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Fecal NIRS equations for predicting diet quality of free-ranging cattle

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Abstract

The usefulness of near infrared reflectance spectroscopy (NIRS) for predicting diet quality of free-ranging cattle through fecal analysis was examined. Diet samples were obtained with esophageal fistulated steers; subsequently, study areas were grazed with nonfistulated lactating and dry cows to provide fecal samples representing differing forage diet quality. Diet samples, which were analyzed by conventional laboratory procedures for in vivo corrected digestible organic matter (DOM) and crude protein (CP), provided dependent variable reference data while fecal sample spectra provided independent variable data for development of NIRS predictive equations by stepwise regression. Equations were developed from a data set at one location with subsequent equation development using expanded data ranges obtained by adding samples from a second location. Standard errors of calibration (SEC) and validation (SEV) for the DOM equation developed from the expanded data range were 1.66 and 1.65, respectively; these values were nearly equivalent to the laboratory standard error (SEL) of 1.68. SEC and SEV for the CP equation developed from the expanded data range were 0.89 and 0.93, respectively, compared to the 0.44 SEL. Coefficients of determination for DOM and CP equations were 0.80 and 0.92, respectively. These statistical parameters developed from fecal spectra to predict forage diet quality are equal to or better than statistics reported in the literature for NIRS equations developed using forage spectra. Furthermore, equation standard errors were within acceptable limits for NIRS calibrations. No effects of physiological stage of animals on calibration were noted in this study. Results are interpreted to indicate that prediction of diet DOM and CP of free-ranging herbivores can be accomplished with NIRS fecal analysis to a degree of precision equivalent to conventional laboratory diet analyses.

Key Words: crude protein, digestible organic matter, near infrared reflectance spectroscopy

Presently there are no rapid reliable methods of determining diet quality of free-ranging herbivores. However, recent investigations indicate potential for application of near infrared reflectance spectroscopy (NIRS) in rangeland diet quality analysis (Holechek et al. 1982b, Stuth et al. 1989). In addition, NIRS prediction of forage quality of free-ranging herbivores through fecal analysis appears to have potential both as a management and research tool (Brooks et al. 1984, Coleman et al. 1989, Stuth et al. 1989). Our hypothesis was that rangeland herbivore feces contains chemical bonds resulting from undigested residues and microbial fermentation and host animal digestion end products which can provide NIRS spectral information highly correlated with dietary crude protein and digestibility. This study examines the potential of NIRS technology to predict diet digestibility and crude protein content of free-ranging cattle.

Study Area and Treatments

This study was conducted at 2 locations. The first location was the La Copita Research Area (27° 40′ N, 98° 12′ W) in northeastern Tamaulipan Province, approximately 30 km W of Corpus Christi, Texas. *Prosopis-Acacia* shrubland sites at this location were characterized (Olson 1984) by a post oak (*Quercus stellata* Wang.) overstory and a herbaceous component dominated by little bluestem (*Schizachyrium scoparium* Michx.) and brownseed paspalum (*Paspalum plicatulum* Michx.). Five trials were conducted at La Copita in June, August, and October of 1988 and in January and June of 1989. Esophageal fistulated 5- to 9-yr old steers (680

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kg) were used to collect diet samples. During each trial, 8 Brahman × Hereford nonfistulated cows (520 kg) selected from a group of 12 cows at various stages of lactation and gestation were utilized to graze study plots and generate fecal samples.

The La Copita grazing scheme involved 2 sites, 4 cows per site, 2 levels of grazing, and 4 days grazing per site for 320 potential fecal samples. At the beginning of each trial, cows were randomly assigned to either an upland or lowland grazing group, including 2 lactating and 2 dry cows each. Sites were ungrazed between trials to allow accumulation of new forage. Within trials, sites were grazed at 2 levels of forage quality (high and low). During the first grazing cycle (high quality), cow groups grazed 1 day in each of 4 paddocks within sites; subsequently, plots were grazed by steers until preferred plants were grazed to mean leaf collar height to create a lower level of forage quality yet adequate standing crop during the second grazing cycle. After cows had grazed each paddock at the high quality level, they were rotated back through paddocks for grazing at the lower forage quality level.

Diet samples were collected at daylight on each day of a trial. Two groups of 3 steers were used to collect diets from each range site each day of a trial. After diet samples were collected on the first day of a trial, cows were turned into sampled paddocks in each site. Fecal sampling began the first morning after grazing was begun and continued until 1 day after each trial ended. Diet and fecal sampling were conducted at the same time each morning during a trial. Fecal samples were taken by fecal aeration, i.e., grab sample. Extrusa samples were dried immediately after collection at 60° C for 48 hours and then ground in a Wiley mill to pass a 2-mm screen to avoid problems with unground sample residue (Lippke et al. 1986). Fecal samples were frozen for later processing.

At College Station, 5 additional trials were conducted in August, October, and December of 1989 and April and May of 1990. These trials were used to collect forage and fecal data which would allow expansion of the La Copita data range at both extremes and provide additional spectral variability.

In College Station trials, sampling sites were selected based on available forage and feasibility of collecting representative diets with esophageal fistulated steers. Each trial was designed to collect data at 1 diet quality level. Diet samples were collected using 3 steers at the beginning of each trial. Eight intact cows then grazed sampled areas for a 72-hour period. Fecal samples were collected at 12-hour intervals beginning at 24 hours after initiation of grazing. Extrusa and fecal samples were processed in the same manner as those collected at La Copita.

Laboratory Methods

Digestibility Determinations

Extrusa sample digestibility was determined by in vitro procedures using a 48-hr fermentation (Tilley and Terry 1963) followed by neutral detergent fiber procedure (Van Soest and Wine 1967). Three forage standards of known in vivo digestibility (values suppled by W.E. Pinchak of the Texas Experimental Ranch, Vernon, Texas) were included in each in vitro run for every 10 unknown samples. Standards were wheat stubble hay, 54.8% in vivo organic matter digestibility (OMD); kleingrass hay, 65.0% OMD; and alfalfa hay, 76.3% OMD.

Forty-eight hour in vitro values were corrected to in vivo values by regression. Resulting OMD values for unknowns were converted to in vivo digestible organic matter (DOM) values using organic matter values for individual samples. Daily extrusa samples were pooled across animals within each site for use as dependent variable reference data in NIRS equation development.

Crude Protein Determinations

Each extrusa sample was analyzed for crude protein (CP) content on a dry matter basis by micro-Kjeldahl procedure using the Hach system (Hach 1987). Daily extrusa samples from each range site were pooled for use as dependent variable reference data in NIRS equation development.

Fecal Sample Processing for NIRS Analysis

Frozen fecal samples were dried in a forced-air oven at 60° C for 48 hours with periodic stirring to eliminate crusting and facilitate drying. Dried samples were ground in a Udy cyclone mill to pass a 1-mm screen to reduce particle size and ensure uniformity of particle dimension for improved precision of NIRS results (Norris et al. 1976). Moisture was stabilized in samples (Lyons 1990) before scanning with a Pacific Scientific NIR Scanner 4250 equipped with 3 tilting filters and a spinning sample cup.

Equation Development

Calibration equation development in this study was accomplished using stored NIRS spectra from fecal samples as independent variable reference data. However, reference data for the dependent variables DOM and CP was obtained from laboratory analysis of esophageal extrusa samples.

To match diets and feces for La Copita samples, an averaging algorithm was used for both DOM and CP data in which reference data for day 1 and day 2 of a trial within each site were averaged. This average was used as the reference data for day 2 fecal samples. The mean reference data for day 2 was added to the value for day 3 and the mean of the 2 values calculated. This mean served as the reference data for day 3. This algorithm was repeated until reference data was calculated for each day in the trial. Because of probable diet transitions onto experimental plots and transitions between levels of diet quality, only information from days 3 and 4 and days 7 and 8 of a trial were used in equation development. Huston et al. (1986) reported mean gastrointestinal tract retention times for cattle grazing native rangeland of 33, 34.7, 40, and 34.8 hours for spring, summer, fall, and winter, respectively. The fecal sample selection procedure described above resulted in 144 samples which composed a calibration data set for the 5 La Copita trials used to develop initial NIRS equations for prediction of DOM and CP.

College Station data which either expanded the La Copita data range or provided additional samples at points within the data range where data were absent or inadequate were selected for use in development of equations using combined College Station/La Copita data sets. College Station fecal samples within 12-hour collection periods with La Copita equation diet quality predictions most closely approximating laboratory diet analysis were selected to provide spectral data for recalibration with the assumption forage selected by fistulated animals may differ from that available to cows used in grazing and fecal sample collection prior to beginning trials.

Equations were developed by modified stepwise regression (Westerhaus 1985a). Equation selection involves consideration of several factors which includes the standard error of calibration (SEC) (Hruschka 1987, Osborne and Fearn 1986); laboratory standard error (SEL) (Hruschka 1987); coefficient of determination (R^2) (Hruschka 1987, Osborne and Fearn 1986); equation wavelength F-statistics (Westerhaus 1985); wavelength coefficient magnitude (Williams 1987); and equation wavelength examination to determine if chemical relationships exist with variables being measured (Hruschka 1987).

Results

Digestibility Equations

In terms of standard error of calibration, DOM equation development was deemed successful. Calibrations using the same diet reference data and spectra data from lactating and dry groups (Table 1) within the La Copita data set resulted in identical calibration statistics, and slopes and bias for validation samples which were not significantly different ($P>0.05$). The SEC for the equa-

Table 1. Comparison of in vivo corrected digestible organic matter (DOM) and crude protein (CP) equations for lactating (LACT) and dry (DRY) cow fecal calibration sets within La Copita calibration set.

Equation	Calibration			Validation				
	n	SEC	R^2	n	SEV(C)	r^2	Bias	Slope
LACT DOM	54	1.70	0.70	18	1.93	0.70	–0.37	1.03
DRY DOM	54	1.70	0.70	18	1.88	0.71	–0.75	0.92
LACT CP	54	0.87	0.63	18	1.21	0.45	0.15	1.14
DRY CP	54	0.87	0.63	18	1.09	0.57	–0.04	1.23

SEC-Standard error of calibration.
R^2-Coefficient of determination.
SEV(C)-Standard error of validation corrected for bias.
r^2-Coefficient of simple correlation.

tion developed from the La Copita calibration set (Table 2) was 1.75 compared to 1.66 for the equation developed from the College Station/La Copita calibration set. These values were nearly equivalent to the SEL (1.68), which indicates procedures used in sample preparation for NIRS scanning introduced little error. Standard errors of calibration obtained in the present study were equivalent to or lower than values reported for digestibility estimates in other studies (Holechek et al. 1982b, Brooks et al. 1984, Brown et al. 1990). Standard error of validation corrected for bias, SEV(C), shown in Table 2 was obtained using an equation developed from odd-numbered samples predicting even-numbered samples (Norris et al. 1976). The College Station/La Copita equation SEV(C) of 1.65 (Table 2) indicates a high degree of precision in predictions. The relationship between reference DOM values and NIRS predicted values for the College Station/La Copita validation set is illustrated in Figure 1.

The La Copita DOM equation R^2 (0.69) (Table 2) was lower than values reported by others. Using fecal material, Brooks et al. (1984) reported a 0.88 R^2 for in vivo dry matter digestibility equations for prediction of extrusa. However, improvement in R^2 (0.80) was achieved with the College Station/La Copita equation (Table 2). The relatively low La Copita equation R^2 is at least partially due to limited data at the extremes of the data set and possibly the narrower range of the data.

The La Copita DOM equation range (54.6–65.3%) had only 12 samples below 56% and 4 samples above 64%. A wider range of values (54–68%), more samples at extremes, and additional samples at points where data was lacking resulted in improvement of R^2 from 0.69 to 0.80. Calibration samples should be numerous enough to accomplish calibration, well distributed over the range and representative of the population (Osborne and Fearn 1986).

Crude Protein Equations

Standard error of calibration for CP did not approach the laboratory standard error as closely as in the DOM equation, yet calibrations were not affected by physiological stage. As with DOM, crude protein calibrations for the lactating and dry groups (Table 1) resulted in identical SEC and R^2 values and no significant bias or differences in slope ($P>0.05$). The standard error of calibration for the La Copita equation was 0.88 compared to a SEL of 0.44. Variation in CP between pooled extrusa samples was used to calculate this SEL. The College Station/La Copita equation SEC was 0.89 (Table 2). These values are nearly 2 times the SEL, which is within acceptable limits for NIRS calibration procedures (Hruschka 1987). Crude protein standard errors of calibration obtained in our study were similar to those reported by others (Holechek et al. 1982b, Brooks et al. 1984, Brown et al. 1990). Higher relative CP standard errors of calibration compared to those for DOM equations could be related to variations in the supply of nitrogen (N) due to rumen recycling and endogenous N. The relationship between reference CP and NIRS predictions for the College Station/La Copita equation validation set is illustrated in Figure 1. The SEV(C) shown in Table 2 indicates a high degree

Table 2. In vivo corrected digestible organic matter (DOM) and crude protein (CP) equations from La Copita (LC) and College Station-La Copita (CSLC) calibration sets.

Equation	Calibration						Validation				
	n	Math	λ	F	SEC	R^2	n	SEV(C)	r^2	Bias	Slope
LC DOM	72	1st	1968	29	1.75	0.69	72	1.73	0.71	–0.30	1.03
			2145	30							
			2297	292							
CSLC DOM	102	2nd	1968	146	1.66	0.80	102	1.65	0.80	0.08	1.02
			2064	208							
			2278	356							
			2297	389							
LC CP	72	2nd	2044	139	0.88	0.64	72	0.89	0.63	–0.12	1.03
			2064	109							
			2219	106							
			2293	85							
CSLC CP	98	2nd	2036	258	0.89	0.92	97	0.86	0.93	0.08	0.97
			2063	220							
			2107	649							
			2210	100							
			2275	427							

Math-1st or 2nd derivative of log (1/R) spectra.
SEC-Standard error of calibration.
R^2-Coefficient of determination.
SEV(C)-Standard error of validation corrected for bias.
r^2-Coefficient of simple correlation.

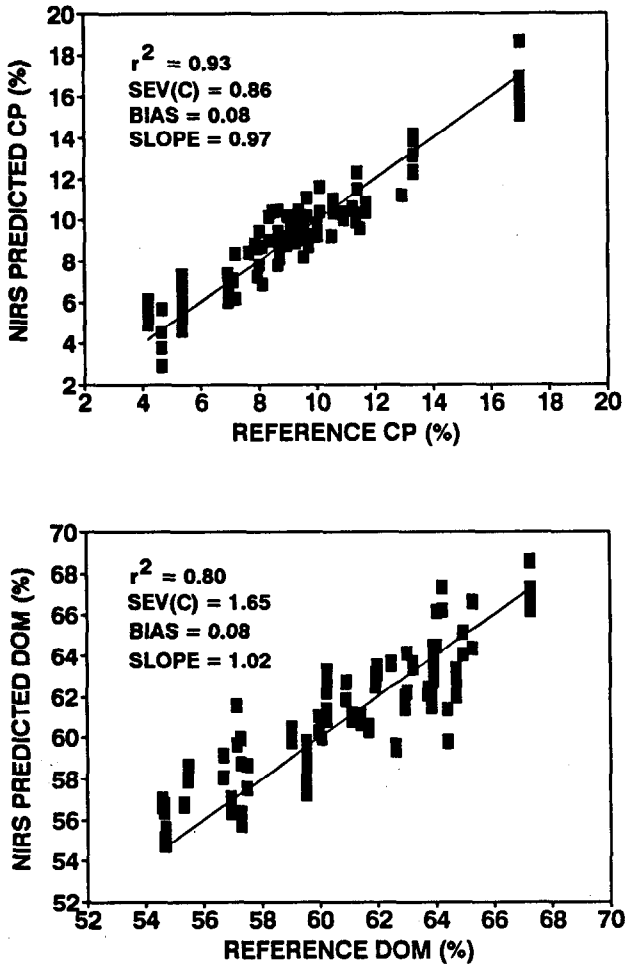

Fig. 1. Reference crude protein (CP) vs. NIRS predicted CP and reference in vivo corrected digestible organic matter (DOM) vs. NIRS predicted DOM for the College Station/La Copita validation set indicating standard error of validation corrected for bias, SEV(C), coefficient of simple correlation (r^2), bias, and slope.

of precision in estimates for CP.

La Copita crude protein equation R^2 (0.64) was lower compared to values reported by others, 0.99 (Brooks et al. 1984) and 0.92 (Holechek et al. 1982b). However, marked improvement (0.92) was obtained with the College Station/La Copita equation (Table 2). As wih DOM, the range in the data appears to be related to the lower R^2 values. The CP range (6.9–12.9%) had only 8 samples below 7% and 4 above 11%. Both a wider range of data (4–17%), as well as more samples at extremes and additional samples where data were lacking, resulted in marked improvement in this statistic for the College Station/La Copita equation. The CP range reported by Brooks et al. (1984) was 3.0–23.3% with a 0.99 R^2, while Holechek et al. (1982b) reported a range of 5.2–14.9% with a 0.92 R^2.

Discussion

Fecal nitrogen indices for use in estimating dietary intake (Gallup and Briggs 1948); digestibility (Lancaster 1949, Holechek et al. 1982a); and CP content (Raymond 1948, Hinnant 1979, Holechek et al. 1982a) have been of interest to many researchers. Fecal analysis is appealing because samples are easily obtained and should theoretically, be representative of the quality of forage selected by grazing animals. These attributes of fecal analysis make the technique appealng both as a research and management tool. Although earlier studies (Gallup and Briggs 1948, Raymond 1948, Lancaster 1949, Hinnant 1979) have dealt primarily with fecal N-dietary N relationships, some studies (Holloway et al. 1981, Holechek et al. 1981, Holechek et al. 1982a, Leite and Stuth 1990) have examined multiple fecal indices. Investigations with both fecal N and multiple fecal indices have met with mixed results.

Consideration should be given to ruminant fecal composition and its theoretical relationship to dietary constituents. Ruminant fecal dry matter consists of undigested dietary materials, undigested cell walls of rumen bacteria, microbial cells from the cecum and large intestine, and residues of endogenous substances including digestive enzymes, mucous and other secretions, and sloughed epithelial cells (Merchen 1988, Van Soest 1982). Morphologically, increased concentrations of bacterial cells were observed in sheep feces as rations became less fibrous, while dietary residues vary with the nature of the ration but include small amorphous pieces of lignified material, pitted xylem vessels, and xylmem fibers (Mason 1969). Undigested plant residues in herbivore feces consist largely of plant cell wall constituents including cellulose, hemicellulose, and lignin (Jarrige 1965, Van Soest and Moore 1965). The proportion of dietary materials to materials of metabolic and endogenous origin is greatest with diets of low quality forage (Jarrige 1965, Merchen 1988). Feces physically become more fibrous as plants age and digestibility decreases (Jarrige 1965). Pond et al. (1987) found larger sheets of cuticle, more parenchyma bundle sheath cells associated with vascular bundles, and more exposed tracheary elements in fecal particles derived from mature versus immature Coastal bermudagrass (*Cynodon dactylon* L.). Bacterial N excretion has been reported to be closely related to amount of energy fermented in the host animal (Mason 1969).

Microbial cells and residues constitute a large proportion of fecal dry matter (Merchen 1988). Indigestible cell walls from rumen bacteria plus cells from fermentation in the lower gastrointestinal tract are the largest sources of microbial fecal matter (Van Soest 1982). Endogenous fecal matter, i.e., nonmicrobial, constitutes 10–15% of the metabolic fraction. About 86% of fecal N is of bacterial and endogenous origin with 74% of this nondietary N being of bacterial origin (Merchen 1988). No evidence exists of potentially digestible feed protein in feces because dietary protein residues are present as keratin or Maillaird products and bound to lignin (Van Soest 1982).

Microbial cell walls contain substituted glucosamine (muramic acid) polymers with attached peptides (Van Soest 1982). The fecal amino acid profile is similar to that of isolated gastrointestinal bacteria (Merchen 1988). Among the amino acids present is diaminopimelic acid (DAPA) (Mason 1969, Van Soest 1982), which is unique to bacteria. Cell walls also contain teichoic acids, polymers of ribitol or glycerol phosphate with alanine side chains (Van Soest 1982). Indole and skatole, ring compounds produced from microbial degradation of tryptophan, also appear in the feces.

As suggested by Hruschka (1987), the final NIRS equation evaluation involves wavelength examination to determine if selected wavelengths appear to have a chemical relationship to the variable being measured. Norris et al. (1976) suggested the first 2 wavelengths in terms of F-statistic rank were most important in NIRS multiple regressions. Windham et al. (1988) also indicated that, although wavelengths of multiterm equations are so interdependent that interpretation of individual wavelengths is often difficult, it is useful to evaluate the first 2 wavelengths in terms of related chemical constituents. For these reasons and because tilting fiber instruments such as the one used in this study provide only approximate wavelength identification, we will briefly discuss the possible biological basis only for primary wavelength selection in the College Station/La Copita CP and DOM equations.

Log (1/R) spectra of fecal samples representative of forage

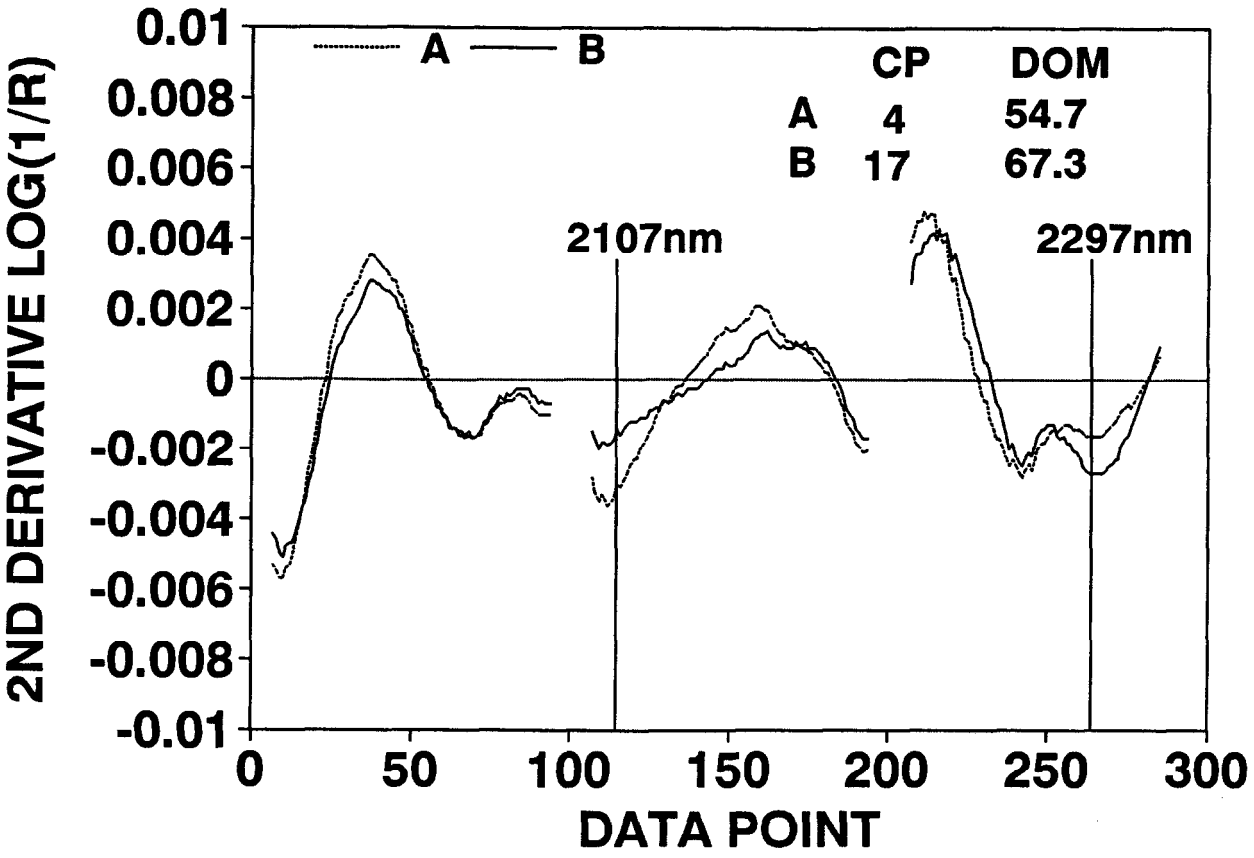

Fig. 2. Comparison of second derivative log (1/R) fecal spectra associated with fermentation and digestion of low (A) and high (B) quality forages illustrating greater absorbance at most significant estimated wavelengths in the College Station/La Copita CP equation (2107 nm) for sample A and in the DOM equation (2297 nm) for sample B. Valleys (minima) in second derivative are analogous to peaks (maxima) in log (1/R) spectra. Gaps indicate filter changes.

quality at extremes of data sets were converted to second derivative spectra to accentuate spectral characteristics (Hruschka 1987). Maxima in log (1/R) spectra correspond to second derivative minima (Barton 1987), i.e., valleys indicate greater absorbance with second derivative spectra. Spectra of fecal samples representing diet quality of 4% CP, 54.7% DOM and 17% CP, 67.3% DOM are illustrated in Figure 2. For the DOM equation, this comparison shows greater absorbance for the high quality sample at the primary wavelength (2297 nm). In NIRS forage applications, this wavelength region has been associated with neutral detergent fiber (Norris et al. 1976, Redshaw et al. 1986). Furthermore, Barton et al. (1986) attributed an observed continual decrease in apparent absorbance intensity at 2290 nm with time of barley straw in vitro incubation to cellulose digestion. Location of primary absorbers may be shifted by the derivative process or distorted by tilting filters and by various combinations of absorbing bonds. The implied discrepancy of greater absorbance for feces from high quality forage observed in our study at 2297 nm and decreasing absorbance observed at 2290 nm by Barton et al. (1986) may be due to shifting by tilting filters of our instrumentation. Regardless of exact wavelength location, we suggest the observed greater absorbance associated with feces from high quality forage may indicate detection of microbial response to diet quality possibily through absorbance associated with chemical bonds in undigested rumen microbial cell walls, whole microbial cells produced in the lower gastrointestinal tract, and aromatic and other by-products of microbial degradation. As indicated above, a direct relationship exists between dietary energy and fecal microbial residues (Mason 1969).

For the spectral region near the primary wavelength (2107 nm) in the CP equation (Fig. 2), absorbance appears to be greater for the low quality sample. Because a filter change occurs in this area, wavelengths are estimated, and intercorrelations between wavelengths exist, it is possible that an artifact could have been produced which correlated well with CP, and the actual wavelength related to CP may be one of the other wavelengths listed in Table 2. However, we suggest that this wavelength is possibly associated with undigested dietary residues of cell wall carbohydrates which would be present in greater portions in feces from lower quality forage (Mason 1969, Merchen 1988). This 2100 nm wavelength region usually represents the very strong OH combination band seen in all starch- and cellulose-containing substances (Murray and Williams 1987). Crude protein and digestibility of range grasses decline with advancing maturity (Burzlaff 1970), which is, of course, associated with increased fiber content.

Average number of equation wavelengths encountered in NIRS studies involving forage and extrusa (Norris et al. 1976, Shenk et al. 1981, Holechek et al. 1982b, Brown et al. 1990) were 5 and 7 for CP and digestibility, respectively. However, Brooks et al. (1984) reported use of 6 and 3 wavelengths in equations developed from forage samples and 5 and 2 wavelengths in equations developed from fecal samples for CP and in vivo dry matter digestibility, respectively. In the present study, College Station/La Copita CP and DOM equations contained 5 and 4 wavelengths, respectively. Although both diet CP and DOM predictions through fecal analysis are indirect estimates, we suggest more wavelengths were required for CP because little measured dietary CP is present in the feces while much of the measured undigested material associated

with measurement of DOM is present.

Differences in levels of forage intake and rates of passage associated with different physiological stages (dry, lactating) of animals providing fecal spectra were thought to be potential sources of error in equation development. However, lack of differences in equation and validation statistics (Table 1) are interpreted to suggest that equation calibrations in this study were not affected by animal physiological stage. This lack of difference may be due to compensatory fermentation and digestion. Deswysen and Ellis (1988) reported evidence of compensatory cecum-colon fermentation in heifers with different voluntary intake potentials.

Interestingly, addition of calibration samples from a second location (College Station) to the original data set (La Copita) improved statistics of DOM and CP equations. Added samples included C_3 and C_4 plants, annual and perennial plants, monocots and dicots, and plants at various phenological stages. These results lend encouragement to the idea that development of broad-based NIRS equations (Abrams et al. 1987) is feasible. We suggest that in the case of fecal analysis, broad-based equations may even improve local equations by expanding the data range increasing spectral diversity within the data set.

Conclusions

Success of NIRS equation calibrations for both DOM and CP suggest that NIRS technology may have potential for nutritional profiling of free-roaming cattle and other herbivores on rangelands. Precision of DOM equations matched that of conventional laboratory methods. Although CP equations lacked relative precision compared with DOM equations, these equations still possess an acceptable level of precision.

To determine broad based applicability of DOM and CP equations developed, field validation is needed. One means of validation would be collection of esophageal extrusa samples from discrete plant communities followed by collection of fecal samples from animals grazing the area and determine correlations between conventional laboratory analysis of diet samples and NIRS fecal predictions of diet quality. Another means of assessing applicability of equations is to examine fecal samples from cattle grazing in various regions for spectral outliers to the calibration data set. This procedure could help identify regions or seasons which require further calibration. If universal equations prove infeasible, regional and animal species-specific equations must be developed.

NIRS continues to exhibit potential for rangeland applications. Its precision, potential accuracy, flexibility, and ability for rapid analysis once calibrations are available establish NIRS as a viable animal monitoring method for the future.

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