prediction equations.**

<b>Statistic</b>	<b>Definition</b>
Standard error of calibration (SEC)	Variability in the difference between predicted values and reference values when the equation is developed from the calibration data set
Standard error of prediction (SEP) <sup>a</sup>	Variability in the difference between predicted values and reference values when the equation is applied to the validation data set
Standard error of cross validation (SECV)	Variability in the difference between predicted values and reference values when the equation is applied to a subset of data from the calibration data set
Coefficient of determination (R <sup>2</sup> )	Proportion of variability in the reference data accounted for by the regression equation; may be adjusted (R <sup>2</sup> <sub>adj</sub> ) to account for the number of degrees of freedom in the regression equation
Correlation coefficient (r)	Degree to which analyte values and log(1/R) values at particular wavelengths are correlated
Bias (D)	The mean difference between the predicted and the reference values

<sup>a</sup> also called “standard deviation of performance” (Williams 1987) or “standard error of the estimate” (Statistical Analysis Systems 1988) or “standard error of analysis (Brown, *et al* 1990) or “standard error of selection” (Smith and Flinn 1991).

The SEC may be calculated as follows (Smith and Flinn 1991):

$$SEC = \{\Sigma(X_i - Y_i)^2 / (N - p - 1)\}^{0.5}$$

where: X<sub>i</sub> = predicted value of the i<sup>th</sup> item in the validation set  
 Y<sub>i</sub> = reference value of i<sup>th</sup> item in the validation set  
 N = number of items in the validation set  
 p = number of independent variables in the prediction equation

Adesogan, *et al* (1998) suggested that equations with the largest R<sup>2</sup>, smallest SEC, and lowest number of spectral terms (to avoid overfitting) should be selected. Stimson, *et al* (1991) recommended the following extra criteria, in reducing order of importance:

- (1) reject equations with any terms with an F value < 10,
- (2) reject equations with terms having F values > 100,000
- (3) select equations which use wavelengths that correspond most closely with those known to associate with the chemical fraction under consideration.

Calibration equations are routinely validated against another data set in which reference analyte values have been determined. The samples in the validation set are normally different to those which were used to develop the prediction equation, and are usually a smaller set than the calibration set. The predicted values will normally differ from the reference values. Much of this is because of random error, but there are two types of systematic error. If the regression coefficient is different from 1.0, Williams (1987) noted that this will introduce a systematic bias at either end of the range of predicted values. Predictions may be biased, i.e. displaced by a constant amount from the reference values. A standard error of prediction corrected for bias (SEP(C)) can be calculated as follows (Smith and Flinn 1991):

$$\text{SEP}(C) = \{\Sigma (X_i - Y_i)^2 - N (\text{bias})^2 / (N - 1)\}^{0.5}$$

where:  $X_i$  = predicted value of the  $i^{\text{th}}$  item in the validation set  
 $Y_i$  = reference value of  $i^{\text{th}}$  item in the validation set  
bias = difference between overall means  
N = number of items in the validation set

Some workers use all the available reference data to construct the calibration set. Validation is then done by taking a series of randomly selected subsets of the calibration data and examining the distribution of differences between the predicted and reference values for each set. The statistic which describes the precision of the prediction is then the standard error of cross validation (SECV).

Williams (1987) has provided rules for interpreting values for bias, SEP and correlation between predicted and reference values. He recommended that the SEP should not be more than 3 % of the mean reference value for that analyte. Westerhaus (1985, cited by Stimson, *et al* 1991) recommended that the SEP should be no greater than twice the SEL. Bias can be assessed by the size of the ratio of  $\text{bias}^2 : \text{SEP}^2$  in relation to the mean of the reference values (Hruschka 1987). This ratio should be small. A uniform bias can be corrected by adjusting the regression intercept. Displacements of predicted values at either end of the reference range can be corrected as described by Williams (1987).

### 3.4. Technical errors in NIR spectroscopy.

Although NIR spectroscopy is rapid and technically simple to carry out, there are nearly 40 sources of error (Williams 1987). These contribute to error in both the calibration and validation processes. As indicated above, between-sample variations in water content and particle size are particularly important.

Williams (1987) listed sources of error (summarised in Table 6) and has discussed ways in which these may be controlled. In practice, NIR spectroscopy laboratories go to lengths to control ambient temperature, sample water content, and vibration.

**Table 6. Procedural sources of error in NIR spectroscopy (selected from Williams 1987).**

<b>Instrument factors</b>	<b>Sample factors</b>
instrument noise	variations in water content
stray light	bulk density, texture, packing characteristics
non-linearity of signal	sample temperature
static electricity	subsampling procedures
instrument temperature control	mean particle size, particle size distribution
fluctuations in power supply	mixing after preparation
instrument geometry	sample storage
cell window characteristics	

### 3.5. Errors in the reference values.

Reference errors arise from using different subsamples to conduct the NIR spectroscopy and reference analyses, and from random and systematic errors in the reference methodology (Hruschka 1987; Sorensen 2002). Systematic errors (bias) should be identified and eliminated. These may include errors relating to the chemistry of the reference determination such as the loss of N from refractory substances in the Kjeldahl N determination or use of an inappropriate factor to convert N to protein. Analytical methods should be standardised as much as possible to reduce random error. The amount of random error in the reference analyses can be expressed as the laboratory standard error (SEL) as described by Smith and Flinn (1991):

$$\text{SEL} = \{\Sigma(X_1 - X_2)^2 / N\}^{0.5}$$

where:  $X_1, X_2$  = duplicate reference analyses  
N = number of samples

When NIR spectroscopy is used to predict more complex outcomes, such as digestibility or intake (e.g. Givens, *et al* 1997; Coleman, *et al* 1989) or animal performance (Lippke, *et al* 1989) then the correction of reference errors becomes much more problematic. Norris, *et al* (1976) used NIR spectroscopy to predict dry matter (DM) and digestible energy (DE) intakes of tropical and temperate hays, silages and fresh forages. They obtained  $R^2$  for DE intake and DM intake of 0.72 and 0.64. Much of the lack of precision appeared to be caused by a subset of the data, and when these were eliminated,  $R^2$  increased to 0.87 for DM intake and 0.90 for DE intake. The result of removing these outliers does not prove that the reference values for these samples were erroneous, but it is possible that the calibration samples may have included material which was corrupted by laboratory errors, or mis-labelled, etc.

Errors in reference values inflate the errors associated with NIR prediction equations. If an estimate of the reference measurement error variance is available Faber and Kowalski (1997) and Sorensen (2002) offer methods of correcting the SEP and/or SECV.

It can be expensive to reduce the incidence of reference error. Wet chemistry methods are consuming of time, labour and experimental materials, including samples. The effect of reducing reference error may be negligible if the variability in the reference material is less than that in the NIR data (Sorensen 2002). He suggests that "It may be better to reduce the number of replicate (reference) analyses and instead introduce more samples to improve the robustness of the calibration." The use of NIR spectroscopy to predict complex animal responses like intake, digestibility and growth involves the preparation of very expensive calibration data sets, and a wide variety of situations must be encompassed to obtain an adequately robust calibration. Sorensen's (2002) recommendations have particular relevance to these situations.

### 3.6. Portability of calibration equations.

A calibration performed on a particular instrument can not be expected to apply to any other instrument. Differences in internal geometry (positioning of mirrors, gratings, sample cell, etc.), and the laboratory environment (temperature, electrical voltage, vibration, sample preparation) lead to differences in the performance of different instruments.

The portability of prediction equations between instruments has been investigated by several authors. Shenk, *et al* (1985) demonstrated that prediction equations for protein, ADF, NDF, lignin and IVDMD of legumes and grasses could be used by other instruments of the same model with acceptable accuracy and precision. On the other hand, Williams and Krischenko (1986) could not duplicate protein determinations while using two similar instruments, and Shenk and Westerhaus (1985) found that quite different instruments produced equations with different numbers of wavelengths and predictive performances. Similar data are presented by Valdes, *et al* (1987). They reported similar  $R^2$  and SEP for IVDMD in whole-plant maize from different instruments, but noted that protein estimations differed. Offer (1993, cited by Givens, *et al* 1997) has warned that even instruments which have been matched by the manufacturer may still behave differently, especially with more complex equations.

Universal calibrations are intended to apply to “all reasonable samples of a product”, and while having broad coverage they may not be as accurate as more narrowly-based calibrations (Shenk 1989). Universal equations have been tested under several circumstances. Two examples are those provided by Williams and Cordeiro (1985) for moisture and protein in wheat grain and Valdes, *et al* (1990) for ADF, protein and IVDMD in whole-plant maize forage. Both groups were able to develop satisfactory a satisfactory universal calibration, provided that this was based on reference data obtained for the whole of the years and locations under consideration. In both cases, single-year calibrations gave unacceptably large biases when they were applied to years other than that for which they were derived. As a third example, Smith and Flinn (1991) developed broad-based calibrations to predict protein, NDF and IVDMD in mixed temperate pasture. The equations successfully predicted these constituents in the validation set (which was a subset of the original samples) but there was some bias when constituents in new samples were analysed. Smith and Flinn (1991) recommended that equations should be recalibrated with a small number of reference values whenever they are used in a new context.

Notwithstanding the reservations expressed above, there are several examples of the use of universal calibrations. The United Kingdom agricultural industries have adopted a “universal” equation to predict the *in vivo* OM digestibility of grass silage (Givens, *et al* 1997), the Norwegian Forage Research Program has used a universal equation since 1982 (Aastveit and Marum 1993), and Givens, *et al* (1997) cite another four examples of European use of universal equations.

# 4. Applications of Near Infrared Reflectance Spectroscopy in Agriculture

## 4.1. NIR spectroscopic analysis of feed chemical composition

Norris and Hart (1965, cited by Norris, *et al* 1976) used NIR spectroscopy to predict the moisture content of grains and oilseeds, and Norris, *et al* (1976) are credited with the first application of NIR spectroscopy to the analysis of forages. In this paper, they reported the results of NIR spectroscopy analyses for chemical constituents, digestibilities and DM intakes of a range of temperate and tropical grasses, and lucerne hay, fed to sheep. Their precisions (SEP for protein, NDF, OM and DM digestibilities, and DM intake of 0.74 %, 2.39 %, 2.5 % and 8.6 g/kg liveweight) were sufficiently high for them to claim that "... infrared reflectance has the potential for use in rapid evaluation of forage quality".

The literature on the use of NIR spectroscopy to predict the chemical composition of animal foods and animal-derived biological materials is now voluminous. Murray (1996), Givens, *et al* (1997) and Givens and Deauville (1999) have reviewed the earlier literature on NIR spectroscopy in the analysis of the chemical composition and digestibility of animal forage and concentrate feeds.

The precision and accuracy of the predictions are critical to the acceptance of NIR spectroscopy as an analytical tool. Generally, SEP are very low in relation to the mean constituent content, bias is negligible, and  $R^2$  values of greater than 0.8 are routinely reported (Table 7). However, these statistics are not informative unless they can be compared to similar values for the reference chemical methods. The standard deviation of Kjeldahl N determinations is 0.43 % (Templeton, *et al* 1983; standard error), 0.41 % (O'Keeffe, *et al* 1987), 0.44 % (Lyons and Stuth 1992), or 0.2 % (cited in Faber and Kowalski 1997). Templeton, *et al* (1983) reported standard errors of 1.40 % for NDF, 1.70 % for ADF and 0.90 % for lignin, and Lyons and Stuth (1992) obtained a standard error for digestible OM determinations of 1.68 %. SEL of conventional methods for DM (0.7 %), protein (5.0 %), ADF (9.1 %), NDF (8.2 %) and lignin (1.9 %) were reported by Stimson, *et al* (1991). The effects of sample type (and thus sampling variation) on reference method precision is evident in the values reported by Melchinger, *et al* (1986). These authors reported standard errors of 0.14 and 0.26 % for protein in maize grain and maize stover, respectively.

Few authors have reported the variability of duplicate NIR spectroscopy measurements. O'Keeffe, *et al* (1993) reported standard deviations for the difference between duplicate NIR values of a similar order to that for the Kjeldahl determination, i.e. 0.22 %. Standard errors of duplicate NIR determinations for protein, fibre and water-soluble carbohydrates in maize grain and protein and ADF in maize stover of 0.16, 0.11, 0.21, 0.28 and 0.46 % respectively (Melchinger, *et al* 1986) were 6 to 22 % higher than those for chemical determinations. Starch in maize grain was determined substantially less precisely by NIR than by laboratory measurement, while the reverse situation applied to predictions of IVDOM and ME. Finally, when estimates of the precision of conventional reference chemical methods are compared with cited NIR spectroscopy SEP it should be remembered that in almost every case, reported SEP have not been corrected for imprecision in the reference data, and that this decreases the precision of NIR predictions.

**Table 7. NIR spectroscopic prediction of the chemical composition of forages.**

Sample type	Constituent	Reference mean (%)	SEP <sup>1</sup> (%)	Bias (%)	R <sup>2</sup>	Author
Lucerne forage	ADF	33.3	1.30	-0.1	0.94	Martens, <i>et al</i> (1984)
	NDF	42.3	1.46	-1.0	0.95	
	Protein	20.8	0.42	-0.3	0.98	
Mixed temperate legumes	ADF	33.5	1.70	0.8	0.98	Martens, <i>et al</i> (1984)
	NDF	41.9	2.23	1.0	0.98	
	Protein	18.0	1.00	-0.3	0.97	
	Lignin	6.1	0.63	-0.2	0.96	
Lucerne forage	Protein	12.6 - 24.8	0.81		0.96	Bertrand, <i>et al</i> (1987)
Stargrass/bermuda grass	Protein	4.3 - 23.9	0.83	0.96	0.95	Brown and Moore (1987)
American joint vetch	Protein	0.6 - 4.6	0.32	0.21	0.82	
Bermuda grass	Protein	4.0 - 17.7	0.92	-0.07	0.92	Brown, <i>et al</i> (1990)
Stargrass	Protein	1.6 - 26.0	0.85	-0.24	0.89	
Digit grass	Protein	2.6 - 14.0	0.90	-0.15	0.87	
Bahia grass	Protein	3.4 - 15.6	0.77	-0.11	0.88	
Maize stover	ADF	34.3	1.55	0.14	0.79	
	Protein	6.9	0.60	-0.06	0.87	
Maize grain	Protein	11.1	0.29	0.05	0.96	Melchinger, <i>et al</i> (1990)
	Crude fibre	4.5	0.25	0.04	0.90	
	Starch	68.3	1.29	0.06	0.94	
	WSC <sup>3</sup>	2.6	0.59	0.17	0.73	
Whole-plant maize forage <sup>2</sup>	ADF	14.0 - 38.8	1.6	0.1	0.88	Valdes, <i>et al</i> (1990)
	Protein	5.1 - 10.0	0.5	0.1	0.8	
Maize stover	ADF	34.9	1.43	0.14	0.94	Zimmer, <i>et al</i> (1990)
	NDF	65.4	1.78	0.30	0.95	
	Lignin	3.3	0.42	-0.10	0.77	
Temperate mixed grasses/legumes	NDF	22.5 - 63.5	2.17	-0.01	0.95	Smith and Flinn (1991)
	Protein	7.3 - 28.7	0.85	0.18	0.97	
Mixed pasture species	ADF	25.4 - 40.8	1.42	-0.19	0.76	Garcia-Cuidad, <i>et al</i> (1993)
	NDF	38.3 - 70.4	2.06	-0.49	0.86	
	Protein	5.8 - 16.4	0.57	0.04	0.90	
	Lignin	1.9 - 8.3	0.45	< 0.01	0.88	
Temperate grass silage	Protein	6.5 - 17.6	0.63		0.90	O'Keefe, <i>et al</i> (1993)
Whole-plant maize forage	DM	87.4 - 97.0	4.25		0.61	Cozzolino, <i>et al</i> (2000)
	ADF	6.8 - 59.0	1.85		0.98	
	NDF	33.1 - 89.7	2.43		0.83	
	Protein	1.2 - 13.8	1.04		0.86	
	Ash	1.5 - 16.0	0.38		0.64	

<sup>1</sup> standard error of prediction

<sup>2</sup> multi-year calibration; equations for single years had R<sup>2</sup> between 0.81 and 0.95 for these variables

<sup>3</sup> water-soluble carbohydrates

<sup>4</sup> equations derived by stepwise regression

<sup>5</sup> constituents expressed on an organic matter basis



Fairbrother and Brink (1990) obtained SEP for pectin, arabinose, xylose and glucose in forage cell walls of 0.94, 1.04, 2.90 and 2.53 %, measured in a range of temperate and tropical grasses and legumes. The SEL for these determinations were 0.6, 0.4, 0.5 and 0.6 %, respectively. Condensed tannins in leucaena forage (Wheeler, *et al* 1996) and lotus (Smith and Kelman 1997) were successfully determined by NIR spectroscopy.  $R^2$  for regression of NIR values on laboratory reference values were 0.84 and 0.82, respectively.

Mineral elements can not be detected directly with NIR spectroscopy; but are detected indirectly because their presence in organic complexes affects H bonds (Shenk 1992, cited by Givens *et al* 1997) or the concentrations of organic constituents (Watson, *et al* 1976). NIR predictions of mineral contents are not generally reliable. Shenk and Westerhaus (1985) obtained  $R^2$  of 0.17 to 0.74 for NIR predictions of P, K, Ca and Mg contents of various forages and Smith, *et al* (1991) were only modestly successful in predicting Mg contents in perennial ryegrass. Cozzolino, *et al* (2000, Table 7) reported variable prediction of the ash content of maize forage. Windham, *et al* (1991) had SEP equivalent to 10 % of the mean reference value for predictions of ash in pasture, oesophageal fistula extrusa and faeces, although their  $R^2$  values were between 0.89 and 0.93. P contents were successfully predicted by Showers (1997) from faeces, possibly because of the P=O bonds in phosphate groups.

The data reviewed in Table 7 suggest that organic analytes in animal feeds can be predicted by NIR spectroscopy with acceptable precision and accuracy. Estimates of protein are usually associated with  $R^2 > 0.9$ , although cell wall constituents (lignin, ADF, NDF) are predicted less precisely. The average SEP and  $R^2$  of NIR predictions in Table 7 are 1.17 % and 0.89, respectively. Bias (average = 0.03 %) is small in relation to the mean constituent contents. Further, NIR spectroscopy appears to be as precise as conventional (wet) chemical methods of analysing organic material. These characteristics will make NIR spectroscopy an acceptable alternative analytical method, in those laboratories which have the capacity to measure and interpret NIR spectra and to perform the wet chemical analyses which are required to provide the reference values for NIR calibration.

## 4.2. Digestibility prediction by NIR spectroscopy.

There is a large data base of forage digestibilities predicted by NIR spectroscopy. Performance in this area is illustrated by the data summarised in Table 8. NIR spectroscopy predicts feed digestibility with somewhat lower precision than for chemical composition, e.g. the SEP for digestibility are about twice the size of those for organic chemical constituents. Nevertheless, other aspects of prediction like bias and  $R^2$  (averages of 0.045 % and 0.906, respectively) are similar between chemical composition and *in vitro* digestibility. Digestibility is influenced by the amounts of food constituents like NDF and ADF, and the patterns of chemical bonding between hemicellulose and lignin (O'Keeffe, *et al* 1987). It is not surprising that with *in vitro* digestibility, where feed-related factors mainly influence the result, the performance of NIR spectroscopy is similar to the Tilley and Terry and pepsin-cellulase methods (Table 8). However, there are noticeable differences between predictions of *in vitro* and *in vivo* digestibilities. For example, Coelho, *et al* (1988) predicted the IVDMD digestibility of bermudagrass with  $R^2 = 0.9$  and bias = 2.1 %; predictions of *in vivo* DM digestibility were less precise ( $R^2 = 0.69$ ) but with a similar bias. Digestibility is also influenced by non-feed factors, such as level of feed intake and the rate of passage of feed through the digestive tract. These may not be predicted well by NIR techniques (or any conventional chemical method). This may explain the lower precision and greater bias of the NIR predictions (average bias and  $R^2$  of -0.77 % and 0.78, respectively). In most cases the biases are small in comparison to the means, and are probably not biologically important.

NIR spectroscopy predicts *in vivo* digestibility at least as precisely as other predictive methods like chemical composition or *in vitro* or enzyme digestibilities. O’Keeffe, *et al* (1987) reported the standard deviation of differences between NIR measurements of silage IVDMD digestibility was 0.77 %, compared to 0.85 % for the Tilley and Terry (1963) method. In the study of Coelho, *et al* (1988), *in vivo* DM digestibility of six forages was predicted from NDF content, 5 different *in vitro* digestion methods, and six different cellulase methods, with similar SEP, i.e. from 0.08 to 0.15 %.  $R^2$  for these procedures ranged from 0.72 to 0.88. Barber, *et al* (1990) predicted *in vivo* OM digestibility from measures of IVOMD obtained by Tilley and Terry and pepsin-cellulase digestions, and predictions from lignin and modified ADF, with an NIR prediction. NIR was the most precise method ( $R^2 = 0.76$ , v. 0.14 to 0.64 for the other methods), and has similar or better accuracy (i.e. absence of bias) as the other methods. Riviere, *et al* (1989) compared *in vitro* (24 h incubation in rumen liquor), *in situ* (24 h incubation) and pepsin-cellulase digestions with NIR spectroscopy to predict *in vivo* OM digestibility of green forages. Pepsin-cellulase performed well with first-cut forages ( $R^2 = 0.88$ ) but not with more mature samples ( $R^2 = 0.35$ ). Rumen digestion procedures gave modest results ( $R^2 = 0.50$ ). NIR predictions had  $R^2 = 0.83$  and performed better than pepsin-cellulase when compared on the same set of forages.

NIR spectroscopy out-performed conventional *in vitro* methods in the study of Givens, *et al* (1991) with ammoniated and untreated cereal straws. *In vivo* OM digestibility was predicted more precisely by NIR spectroscopy ( $R^2 = 0.647$ ) than by the Tilley and Terry *in vitro* method ( $R^2 = 0.600$ ), or cellulase methods ( $R^2 = 0.48$  to  $0.509$ ). De Boever, *et al* (1996) used a data set of 64 grass silages to compare the predictive capacities of NIR spectroscopy with *in vitro* digestion and treatment with cellulases. *In vivo* OM digestibility was predicted relatively poorly ( $R^2 = 0.528$  to  $0.646$ ) by chemical composition, but somewhat better by the *in vitro* and cellulase techniques either alone ( $R^2 = 0.644$ ,  $0.684$ ) or *in vitro* data together with DM and protein ( $R^2 = 0.779$ ), or cellulase digestion data with protein, fibre and DM ( $R^2 = 0.841$ ). NIR spectroscopy was similarly precise ( $R^2 = 0.792$ ). Adesogan, *et al* (1998) found that predictions of organic matter digestibility of whole-crop wheat by *in vitro*, *in situ*, neutral detergent/cellulase digestions ( $R^2 = 0.41$ ,  $0.44$ ,  $0.41$ ), and gas production ( $R^2 = 0.26$ ), were substantially poorer than the prediction from NIR spectroscopy ( $R^2 = 0.87$ ).

Flinn and Heazlewood (2000) used 16 Australian forages – seven legumes, four temperate cereal hays, two sorghum hays, and three temperate pasture hays – to compare NIR spectroscopy with other predictive methods. NIR predicted *in vivo* DM digestibility as efficiently as pepsin-cellulase digestion ( $r = 0.95$ ), and better than equations using ADF and N contents. The performance of NIR was surprisingly good, as the calibration was based on reference values not directly determined but predicted from a regression of *in vivo* DM digestibility on pepsin-cellulase digestion.

### 4.3 Prediction of food intake and animal growth rate from forage samples

There appear to be few predictions of food intake or animal performance from the NIR spectra of forages. Norris, *et al* (1976) correlated the DM intakes of 79 tropical and temperate forages with their NIR spectra ( $r = 0.79$ , SEP = 7.8 g). DM intake of bermudagrass forage was predicted by Coelho, *et al* (1988) with  $R^2 = 0.84$  and a bias of  $-5.7 \text{ g/kgW}^{0.75} \cdot \text{d}^{-1}$  (8.5 % of the mean reference DM intake). Lippke, *et al* (1989) predicted heifer growth from NIR analysis of bermudagrass, sorghum hybrids, paspalum and ryegrass forages. They identified five wavelengths which were highly correlated ( $r = 0.976$ ) with growth. Two of these (1696 and

2298 nm) were correlated ( $r = -0.928, -0.909$ ) with digestible OM intake, and 1696 nm was also correlated ( $r = 0.927$ ) with forage ADF content. More recently, Steen, *et al* (1995) showed that silage consumption was correlated with the concentrations of forage N and fibre fractions ( $r = 0.19$  to  $0.49$ ), but more highly correlated with NIR predictions ( $r = 0.85$ ). DM intakes were predicted by NIR spectroscopy for the set of 16 Australian forages described above (Flinn and Heazlewood 2000). NIR ( $r = 0.60$ ) gave the best results. Neither ADF or NDF contents, pepsin-cellulase digestion, or shear energy predicted DM intake precisely ( $r = 0.05$  to  $0.32$ ).

**Table 8. Prediction of digestibility and available energy contents.**

Sample type	Parameter <sup>1</sup>	Reference mean (%)	SEP <sup>2</sup> (%)	Bias (%)	R <sup>2</sup>	Author
<b><i>In vitro digestibility:</i></b>						
Lucerne forage <sup>3</sup>	IVDMD	53.3 - 74.3	2.22		0.89	Bertrand, <i>et al</i> (1987)
Temperate grass silage	IVDMD	40.7 - 69.4	2.96		0.72	O'Keeffe, <i>et al</i> (1987)
Smooth bromegrass	IVDMD	66.3	1.62	-0.44	0.92	Gabrielsen, <i>et al</i> (1988)
	CDMD	67.7	1.66	0.09	0.97	
Crested wheatgrass	IVDMD	61.5	1.62	-0.29	0.97	Gabrielsen, <i>et al</i> (1988)
	CDMD	70.1	1.69	0.50	0.98	
Lucerne forage	IVDMD(t)	76.0	2.07	0.1	0.96	Bughrara, <i>et al</i> (1989)
Maize stover	IVDOM	61.3	2.24	-0.12	0.88	Zimmer, <i>et al</i> (1990)
Mixed temperate grasses and legumes	CDMD	53.7 - 82.0	2.53	0.48	0.86	Smith and Flinn (1991)
Kikuyu grass	gas prodn. <sup>4</sup>	0.038 - 0.047	> 0.002	-0.001 - 0.0	0.52	Herrero, <i>et al</i> (1996)
<b><i>In vivo digestibility:</i></b>						
Temperate pasture grasses and legumes <sup>7</sup>	OMD	55.5 - 80.8	2.51		0.90	Robert, <i>et al</i> (1986)
Grass and lucerne hays	DMD	56.9		-0.3		Coelho, <i>et al</i> (1988)
Cereal straw <sup>5</sup>	OMD	31.6 - 64.9	3.71	-1.24	0.647	Givens, <i>et al</i> (1991)
Temperate grass silage <sup>6</sup>	OMD	61.0 - 80.8	2.35		0.82	Baker, <i>et al</i> (1994)
Mixed pig feeds	OMD	69.5 - 93.5	1.79			Aufrere, <i>et al</i> (1996)
Mixed ruminant feeds	OMD	65.2 - 90.6	2.15			
Grass silage <sup>8</sup>	OMD	61.6 - 83.7	2.9		0.753	de Boever, <i>et al</i> (1996)
Whole-crop wheat forage	OMD	59.8 - 74.2			0.72	Adesogan, <i>et al</i> (1998)
	DOMD	55.8 - 70.8			0.87	
<b><i>Digestible, metabolisable and net energy:</i></b>						
Grass hay	ME	} 8.1 - 11.8	0.45	-0.15		Berglund, <i>et al</i> (1990)
Grass silage	ME		0.61	-0.16		
Legume hay	ME		0.55	-0.18		
Legume silage	ME		0.70	-0.12		
Grass silage <sup>8</sup>	ME	6.67 - 11.88	0.31		0.77	de Boever, <i>et al</i> (1996)
	NEL	3.76 - 7.15	0.26		0.88	
Mixed pig feeds	DE	11.96 - 17.11	0.37			Aufrere, <i>et al</i> (1996)
	ME	11.61 - 16.72	0.39			
Mixed ruminant feeds	DE	13.23 - 18.85	0.57			Aufrere, <i>et al</i> (1996)
	ME	10.68 - 15.04	0.42			

<sup>1</sup> IVDMD, *in vitro* dry matter digestibility by the Tilley and Terry method; IVDOM, *in vitro* digestibility of organic matter; IVDMD(t), IVDMD determined by a final extraction with neutral detergent solution; CDMD, DM digestibility determined by cellulase; OMD, *in vivo* organic matter digestibility; DOMD, digestible organic matter content (DM basis); ME, metabolisable energy content (MJ/kg DM, pigs; /kg OM, ruminants); NEL, net energy for lactation content (MJ/kg DM, pigs; /kg OM, ruminants)

<sup>2</sup> standard error of prediction

<sup>3</sup> equations derived from log<sub>10</sub>(1/R) values

<sup>4</sup> gas production rate (/h) calculated from the McDonald (1981) equation

<sup>5</sup> pooled data for ammoniated and untreated straws

<sup>6</sup> best of the 20 equations examined

<sup>7</sup> equations derived by principal components analysis

<sup>8</sup> after correction for bias

# 5. Predictions of Animal Performance

## 5.1. Predicting animal performance from faecal chemistry

Relationships between faecal chemistry and diet composition have been investigated, and used, since the 1940s. For example, faecal N determined by conventional wet chemistry was used to predict the digestibility of pasture by Lancaster (1949). Various aspects of the use of faecal indices for predicting diet digestibility, and protein and mineral (especially P) contents, have been reviewed by Van Soest (1994, pp. 111-113) and Wehausen (1995). Howery and Pfister (1990) have discussed the use of faecal indices with particular attention to their use in deer. A continuing theme running through this work has, however, been warnings against the uncritical use of faecal/diet relationships. Several authors have warned about the problems caused by tannins, pasture species composition, fertilisation practice, inappropriate application of regression relationships, etc. (e.g. Hobbs 1987; Wehausen 1995; and the review by Corbett 1978).

The difficulties inherent in accurately predicting diet characteristics from faecal composition are illustrated in the investigation of Leite and Stuth (1990). These workers attempted to predict dietary protein, IVDOM and feed constituent intakes of cattle from faecal OM and total N, N fractions, tannins, and proportions of monocotyledonous plant fragments. No single component satisfactorily predicted diet quality. Predictions of diet protein content from faecal total N had  $R^2 = 0.35$ . When other faecal constituents were added to the prediction equations,  $R^2$  values increased, but not to levels which would give confidence in the use of these variables to predict diet protein or DOM contents ( $R^2 = 0.51$  to  $0.57$  for protein,  $0.34$  to  $0.37$  for DOM), or any measure of nutrient intake ( $R^2 = 0.35$  to  $0.51$ ). Leite and Stuth (1990) suggested that difficulties in measuring condensed tannins and the relatively uniform amount of monocotyledonous plants in the diets may have impaired the predictive ability of these equations. They emphasised the role of plant tannins in confounding relationships between faecal N and diet protein contents.

Notwithstanding the difficulties of quantitatively predicting diet nutritive value, faecal indices (especially total N) give good qualitative descriptions of the quality of a grazing animal's diet. Many North American deer researchers have used faecal indices (determined by conventional chemistry) of dietary protein, P and energy content, perhaps because of the greater difficulty in obtaining blood, bone or oesophageal extrusa samples from wild deer than from domestic grazing animals.

Faecal total N has been used in several instances to monitor the N content of deer diets (e.g. Leslie and Starkey 1985; Leslie, *et al* 1989; Osborn and Jenks 1998). However, there have been relatively few controlled studies to attempt to validate this approach. In elk (wapiti, *Cervus elaphus nelsoni*) faecal N content was closely correlated ( $R^2 = 0.97$ ) with diet protein content (Mould and Robbins 1981), provided that the diet was free of tannins. Brown, *et al* (1995), in two trials, obtained similar results, but in their experiments the response to diet protein (which varied between 6.8 and 18.5 %) was influenced by greater and more consistent changes in response to diet digestible energy (5.9 to 13 MJ/kg). This is consistent with the catabolism of body tissue under conditions of energy deficiency, and with the results of Mould and Robbins (1981) who showed that elk required  $0.64 \text{ MJ digestible energy/kg } W^{0.75} \cdot d^{-1}$  to maintain a positive N balance. This is similar to estimates of the ME requirements for maintenance of deer (e.g. Dryden, *et al* 2002).

Other faecal N fractions than total N change with changing diet protein content. Howery and Pfister (1990) showed that white-tailed does fed 16.5 % v. 7.4 % protein diets excreted faeces with significantly different total, NDF-insoluble and metabolic N and P contents.

While the positive effect of diet protein content on faecal N has been demonstrated several times, Mould and Robbins (1981) appear to be the only workers who have attempted to calibrate the response in deer, i.e. to report a regression coefficient. The low-tannin diets (protein contents of 3.8 to 29.3 %, DM basis) of Mould and Robbins (1981) dietary N content was related to faecal N content:

$$N_{\text{diet}} = 0.77 + 0.49 \times N_{\text{faeces}}$$

where:  $N_{\text{diet}}$  = dietary N content (%)  
 $N_{\text{faeces}}$  = faecal N content

Tomkins and McMeniman (1996) fed four diets of 9.1 to 20.5 % protein to rusa deer, and Osborn and Ginnett (2001) fed five diets with protein contents between 8.1 and 25.6 % to white-tailed deer. None of these diets contained tannins and within experiments the diets varied little in digestibility. In both cases, deer fed higher-protein content diets either excreted more faecal N (Tomkins and McMeniman 1996) or had higher faecal N contents ( $R_{\text{adj}}^2 = 0.81$  for regression of faecal N content on dietary N content, Osborn and Ginnett 2001). Neither of these authors reported a regression equation.

Deer saliva contains a protein which precipitates tannins (Austin, *et al* 1989), and this might be expected to protect the dietary/faecal N relationship of deer from the influence of tannins more than for other ruminants. Hobbs (1987) and others have warned against the confounding effects of food tannins. Mould and Robbins (1981) data showed clearly elevated faecal N contents when tannin-containing diets were fed. Diets with different tannin contents fed by Osborn and Ginnett (2001) had no effect on faecal N at low diet protein contents, but significantly increased faecal N when high-protein diets were fed.

Faecal indices of energy status include 2,6-diaminopimelic acid (DAPA) and NDF. DAPA is found in some species of bacteria. It is not commonly present in plant food constituents, and does not occur in mammalian tissue. Increased faecal DAPA indicates increased growth of hindgut bacteria. This population is limited by the availability of energy (e.g. Thornton 1970) and elevated faecal DAPA suggests an increased supply of bypass energy, originating from the diet. Relationships between faecal DAPA concentration and diet quality have been investigated in several situations. Leslie, *et al* (1989) showed that DAPA and total N increased through spring, summer and autumn as the nutritive value of feed in the white tailed deer and moose range they examined presumably improved. Brown, *et al* (1995) found that faecal DAPA consistently changed with the digestible energy and protein contents described above. The effects were large (up to 50 % change) but in different directions – highest faecal DAPA was associated with highest DE but lowest diet protein. Similar results were reported by Osborn and Ginnett (2001). These workers also demonstrated that faecal DAPA was not influenced by diet tannin content.

Plant fibre is negatively correlated with food digestibility (e.g. Van Soest 1994, pp. 351, 408), and its concentration in faeces should be consistent with diet quality. Brown, *et al* (1995) showed that faecal NDF content could be related to the diets described above. NDF was

higher in the faeces of deer fed lower-DE and lower protein diets. N is required by the rumen fibrolytic bacteria, and the excretion of more undigested NDF when diets are low in protein is thus to be expected.

Phosphorus in white-tailed deer faeces responds to changes in diet P contents. For example, white-tailed does fed diets with 0.5 v. 0.3 % P excreted faeces with significantly different P contents (Howery and Pfister 1990). A similar behaviour of faecal total P in white-tailed deer was reported by Osborn and Jenks (1998).

## 5.2. Applications of faecal NIR analysis to monitoring the nutritional environment and performance of grazing animals

Lyons and Stuth (1992) and Showers (1997) have described the components of faeces and discussed their possible relationships with the NIR spectra and the diet. The primary wavelengths identified by Showers (1997; Table 9) are similar to those listed by Norris, *et al* (1976) and in Givens and Deauville (1999) as being important in predicting forage quality from NIR analysis of the forages themselves. Further details on the associations between wavelengths and chemical bonds and groups are given by Showers (1997).

**Table 9. Relationships between faecal NIR spectra and the chemical constituents in the faeces of deer fed forages; primary wavelength listed first (from Norris, *et al* 1976; Showers 1997; Givens and Deauville 1999).**

Diet attribute predicted	Matrix	Wavelengths (nm)	Chemical entities
<i>Predicted from faecal spectra:</i>			
Protein	faeces	2324, 1884, 1972, 1356	Aromatics, aliphatics (anti-quality factors), nitrites, amino groups, carbonyl groups (protein)
DOM	faeces	2136, 1656, 1540, 1276	Phenolics, aromatic substances (lignin), alcohols (waxes), amines (ammonia), cellulose, protein
<i>Predicted from forage spectra:</i>			
Protein	forages	2164, 2084, 2254, 1610	
Lignin	grass silage	1658, 2286	
Lignin	forages	1552, 1642, 2030, 1694	
NDF	forages	2294, 2072, 1902, 1558	
<i>In vivo</i> DMD	mixed grass	2266, 1662	
<i>In vivo</i> DMD	hays	1326, 2266	
<i>In vivo</i> DMD	forages	1666, 1992, 2266, 1596	

**Prediction of diet quality and feed intake:** Stuth and his co-workers have developed relationships between faecal NIR spectra and the quality (i.e. the IVDOM and protein contents) of grazing cattle diets. These relationships were developed from calibration data sets obtained by analysing oesophageal extrusa by the Tilley and Terry (1963) *in vitro* digestion method, and measurements of N content by wet chemistry. Lyons and Stuth (1992) reported variable success in predicting the quality of diets selected by lactating and dry cows.

Protein predictions were unsatisfactory ( $R^2 = 0.63$ ) to good ( $R^2 = 0.93$ ), although they obtained better predictions of DOM content ( $R^2 = 0.71$  to  $0.80$ ). There was little bias associated with either prediction. The error in predicting protein contents may have been associated with different chemistries in the faeces of lactating, rather than dry, cows. Similar results were reported by Coleman, *et al* (1989) for two trials in which NIR predictions of DM digestibilities and intakes of pen-fed cattle had  $R^2$  of 0.23 to 0.54 for DM digestibility and 0.55 for DM intake. These results may illustrate the difficulties in obtaining good predictions when the reference values are not reliable (markers were used to obtain the digestibility data in one trial) and when calibration equations are used in widely different situations.

Lyons, *et al* (1995) more successfully predicted diet protein and DOM contents with  $R^2 = 0.98$  and  $0.87$ , respectively, and SEP of 0.49 and 1.12 %, when they applied their earlier equations (Lyons and Stuth 1992) to four different native pastures and a ryegrass pasture.

In northern Australia, using mainly tropical pastures species, Coates (1989) derived NIR prediction equations for cattle with data obtained from grazing oesophageal fistulated steers or pen trials. SECV for diet protein content, DMD, and digestible DM intake were 0.18 %, 0.03, and 1.65 g/kg W.d<sup>-1</sup>, respectively.  $R^2$  for these variables were 0.95, 0.86, and 0.73. The values for protein and DDM are similar to those reported by North American workers. Interestingly, Coates (1989) was better able to predict DM digestibility from NIR spectroscopy than from the pepsin-cellulase *in vitro* method which predicted *in vivo* DMD poorly ( $R^2 = 0.64$ , bias = 0.308 %).

Prediction equations were developed for goats grazing a variety of Texan pasture species and types by Leite and Stuth (1995). Diet IVDOM and protein contents were predicted precisely and accurately ( $R^2 = 0.94$  and  $0.92$ , bias = 0.16 and 0.18 %, and SEP corrected for bias = 1.28 and 2.12 % for protein and IVDOM, respectively).

Nutritional physiology can also be predicted from faecal NIR spectra. Whitley and Stuth (1996, cited in Stuth and Tolleson 2000) predicted rumen degradable, bypass and indigestible protein fractions in cattle diets. Changes in NIR spectra can indicate digesta whole-tract passage rates. Walker, *et al* (1998) found that the prediction of diet leafy spurge content improved (e.g. for goats,  $R^2$  increased from 0.04 to 0.54 then fell to 0.15) when faeces collected at 48 h after feeding were examined, rather than those collected at either 24 or 72 h. Using a similar approach, Lyons, *et al* (1995) obtained best prediction of oesophageal extrusa protein content from faecal measurements made 72 h after the extrusa were collected. Lyons, *et al* (1993) showed that the NIR-predicted diet protein and IVDOM contents of their supplemented and unsupplemented cattle became similar at 40 to 60 h after the supplements were withdrawn.

Coates (1999) was able to predict the growth of *Bos taurus* × *indicus* heifers grazing a *Urochloa/Stylosanthes* pasture in north Queensland. His precision was relatively high ( $R^2 = 0.89$ , SECV = 1.16 g/kg W).

**Prediction of plant species composition in animal diets:** Diet species composition of goats and cattle grazing rangelands has been identified by NIR spectroscopy. Walker, *et al* (1998) successfully monitored the percentage of leafy spurge (*Euphorbia esula*) in sheep and goat diets, and reported substantially more accurate predictions from the best NIR equation ( $R^2 = 0.96$ , SEP = 4.75 %, 5.01, bias = -0.94, 0.73 %) than from microhistological examination of faeces ( $R^2 = 0.22$ , 0.32, SEP = 21.72 %, 20.23, bias = 7.15, 7.64 %). The



relative proportions of the  $^{12}\text{C}$  and  $^{13}\text{C}$  isotopes in faeces give information on the proportions of dicotyledonous and monocotyledonous plants in an animal's diet. Coates (1999) was able to predict quite accurately the proportions of *Stylosanthes hamata* (a legume) and *Urochloa mosambicensis* (a grass) in the diets of grazing cattle ( $R^2 = 0.96$ ,  $\text{SECV} = 0.82 \delta^{13}\text{C}$  units).

**Identification of gender, species, pregnancy and parasite burden:** NIR analysis of faeces has also been used to predict attributes unrelated to diet quality, but which give very useful animal management information. These include predictions of growth rate, pregnancy, intestinal parasite burden, animal species and gender.

Godfrey, *et al* (2001) were able to class ewes as pregnant or non-pregnant with 75 to 95 % success. Tolleson, *et al* (2001a) developed an equation which worked well with cattle grazing tropical forages but did not satisfactorily discriminate between pregnant and non-pregnant cows grazing temperate grasses. It seems that present NIR equations will identify animals in mid to late pregnancy but not those in early pregnancy (Tolleson, *et al* 2000, 2001b). Tolleson, *et al* (2000, 2001c, cited in Tolleson and Stuth 2002), Godfrey, *et al* (2001) and Osborn, *et al* (2002, cited in Tolleson and Stuth 2002) have successfully (70 to 100 % accuracy) identified bulls v. heifers, ovariectomised v. intact cows, rams v. ewes, and gender in red, white-tailed and fallow deer, and African elephants.

Bovine (cattle v. *Bison bison*) and cervine (*Odocoileus hemionus* v. *O. virginianus*, and *Cervus elaphus* v. *Dama dama*) species were successfully differentiated by Tolleson (unpublished, cited in Tolleson and Stuth 2002).

Tick burdens apparently cause unique changes in the faecal NIR spectrum (Tolleson and Stuth 2002) which allows the identification of animals at different stages of infestation. The NIR spectra of heifers in the pre-infestation and pre-attachment stages differed from those obtained when the ticks were attached. Predicted diet quality (contents of total protein, protein degradability fractions and DOM) differed between these periods (Tolleson, *et al* 2002).

It is remarkable that NIR-based relationships have been equally, or more, successful than those derived from conventional methodologies. While NIR spectroscopy ostensibly predicts the concentrations of analytes like N, the faecal NIR spectrum probably contains more information than is given by chemical determinations of specific analytes. Faeces is a complex mixture of undigested feed residues, microbial cells and endogenous substances (Dryden 1982; Lyons and Stuth 1992). This mixture may be more completely characterised by an NIR spectrum than by specific chemical analyses.

### **5.3. Faecal profiling using NIR technology**

Faecal profiling is the technique of regularly measuring the composition of animal faeces and from this, predicting aspects of diet quality and animal performance. The Grazingland Animal Nutrition Laboratory at Texas A&M University has provided a commercial faecal profiling service to beef cattle producers in North America since 1994 (Stuth and Tolleson 2000). The purpose of the service is to give producers a way of monitoring the quality of their pastures (especially protein and digestible OM contents) and so to identify periods of nutritional stress. User of the service provide faecal samples at regular intervals. From these, the GAN Lab predicts diet quality from a calibration data set of 300 to 600 diet faecal pairs obtained from 8 United States and 1 Canadian location, and encompassing warm- and cool-season plants (Stuth and Tolleson 2000). The predicted diet quality information is linked with

a nutritional modelling program (NUTBAL Pro) to predict animal performance (Stuth, *et al* 1999). Details of the service and the methods that producers use to collect and send faecal samples are in Stuth and Tolleson (2000).

The NIRS-NUTBAL Pro performance monitoring system predicted body condition of droughted cattle to within 0.5 score (Tolleson 2002). Eilers (1999) surveyed the adoption and effectiveness of the NIRS-NUTBAL Pro program throughout the United States. Four percent of ranchers had adopted the service within 5 years of its first commercial release. Users were satisfied with the service and most intended to continue with it. Use of the NIRS-NUTBAL Pro service increased producers' awareness of forage quality (86 % of cases), nutrient requirements of their animals (72 %), feed efficiency (56 %), and the timing of marketing (27 %). Animal performance, and the cost of supplementary feed, were positively affected. In 35 % of cases the annual cost of supplementary feed decreased, by an average of 15.5 %, and 34 % of producers who managed a breeding property reported increased returns of USD26.50/year per breeder mated.

In northern Australia, Coates (1999) found that NIR spectrometry was able to identify seasonal changes in the quality of diets eaten by weaner cattle grazing *Urochloa/Stylosanthes* pastures. Predictions about IVDMD and protein contents, and digestible DM intake, were consistent with the observed rainfall and changes in the pasture species composition. Cattle liveweight changes were also consistent with these NIR predictions.

Currency is important in any nutritional monitoring system. Expected changes in pasture condition should not be assumed to apply in all circumstances. For example, Coates (2000) showed that the protein content of diets eaten by cattle grazing a variety of tropical grasses on seven properties in north central Queensland varied from 8.1 % to 2.5 % during March to June, 1999, but that in the previous year diet protein contents peaked in January and did not fall below 8 % until July. Differences in pasture quality in different years are expected, but they have not been easy to identify in time for managers to take remedial action by providing N supplements or moving cattle to other pastures. Faecal profiling is recommended by Coates (2000) as a means of obtaining timely information on diet nutritive value, species composition, leading to improved decision-making. About 300 producers have adopted NIRS profiling in northern Australia (T. McCosker, personal communication). Coates (2000) also recognises some limitations of NIRS profiling. These include limited capacity to determine diet mineral contents (in a situation where P nutrition may be an important constraint on animal performance), limitations in the size of the calibration data set, and difficulties in monitoring the performance of NIRS predictions for parameters like growth and diet quality.

In regions which are subject to seasonal dry periods pastures, especially those based on tropical grasses, become N deficient. A commonly-used method of combating the adverse effects of pasture N deficiency is to give a N or protein supplement. Timing of the start of supplementation influences the costs and responses to the supplement. It has been shown (see references above) that NIR technology can assist producers to identify the best time to begin supplementation. However, this is not the same as assessing the effectiveness of supplementation (which includes assessing consumption rates and uniformity of consumption) or identifying when supplementation could stop. Lyons, *et al* (1993) made a first investigation into this problem by monitoring NIR predictions of diet protein and IVDOM contents of cattle grazing native Texan pasture and given a concentrate-based energy/protein supplement. They were able to identify diet protein content differences between the supplemented and unsupplemented cattle, and to detect when the effects of the supplement on

these predictions ceased after the supplement was withdrawn. The authors could not separate the direct effects of supplement consumption on the faecal spectra or the indirect effects which may have occurred if the supplement had altered diet selection behaviour. However, for both protein and IVDOM the lag after withdrawal of the supplement and the disappearance of effects on the predicted diet composition were similar (about 40 to 50 hours) to accepted whole-tract transit times for cattle.

NIRS measurements of pasture and silage quality have been used to give rapid and reliable predictions of the quality of intensively-managed, temperate pastures and silages. Cosgrove, *et al* (1998) used NIRS as a way of rapidly describing the stratification of nutrients in a dairy pasture. Such measurements will be valuable in developing decision systems for pasture-fed dairy cattle (e.g. Parker, *et al* 1995, cited in Cosgrove, *et al* 1998). Corson, *et al* (1999) describe the use of rapid NIRS measurements of forage nutritive value coupled with a dairy cow nutritional modelling system to provide decision support for New Zealand dairy farmers.

#### **5.4. Application of NIR analysis to monitoring the performance of grazing deer**

**NIR prediction equations:** Showers (1997) developed equations based on faecal NIR spectra to monitor the nutritional status of deer. He obtained equations which predicted dietary protein ( $R^2 = 0.94$ ,  $b = 0.905$ ,  $SEP = 0.87\%$ ,  $bias = -0.5\%$ ), phosphorus ( $R^2 = 0.91$ ,  $b = 0.93$ ,  $SEP = 0.02\%$ ,  $bias = 0\%$ ) and digestible OM ( $R^2 = 0.85$ ,  $b = 0.89$ ,  $SEP = 2.89\%$ ,  $bias = -2.2\%$ ). Tests of the stability of these equations when applied to different geographical and animal species showed that the protein and digestible OM could be applied to mule deer as well as white-tailed deer, and in several different localities in Texas and Oregon.

**Faecal sampling methods:** Faecal samples must be representative of the mass of faeces voided by the animals under test. The questions which must be addressed here are that the samples (1) encompass the full range of animal responses, (2) provide information on the range of animal responses range, (3) accurately predict the mean response, and (4) are free from bias associated with the time of sample collection. It is probable that the first requirement is situation-specific, and sampling regimes must be devised and tested for each of these. The second and third requirements can be obtained by analysing each individual sample and calculating the mean and a measure of dispersion. Analytical economy can be obtained by compositing (pooling) all samples and conducting a single analysis. Although NIR spectroscopy is rapid and simple, commercial analyses can be quite expensive and the economies allowed by sample pooling may be worthwhile. Jenks, *et al* (1989) demonstrated with white tailed deer faeces that pooling a number of samples gave the same mean value as analysing each sample separately, provided that each sample was represented equally in the pooled material. They pooled between 12 and 19 samples collected at each of 4 localities, and the standard error of the mean of the 4 localities was a good estimate of the standard error calculated from analysing all samples individually. Osborn and Jenks (1998) also reported similar means and standard errors for faecal N and P contents obtained from analyses of pooled and individual samples. It may be important for farmers to know the range of animal responses as well as the mean. The data reported by Jenks, *et al* (1989) suggest that pooling samples within paddocks would give a satisfactory estimation of both the mean and the standard error for a response, provided that the animals and pastures were similar. Lyons, *et al* (1993) observed a treatment  $\times$  day  $\times$  sampling hour interaction when they predicted diet

protein and digestible OM contents by NIRS. This shows that faecal samples should be collected over several different days and composited before they are sent for analysis.

Deer faecal pellets resist weathering. Even when exposed to temperatures between 2 and 31 °C, and rainfall of up to 5 mm on half of the test days (although covered against most of the rain), whole faecal pellets which remained had N, ADF and NDF contents after 24 days which were not significantly different from concentrations determined immediately after voiding (Jenks, *et al* 1990). Leite and Stuth (1994) reported a similar stability in diet protein and digestible OM contents predicted by NIR spectroscopy from goat faeces which were exposed for up to 7 days (but protected from rainfall) in winter, spring and summer (temperatures 2 to 24, 3 to 29, 23 to 36 °C, respectively).

The factors which will influence the apparent composition of faecal samples are sampling technique (i.e. obtaining a representative sample), sample stability during delivery to the laboratory (i.e. elapsed time and method of preservation during delivery), and sample water content and particle size.

Pearce, *et al* (1993) showed that predictions of cattle diet protein and digestible OM contents remained stable for up to 12 days of sample storage, and the changes (positive in the case of predicted diet protein, negative for DOM) although statistically significant, were small (5 and 1 %, respectively). On the other hand, Coates (unpublished, cited in Coates 1998) found that the NIR predictions may change when samples are transported for long periods under tropical conditions.

Post-receival processing may not be important provided that the ultimate particle size and water content are standardised. However, if water content is not standardised, e.g. by allowing rehydration through exposure to ambient humidity (e.g. Baker, *et al* 1994), predictions of diet protein and IVDMD fluctuate widely. Lyons and Stuth (1991) recorded increases in predicted diet protein and IVDMD contents of 47 and 7 % respectively. These variations are clearly biologically as well as statistically significant.

## 6. Conclusions and Recommendations

NIR has been used over the last forty years to analyse accurately (i.e. with standard errors of prediction not more than twice the laboratory standard errors of reference methods) protein, cell wall constituents, and other organic components in animal foods. It is rapid, non-destructive, and non-polluting.

NIR spectral information can not be used to determine analyte concentrations directly because of the way in which near infrared radiation passes into, through, and is reflected from, the sample. Rather, analyte concentrations, or other characteristics of the sample, are predicted from relationships which have been developed between reflectance and reference data, i.e. from prediction equations. Robust prediction equations are based on calibration data sets which encompass the range of sample characteristics expected to be encountered when the equation is used. Appropriate mathematical techniques (e.g. smoothing and derivatisation) should be used, and the samples must be uniform in particle size and water content.

“Universal” equations have been developed to predict the nutrient composition of a wide range of foods of that type. There are several examples of European universal equations for grains and forages, and an equation for Australian mixed temperate pasture. It may be necessary to calculate “local corrections” before universal equations are used in any new context.

When properly calibrated, NIR spectroscopy predicts protein contents with great accuracy. Other constituents are predicted less precisely, although the standard errors of prediction are similar to the standard errors of duplicate laboratory determinations. NIR spectroscopy is used successfully with both concentrate and forage foods. NIR information is obtained from the interactions of near infrared radiation with chemical bonds between non-mineral elements and so does not always accurately predict food mineral contents. NIR methods predict *in vitro* digestibility accurately and precisely, and can predict *in vivo* digestibility at least as well as conventional “wet chemistry” methods such as *in vitro* digestion or the pepsin-cellulase method, and much more rapidly. DM intake can also be predicted, although with less precision than chemical composition or digestibility.

Faecal indices have been used to monitor the nutritional status of grazing animals, and have been often used to monitor wild deer in North America. Faecal indices determined by wet chemistry have given mixed success, but substantially better results have been obtained with NIR spectroscopy. NIR spectroscopy may measure characteristics of faeces which integrate several different aspects of faecal chemistry, while wet chemical analyses focus on single entities.

NIR technology has been used to routinely monitor (through analysis of faecal samples) the nutritional status of cattle, and appears to have potential for identifying tick infestation, pregnancy, gender and animal species. Nutritional status data obtained by NIR analysis of grazing cattle faeces is used as an input to the NUTBAL Pro expert system for North American ranchers. The combination of NIR analysis and nutritional profiling with the NUTBAL Pro program has improved yearly economic returns to American cattle ranchers by up to USD26.50 per cow mated. These results, the preliminary evidence from similar attempts in northern Australia, and preliminary results of a NIR-based nutritional profiling

program for deer in Texas, suggest that a similar technology could be developed to monitor the nutritional status of deer herds and predict the performance of farmed deer.

**Recommendation:** NIR spectroscopy should be developed as a tool for the Australian deer farming industry. This should include development of methods to rapidly analyse deer foods and for faecal profiling. An NIR-based nutritional expert system should be developed to give information which is timely and relevant to individual deer farms.

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