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Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy

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Abstract

The advent of NIRS technology for assessing quality of plant/forage/feed tissue and predicting diet quality from feces offers livestock nutritionists, researchers, farm advisors and resource consultants a rapid mechanism to acquire nutritional information. The portability and low long-term maintenance costs of this technology coupled with rapid turn around time on processing offers a mechanism for nutrition programs to address forage and dietary quality issues that were limited due to high maintenance costs of wet chemistry laboratories. The fundamentals of NIRS technology and associated development of calibration equations are discussed along with methods to validate equations. Direct methods for tissue analysis and indirect methods to predict diet quality from feces are reviewed for major constituents found in forages, including crude protein, digestibility, tannins and minerals.

Keywords: NIRS; Fecal; Calibration; Protein; Digestibility; Minerals

1. Introduction

Animal nutritionists have long recognized the importance of measuring nutritive value in feeds and forages provided to livestock. Various analytical chemistry techniques to quantify such forage quality parameters as protein, energy, or mineral content are well established, though sometimes costly and time consuming. The characterization of feeds or forages fed by a human to an animal is thus relatively simple. More problematic, however, is the task of quantifying the nutritional value of the diet obtained by a grazing animal.

The increased power and widespread use of personal computers and the concomitant development of multivariate statistical procedures in the field of chemometrics have resulted in an increased use of near infrared reflectance spectroscopy (NIRS) as an alternative to traditional analytical methods for determining nutritive value of forages. The NIRS technique is rapid, and does not usually require labor-intensive sample processing, allowing for large-scale sampling. Timely decision making on strategic use of nutritional supplements or adjustments in ration formulation to efficiently sustain milk, meat, or fiber production is also facilitated by this method. Although development of an NIRS laboratory entails significant initial start-up costs, it is relatively inexpensive in the long term. There are other advantages to NIRS over conventional laboratory analytical methods, namely: (1) it is non-destructive; (2) requires no reagents; and (3) allows for the determination of multiple values (e.g. crude protein (CP), digestible organic matter (DOM),

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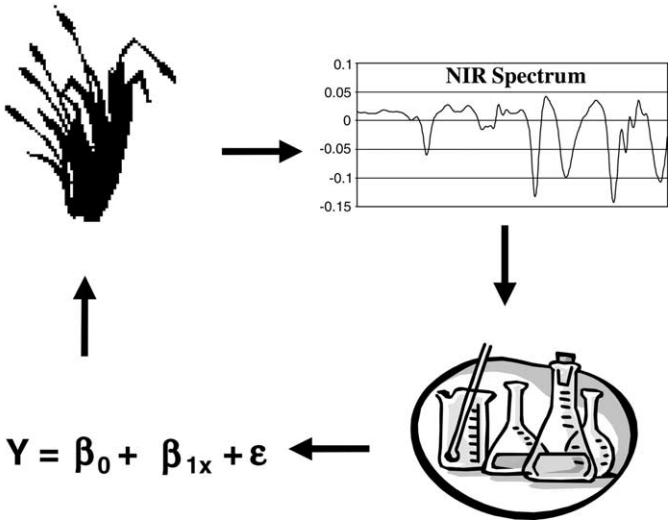

Fig. 1. Traditional direct method of applying NIRS is to analyze a particular product with both NIRS and traditional wet chemistry and pair this information in the calibration set to calculate a predictive equation. We can then use only the NIRS and bypass the need for wet chemistry. NIRS if usually faster, less expensive, and is non-destructive.

acid detergent fiber (ADF), and neutral detergent fiber (NDF) etc.) in a single analytical procedure.

Direct determination of a constituent by obtaining both the near infrared (NIR) spectrum and a traditional chemical analysis on a number of individual samples, and then using this information to develop a predictive equation, is the manner in which NIRS is most often used to quantify forage quality (Fig. 1). Indirect measurements, i.e. obtaining spectra from one material and chemically analyzing the content of a byproduct, or even a precursor, of that material are also possible. For instance, end products of digestion or fermentation can be scanned by NIRS and the primary constituents that contributed to the formation of that product can be predicted. This method is employed in the determination of grazing animal diet quality via NIRS of feces (Fig. 2). A discussion of both approaches, as related to determination of forage and diet quality for livestock, is the objective of this paper.

2. The electromagnetic spectrum

The electromagnetic spectrum (EMS) consists of photons of different energies which, because of these differences, interact with matter in very different ways. For example, higher energy photons cause electron shifts, while lower energy photons result in molecular vibrations (Birth and Hecht, 1987; Murray and Williams, 1987). The EMS ranges from short (<1.0 pm), high frequency (10^{20} to 10^{24} Hz) gamma-rays to long (>1.0 mm), low frequency (<3×10^{11} Hz), radio waves. The visible spectrum resides in the center of this range extending from 400 to 700 nm and is the most biologically significant as it supplies the energy to support life on this planet.

White light is made up of all colors, so the human eye perceives a substance to be blue because that substance absorbs all but the wavelengths corresponding to blue. Structures called cones in the retina detect this reflected light and send the information to the brain. An NIR spectrophotometer works in a similar fashion.

Just outside the red band in the visible region of the EMS is the infrared region, of which, the NIR is immediately adjacent (800–2500 nm, 10^4 to 4×10^{14} Hz). Infrared (IR) light is emitted by the sun and absorbed by all biological compounds but is not visible to the human eye. Chemical bonds in organic molecules vibrate, stretch, and bend at frequencies similar to the vibrations of the EMS found in the IR region when struck by IR light. Wavelengths which correspond to the frequency at which a particular bond is vibrating are absorbed, others are reflected. The NIR spectrometer projects a known quantity of NIR light onto a

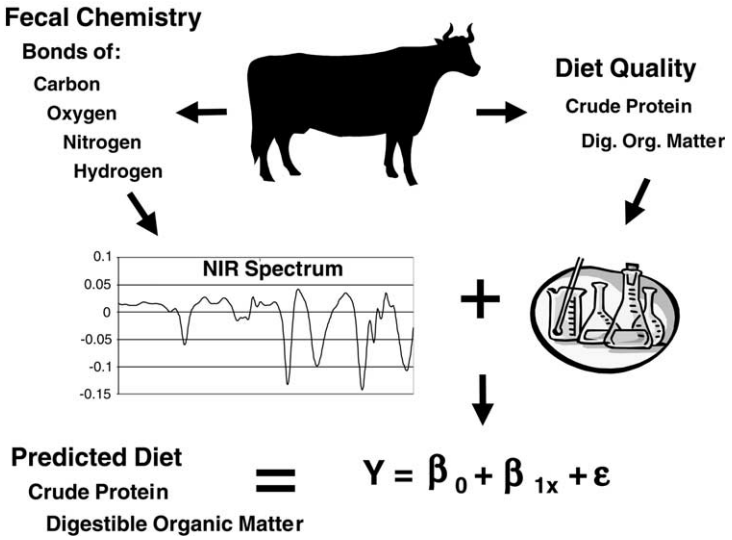

Fig. 2. Over the past 12 years, indirect methods for estimation of diet quality from fecal NIRS scans using diet:fecal pairs of known diet quality and spectral characteristics of the associated feces has allowed estimation of the diet of the animal in free-ranging conditions.

substance and then records the reflectance from that substance, storing the information in a computer. The stretching and bending of primarily CH, NH, OH, CO, and CC bonds as a result of the interaction between this radiation and a biological material yields an abundance of chemical information about that material. In addition to chemical features of a substance, physical attributes, notably particle size, also affect NIR spectra by creating "scatter". Scatter is the dispersion of reflected light from the surface of sample particles without penetrating the sample, and can be a significant portion of the observed variation in NIR spectra. Therefore, scattered light contains no information concerning the chemistry of the sample, but may have implications of the physics related to particle size. It should be noted, however, that particle size and shape can be an indictor of chemical characteristics due to the interrelationships between such parameters as lignification, stage of maturity, and the grinding process. This spectral information can then be used to create calibration data and develop predictive equations.

3. Creation of calibration equations

Calibration is the process of creating a spectrochemical prediction model (Shenk and Westerhaus, 1996). In essence, the process relates chemical information contained in the spectral properties of a substance to chemical (or physical) information revealed by reference laboratory methods. The goal is to derive a predictive equation such that the user can quantify the constituent of interest using NIRS alone, bypassing the laboratory reference method. Chemometrics provides the vehicle to efficiently accomplish this task. Chemometrics exploits the volume and speed of data handling by computers to extract useful chemical information from samples using sophisticated mathematical and statistical methods (Geladi, 1996). A detailed accounting of the calibration process is beyond the scope of this manuscript. What follows is an attempt to familiarize the reader with the concept. For a more complete narrative, the authors suggest Shenk and Westerhaus (1994).

Calibration consists of both physical and electronic steps. The process begins with obtaining a sample set of the desired material, i.e. hay, silage, or feces. The calibration set should be well distributed, representing the range of expected variation in the constituent of interest. This variation could be (1) temporal, e.g. date or time of collection; (2) spatial, e.g. range site or geographic location; or (3) biological, e.g. cultivar or stage of growth.

Processing of the calibration set for NIRS should be consistent with the methods to be used in routine

analysis. Drying and grinding procedures are especially important due to the fact that water is a strong absorber of NIR light and, particle size affects the shape of the spectrum. The conditions under which samples are scanned (NIR spectra obtained) should also be as uniform as possible with respect to ambient temperature. As with particle size, temperature affects the shape of the spectra, shifting the expression of absorption peaks, possibly altering the interpretation of a spectrum.

Once spectra and laboratory reference data are acquired and matched, mathematical and statistical procedures are performed. Data pre-treatment steps such as multiplicative scatter corrections (Martens and Naes, 1991) or detrending and standard normal variate transformations (Barnes et al., 1989) reduce the effect of particle size (scatter) on the calibration set. The effects of baseline shifts and overlapping absorption bands can be removed by calculating derivatives of the spectra (Hruschka, 1987). There have been many multivariate regression procedures developed and applied to spectral data. Two general categories involve either utilizing data from (1) individual wavelengths, usually by stepwise regression; or (2) the full spectrum (or any user defined segment), by the use of some form of principal components (Shenk and Westerhaus, 1991).

4. Validation and monitoring

Equation validation is conducted to assess the predictive ability of the selected calibration equation. Validation entails prediction of either an independent set of samples, i.e. from a different population than the calibration set, with known reference values, or removing a certain number of samples from the calibration set, and not using them in the calibration process. The standard error of prediction (SEP) is used to judge the predictive ability of a calibration equation. As with SEC, this should be as small as possible and similar to the SEC. Cross validation, whereby each sample or a pre-determined proportion of samples, is sequentially removed from the calibration set and predicted by an equation developed with the remaining samples is another method of evaluating equation performance. This method has been described as the single best estimate of the predictive capability of an NIRS equation (Shenk and Westerhaus, 1996). If the defined population represents the expected diversity (species, season, quality, etc.) in diet experienced by the animals in question, then cross validation would legitimately be the ''best'' validation. We know in real life that NIRS predictive equations are always a ''work in progress'', so any point in time validation, of any equation, no matter how robust, could be improved upon at a later date. What is reported is the best at that point in time. Slope and bias are also important considerations in evaluating equation performance (Williams, 1987), systematic over or under prediction of reference values should be avoided. Monitoring and quality control are needed to check both the accuracy of the instrument and the calibration.

Monitoring can be accomplished by tracking the global H values as new samples are predicted, identifying samples which should be added to the equation. In the case of fecal equations to predict diet quality, this is not that simple. Creating new diet:fecal pairs is costly and time consuming. So monitoring is still carried out, but cannot be responded to as quickly or easily as with direct predictions of forage tissue. Fecal samples with problematic predictions require that diets identified for a given condition, find a cooperator to collect forage or feed the diets, or sample with fistulated animals, etc.; standardize lab procedures; then add the new diet:fecal pairs to existing equations.

Monitoring quality control on the instrument is done with the daily diagnostics and check cell reading to ensure that the machine reads consistently. Instrument response, repeatability and wavelength alignment tests should be run at least two times a week as per manufacturer instructions.

5. Direct tissue analysis with NIRS

An extensive review of direct tissue analysis has been provided by Roberts et al. (2003) which is part of a larger book on the use of NIRS technology in agriculture. The reader is urged to review this landmark document if they pursue the use of NIRS technology in their research. This section represents key excerpts from Roberts et al. (2003) more complete handling of the subject. NIRS has been successfully used to predict the nutritive value of forages and hays through direct scanning of the forage samples or the

extrusa obtained from esophageally fistulated animals (Holechek et al., 1982; Volesky and Coleman, 1996). NIRS has also been used in the analysis of anti-nutritive factors in forage (Windham et al., 1988; Roberts et al., 1993; Goodchild et al., 1998).

5.1. Ash content

Ash content represents the minerals both available and unavailable to the animal. Ash measurements by NIR has occurred for common hay crops such as alfalfa (*Medicago sativa* L.) (Redshaw et al., 1986; Reeves, 1988a,b; Windham et al., 1991) and timothy (*Phelum pratense* L.) (Redshaw et al., 1986). Pasture and range crops such as smooth bromegrass (*Bromus inermis* Leyss) (Reich and Casler, 1985; Redshaw et al., 1986), reed canarygrass (*Phalaris arundinacea* L.) (Redshaw et al., 1986), and meadow foxtail (*Alopecurus pratensis* L.) (Redshaw et al., 1986) have also been successfully measured with NIRS. Vazquez de Aldana (1996) used NIRS to determine the ash content in pasture samples collected from different sites in a semi-arid grassland communities with a standard error of calibration (SEC) of 4.6 g kg^{-1} and R^2 of 0.88. The standard error of prediction (SEP) was 5.1 g kg^{-1} and R^2 was 0.94.

5.2. Protein, nitrogen, and related compounds

Total nitrogen (N) or crude protein (usually N × 6.25) is one of the most commonly measured components of forages and feedstuffs. R^2 values of 0.95 or higher and standard errors well within lab errors have been reported. Strong –N–H absorptions in the NIR region are the primary cause for these good relationships. The relatively high concentrations of crude protein, which in forages and feeds can range from 30 to 500 g kg^{-1} DM, is also another contributing factor (Roberts et al., 2003).

5.3. Fiber and lignin

Next to the nitrogen components, the most common constituent to be estimated by NIRS in forages/crop residues is fiber. Roberts et al. (2003) summarized a large variety of studies on forage resources, including pasture, hay, native herbage, straw, digested residues and silage that have successfully developed NIRS calibration equations for fiber. Both acid detergent fiber (ADF) and neutral detergent fiber (NDF) are the commonly reported fiber components. Although NDF is a ''property'' of forages and not a constituent, it can still be estimated due to variations in –C–H and –O–H bonds in the range from 300 to 800 g kg^{-1} DM. Lignin calibrations have been reported mostly for grasses and legume species common to hay and pasture in sub-tropical regions (Reeves, 1988a,b; Jenisch et al., 1994). Precision in estimation is generally lower than protein or ADF (Reeves, 1988b).

5.4. Lipids/ether extract

Measurement of lipids with NIRS is less common. NIR can be used because of the characteristic aliphatic –CH absorptions seen at around 2310 and 1725 nm, with weaker overtone bands at 1400 and 1210 nm. Forage measures of lipids have had mixed results (Amari and Abe, 1997; Berardo et al., 1997; Park et al., 1998), primarily because forage contains relatively small concentrations with low variance leading to poorer correlations.

5.5. Minerals

Analysis of mineral concentration with NIRS has produced mixed results since minerals do not absorb in the near infrared region and they are low in concentration in the tissue. However, their detection by NIR can be possible due to complexes formed with organic compounds, many of which vary among species (Clark et al., 1987). Low levels and narrow ranges in forage plants also hamper the estimation of minerals by NIR. Because of this narrow range, some authors argue that mineral equations should be evaluated by coefficient of variation (CV) rather than R^2, as the narrow range in concentration could render R^2 values misleading (Roberts et al., 2003).

Initial attempts to estimate minerals by NIR spectroscopy were focused on Ca, P, Mg, K, Cu, Zn, Mn, Na, and Fe in crested wheatgrass and alfalfa (Clark et al., 1987). Of these, calibrations only for the macro elements Ca, P, Mg and K were acceptable. Flinn et al. (1996) reported similar results. Follow-up studies attempted calibrations for Ba, Li, Mo, Ni, Pb, V, Al, S, Si, and Se in tall fescue, crested wheatgrass, and alfalfa (Clark et al., 1989). Researchers in these

studies reported acceptable equations for only Li and V in tall fescue, but not in the other two forages.

5.6. Digestibility

Digestibility is a functional component of plant tissue that reflects the nutritive value to the animal and significantly influences intake levels of ruminants. Like protein, digestibility of plant tissue is commonly measured with NIR spectroscopy and is associated with absorptions in the region of 2270 nm corresponding to cellulose and lignin, as well as the aromatic –CH first overtone band at around 1670 nm unique to lignin (Murray, 1990). Digestibility calibrations are based on a diverse set of reference procedures, including both in vivo and in vitro techniques (Jones and Hayward, 1975; Clarke et al., 1982; De Boever et al., 1986, 1994, 1996; Reeves, 1988a,b; Barber et al., 1990; Dhillon et al., 1990; Bughara et al., 1992; Dardenne et al., 1993; Baker et al., 1994; Albanell et al., 1995, 1997; Park et al., 1997, 1998), creating a problem of cross transferability of samples between labs. Digestibility calibrations can be sensitive to residual moisture in samples (Baker et al., 1994; Griggs et al., 1999). Equations developed with samples containing moisture of less than 80 g kg^{-1} DM have been more accurate than those with higher moisture.

There is increasing interest in direct calibration of NIR against in vivo digestibility values for forages (Norris et al., 1976; Barber et al., 1990; Baker et al., 1994; Park et al., 1997, 1998; Flinn et al., 2000). This has the advantage of eliminating intermediate laboratory techniques, but requires data from a large number of in vivo digestion trials conducted under standardized conditions.

Because digestibility is a property of forage or feed and not a chemical parameter, prediction by NIRS can be more difficult. To measure digestibility animals must be fed (in vivo) or digestion simulated (in vitro), introducing an ‘‘animal factor’’ into the calibration process. The additional error must be overcome by feeding enough different animals to incorporate the variability or, due to previously mentioned logistic problems, accept lower equation performance. It becomes a question of cost:benefit of reducing error. Essentially, digestibility is a characteristic, while protein is a chemical entity. Hence, NIRS prediction of digestibility represents a prediction of a predicted component of feed.

5.7. Anti-quality components

In addition to its use in forage quality analysis, NIR spectroscopy is also used in analysis of anti-quality components. Some anti-quality components are measured as a class of compounds, while others are quantified as single compounds.

One class of anti-quality components predicted by NIR spectroscopy is total alkaloid concentration in poisonous plants with $R^2 > 0.90$ (Clark et al., 1987; Roberts et al., 1997). In these studies, concentrations were low, and calibrations were probably successful because: (1) alkaloid(s) occurred in wide ranges and reference data were collected in triplicate; and (2) alkaloids have strong, specific absorbers.

Relative condensed tannins have been quantified in *Leucaena* forage tree (Wheeler et al., 1996), sericea lespedeza (*Lespedeza cuneata* [Dum.-Cours. (G. Don)] (Windham et al., 1988; Petersen et al., 1991), and birdsfoot trefoil (*Lotus corniculatus* L.) (Roberts et al., 1993). Sample preparation can have a profound effect on determination of tannin by reference methods, which is also translated to NIRS through the correlation procedures (Petersen et al., 1991). Total phenolics (expressed as tannic acid equivalents) were also estimated in the fodder shrub tagasaste (*Chamaecytisus proliferus*) by NIR with an SEC of 12.4 and R^2 of 0.96 (range 14–254 g kg^{-1}) (Flinn et al., 1996; Goodchild et al., 1998).

6. Indirect methods of measuring forage quality: fecal profiling

Lyons and Stuth (1992) pioneered the application of NIRS technology to indirectly predict the quality of forage ingested by free-ranging animals via fecal scans. As diet chemistry changes, the by-products of digestion (plant residue, microbial bodies, secondary metabolites, slough tissue, etc.) also change. The behavior of these secondary products in the feces may be related to the characteristics of the primary product (i.e. ingested diet).

Fig. 3 illustrates that certain regions of the representative fecal spectra have obvious differences due to diet quality. In general, NIRS fecal calibration equations have identified approximately 8–12 wavelengths explaining 85–95% of the variation in diet quality with

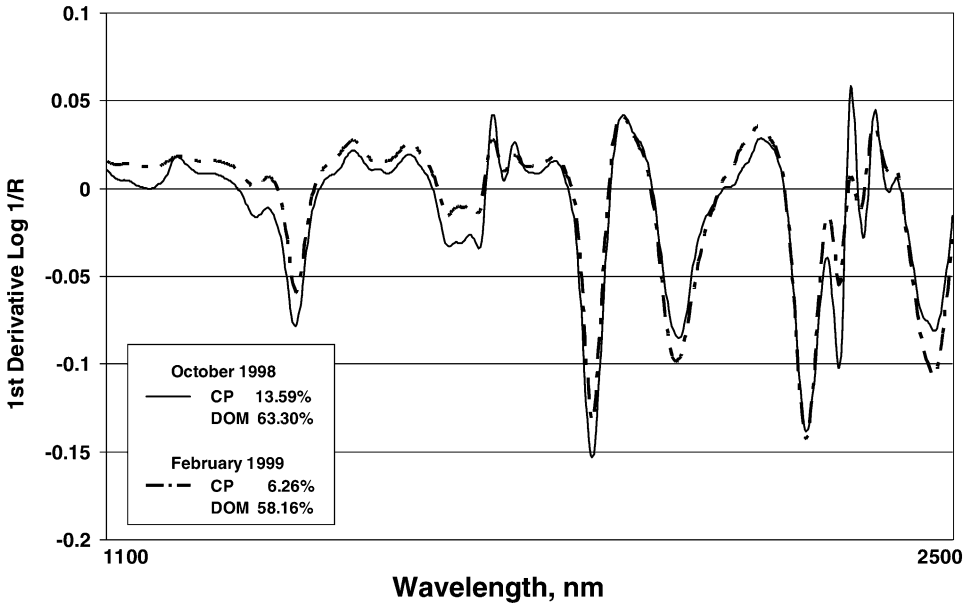

Fig. 3. Comparison of fecal NIRS spectra from animals grazing a coastal bermudagrass pasture of high and low diet quality.

standard errors well within the range of traditional laboratory techniques for either esophageal or stall-fed animals.

Calibration equations are developed by the creation of diet:fecal pairs. In this process, diet samples are collected from either esophageal fistulated or stall-fed animals. Fecal samples from intact animals are paired with twice-daily esophageal collections from a minimum of two animals over a 3-day period to create a single diet:fecal pair for a pasture/paddock. If stall-fed, the animals are offered an array of complex diets and diets of single forage to create the variability across a variety of species and maturity stages. The goal is to create a wide variety of signals and background noise for the math treatments to sort through and isolate those spectra most important to prediction of the parameter of interest. Normally, a wide variety of species are fed over 7 days for deer, elk, goats and donkeys, 10 days for sheep and 14 days for cattle. Fecal samples are then collected on the last 2 days in each case. The resulting fecal NIR spectra are paired with the known dietary nutritional quality (CP, DOM, DMD, P, etc.) and subjected to standard calibration techniques (Shenk and Westerhaus, 1994). Once an equation is developed, it is validated by predicting other fecal samples from animals with known diet quality, which were not used in the calibration process.

Robust equation development requires inclusion of a broad spectrum of plant species representing the diversity of temporal, spatial, species, environmental and landscape conditions that the animals in question would experience. Dietary items such as evergreen species with high volatile oils, or high tannin shrubs, bark, fine twigs, lush cool-season grasses, early/mid/late maturity warm-season grasses, high quality legumes, hays, crop residues, grains, mast, fungi, and (or) animal by-products should be included.

The validity and usefulness of fecal NIRS profiling to determine the nutritional status of animals has been demonstrated for different purposes and different livestock and wildlife species (Table 1). Lyons and Stuth (1992) were able to successfully obtain predictive equations for DOM and CP ($R^2 = 0.80$ and 0.92), respectively. An SEC and SEV (standard error of validation) of 1.66 and 1.65 for DOM which were comparable to an SEL of 1.68. An SEC and SEV of 0.89 and 0.92, respectively, were acceptable for an SEL of 0.44 for CP. No effects of physiological stage of animals on calibration were noted. Lyons et al. (1993) found that supplementation did not affect prediction of the diet quality of the base range forage

Table 1
Expected error reported by authors involved in development of calibration of NIRS fecal profiling equations for predicting dietary crude protein and digestibility of ruminants

Reference	Species	Crude protein		Digestibility		
		R^2	SEC	Units	R^2	SEC
Lyons et al. (1992)	Cattle	0.64	0.88	In vitro DOM	0.69	1.66
Lyons et al. (1992)	Cattle	0.92	0.89	In vitro DOM	0.80	1.75
Leite and Stuth (1995)	Goats	0.94	1.12	In vitro DOM	0.93	2.02
Purnomoadi et al. (1996)	Cattle	0.98	0.70	–	–	–
Showers (1997)	Deer	0.94	0.70	In vitro DOM	0.89	2.64
Coates (1998)	Cattle	0.99	0.54	In vivo DMD	0.89	2.50
Coates (1998)	Cattle	–	–	In vitro DMD	0.97	2.20
Ossiya (1999)	Cattle	0.88	0.85	In vitro DOM	0.83	3.39
Ossiya (1999)	Cattle	–	–	In vivo DOM	0.89	1.82
Krachounov et al. (2000)	Sheep	–	–	In vivo DMD	0.94	2.26
Gibbs et al. (2002)	Cattle	0.99	1.28	In vitro DMD	0.87	2.63
Awuma (2003)	Cattle	0.95	0.87	In vitro DOM	0.90	3.02
Awuma (2003)	Sheep	0.97	0.78	In vitro DOM	0.94	2.26
Awuma (2003)	Goats	0.97	0.79	In vitro DOM	0.95	2.86
Awuma (2003)	Cattle	0.93	0.77	In vitro DOM	0.90	1.90
Awuma (2003)	Goats	0.97	0.72	In vitro DOM	0.95	2.44
Keating (in press)	Elk	0.95	1.13	In vitro DOM	0.74	1.81

by fecal NIRS profiling if fecal sampling occurred 36 and 56 h after supplemental feeding had ceased for CP and DOM, respectively. Recent work by Gibbs et al. (2002) indicated that fecal NIRS profiling can be developed to distinguish forage quality and total diet quality of animals fed forage resources of varying quality and concentrates of different amounts.

Coates (1998) examined the application of fecal NIRS profiling for predicting diet quality of cattle in the tropical region of North Australia under both grazing and stall-fed conditions. He found that stall experiments gave better calibration statistics than esophageal grazing experiments (nitrogen stall: $R^2 = 0.99$, SEP = 0.087; esophageal: $R^2 = 0.94$, SEP = 0.133; digestible organic matter: $R^2 = 0.97$, SEP = 2.2; esophageal: $R^2 = 0.80$, SEP = 0.33). He suggested that the difference might reflect the greater accuracy in derivation of reference values for stall experiments. However, a combination of stall and grazing experiments still gave satisfactory calibration equations for practical application in ranching situations. As with other authors (Lyons and Stuth, 1992; Leite and Stuth, 1995; Showers, 1997), Coates (1998) found better predictive statistics for CP than DOM.

With stall-fed forages, Coates (1998) was better able to predict in vitro digestibility ($R^2 = 0.97$) than that obtained in vivo digestibility ($R^2 = 0.89$). He suggested that this was due to the fact that in vivo values incorporated animal variation in this limited dataset. In vitro digestibility was easier to predict in this case than in vivo digestibility because of the greater amount of explained error within in vitro measurements. Coates (1998) also found that neither in vitro values of feed nor extrusa were closely related to in vivo values ($R^2 = 0.73$ (extrusa) compared to $R^2 = 0.64$ (feed)). These results indicate that estimates of in vivo digestibility derived from in vitro analysis of tropical forages may not be accurate using the techniques of Coates (1998).

Coates (1998) also developed equations to predict intake by NIRS fecal analysis with better statistics ($R^2 = 0.79$) than Lyons (1990) ($R^2 = 0.67$). However, in vivo digestibility was also not well correlated to forage intake. Lyons (1990) was unsuccessful in predicting intake by NIRS fecal analysis when intake was estimated with a marker (ytterbium acetate). However, Flinn et al. (1992) successfully calibrated for intake determined with fecal alkanes as a marker. Perhaps the most significant results of Coates (1998) were the successful prediction of digestible dry matter intake (DDMI, g/kg LW) ($R^2 = 0.89$) which integrates digestibility and intake to give a measure of

digestible energy, a measure closely related to animal performance (Lippke, 1980). Fecal material derived from cattle grazing in *Acacia senegal* and *Commiphera* sp. savanna rangelands in southern Kenya, *Acacia* spp./*Panicum maximum* rangelands in southwestern Uganda, *Acacia tortillas*/*Penisetum* spp. savanna in Ethiopia and *A. tortillas*/*Themeda-Digitaria* spp. savanna in Tanzania indicate the potential of the USA equation developed by Stuth et al. (1999) for predicting diets in these tropical regions (Stuth, 1999). Subsequent work by Ossiya (1999) and Awuma (2003) has shown that species chemistry and metabolic end products in the feces of animals in West Africa require additional diet:fecal pairs to allow the USA derived equation to work effectively in that environment.

Purnomoadi et al. (1996, 1997), working with dairy cattle, used fecal NIRS analysis in a secondary mode to predict the chemical composition of feces, and then to estimate digestibility based on a lignin indicator method. Chemical analysis of the lignin indicator was determined both by conventional laboratory procedures (Purnomoadi et al., 1996) and by NIRS prediction. Separate calibrations equations for feed and fecal samples were developed to predict the concentration of lignin. The in vivo digestibility values were more closely related to the lignin laboratory estimates than to those obtained by NIRS. The reason is that the NIRS method incorporated errors of both the lignin: in vivo relationship as well as the NIR: reference method error. These data point out the problems associated with using NIRS to predict imprecise chemistry values which are later used to predict diet nutritional characteristics. Purnomoadi et al. (1996) suggested that the differences between the lignin indicator methods and the in vivo method was related to the fact that lignin was partially digestible. They concluded that digestion estimation by NIRS shows the potential for individual and routine measurement on a practical farm basis, provided some correction factors or equations are devised to minimize the difference between in vivo and that estimated by NIRS. The method requires double NIRS calibrations for both the feeds and the fecal samples, and a reliable indicator to estimate digestibility.

Recently, Krachounov et al. (2000) developed a calibration equation for predicting in vivo dry matter digestibility for sheep based on an array of stall-fed forages ($n = 119$) in Germany and Bulgaria. The SEC for in vivo dry matter digestibility SEC = 2.26, and $R^2 = 0.94$. Application of cattle fecal profiling equations developed by Lyons and Stuth (1992) for cattle were unsuccessful for goats, indicating that goat feces are biochemically different from cattle feces. However, Leite and Stuth (1995) successfully formulated separate equations to predict CP and DOM in goat diets and suggested that the differences in digestive physiology and diet selection may result in spectral diversity precluding prediction across animal species. Kadine (personal communication) is currently developing calibration equations to predict dietary crude protein and in vivo digestible organic matter donkeys.

Showers (1997) working with white-tailed deer and with rocky mountain elk demonstrated the feasibility of using fecal NIRS profiling for predicting the nutritional status of wildlife, a breakthrough that could have implications for habitat management. As discussed previously, NIRS has been utilized to directly determine tannin concentration in forages (Windham et al., 1988; Roberts et al., 1993; Goodchild et al., 1998).

Ossiya (1999) employed the indirect method and used NIRS of feces to develop a predictive equation ($R^2 = 0.91$, SEC = 9.02 g/kg) for dietary tannin content in sheep. Validation of this equation with an independent set of samples, however, yielded only moderately successful ($R^2 = 0.61$) results. Additionally, Tolleson et al. (2000) successfully discriminated between three levels of dietary tannin content in white-tailed deer with fecal NIRS. Using NIRS for determination of dietary tannin content shows promise, but needs to be tested more thoroughly.

Feces are subjected to individual animal variation in terms of metabolism, diet preference patterns and relative adaptation to environmental conditions. Stuth (personal communication) recently reported SE values for a group of 10 steers fed five different hays for 5 days and fecal samples collected at the start of the next feeding (day 6). Hay crude protein values varied from 5.5 to 16.4% and digestible organic matter from 55.6 to 64.2%. Resulting S.E. values due to individual animal variation was 0.17–0.34 for crude protein and 0.22–0.35 for digestible organic matter.

7. Summary and conclusions

The advent of NIRS technology for assessing quality of plant/forage/feed tissue and predicting diet

quality from feces offers livestock nutritionists, researchers, farm advisors and resource consultants a rapid mechanism to acquire nutritional information. The portability and low long-term maintenance costs of this technology coupled with rapid turn around time on processing offers a mechanism for nutrition programs in developing countries to address forage and dietary quality issues that was not possible previously due to high maintenance costs of wet chemistry laboratories. When the predictions of diet quality are linked with decision support tools such as NUTBAL PRO (Stuth et al., 1999), a comprehensive suite of nutritional assessment technology can be applied to a wide array of grazing, forage and feed conditions.

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