

# Predicting Diet Quality of Donkeys via Fecal-NIRS Calibrations

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## Abstract

Successful applications of fecal-near infrared reflectance spectroscopy (fecal-NIRS) techniques have been reported for ruminant animals. Information on the ability of fecal-NIRS to characterize diet quality in equines is lacking. The objective of these studies was to determine the potential of fecal-NIRS to predict diet quality of free-grazing equines. Two independent in vivo feeding trials, one in Texas (United States) and one in Kenya, were conducted to generate paired samples of diet chemistry: fecal spectrum (D:F). Using 20 female donkeys (*Equus asinus*), 14 (10 US, 4 Kenya) in vivo pen feeding trials were conducted to generate 140 (100 US, 40 Kenya) D:F paired samples. Over 25 species of forage and crop residues ranging from 3.3% to 21.4% crude protein (CP) were used to blend unique diets. Three CP predictive equations based on paired samples from US alone, Kenya alone, US+Kenya combined, and one predictive equation for digestible organic matter (DOM) from US alone were developed. The standard errors of calibration (SEC) and  $R^2$  values were 0.77 and 0.97, 0.97 and 0.95, and 0.88 and 0.90, respectively, for the US, US+Kenya, and Kenya CP equations. The US DOM equation resulted in an SEC of 2.58 with a corresponding  $R^2$  of 0.60. Validation of the US CP equation using an independent dataset resulted in standard error of prediction (SEP) and  $R^2$  of 1.79 and 0.82, respectively, indicating acceptable predictive ability. The validation results (SEP = 15.56) for the US DOM equation were not satisfactory. We calibrated and validated fecal-NIRS equations to predict the DOM and CP contents of diets for donkeys. Crude protein content of diets was predicted with acceptable levels of accuracy, but prediction of diet digestibility was less successful. The degree of accuracy obtained for CP equations indicated that fecal-NIRS can be considered as a tool for routine nutritional management of donkeys.

## Resumen

El uso adecuado de la técnica de espectroscopia de reflexión infrarroja (fecal-NIRS) ha sido tradicionalmente utilizado para rumiantes, mientras que se ha tenido un uso restringido para caracterizar la dieta de equinos. El objetivo de estos estudios fue determinar el potencial de la técnica fecal-NIRS para predecir la calidad de la dieta de equinos en libre pastoreo. Se llevaron a cabo dos pruebas independientes de alimentación (en Texas [USA] y Kenia) para generar muestras apareadas de la composición química de la dieta: espectro fecal (D:F). Teniendo como sujetos de estudio 20 burras (*Equus asinus*), 14 de USA y 4 de Kenia, se realizaron pruebas de alimentación in vivo para generar 140 (100 de USA, 40 de Kenia) muestras apareadas D:F. Para elaborar dietas únicas, se utilizaron más de 25 especies de forraje y residuos de cosecha con un contenido de proteína cruda (CP) que fluctuó de 3.3 a 21.4%. Se desarrollaron tres ecuaciones para predecir PC basadas en muestras apareadas de USA, Kenia, y una combinación de USA+Kenia, y una ecuación de predicción de la materia orgánica digestible (MOD) con muestras de USA. Los errores estándar de la calibración (SEC) y los valores  $R^2$  fueron 0.77 y 0.97, 0.97 y 0.95, y 0.88 y 0.90, respectivamente, para las ecuaciones de predicción de PC de USA, USA+Kenia, y Kenia. La ecuación para predecir MOD de USA resultó en un SEC de 2.58 con una  $R^2$  de 0.60. La validación de la ecuación para PC de USA que uso un banco de datos independiente dio lugar al SE de la predicción (SEP) y un  $R^2$  de 1.79 y 0.82, respectivamente, indicando aceptable habilidad predictiva de la ecuación. Los resultados de la validación (SEP = 15.56) para la ecuación MOD de USA no fueron satisfactorios. Se calibró y validó las ecuaciones para determinar MOD and CP de la dieta de burros mediante la técnica fecal-NIRS. El contenido de proteína cruda de la dieta fue predicho con niveles aceptables de exactitud, mientras que la predicción de la digestibilidad de la dieta fue menos acertada. El grado de exactitud obtenido para las ecuaciones de CP indicó que fecal-NIRS puede ser considerado como herramienta para el manejo alimenticio de rutina en burros.

**Key Words:** crude protein, digestible organic matter, *Equus asinus*, free-grazing, near infrared reflectance spectroscopy

## INTRODUCTION

Donkeys (*Equus asinus*) are important sources of draft power for transport and crop production in smallholder agriculture (Pearson et al. 2001). They play a significant role in the socioeconomic life of millions of resource-poor people in

developing countries (Ghebream et al. 1999). According to the Food and Agriculture Organization (2003), there are more than 40 million donkeys worldwide, of which about 13 million are found in Africa. The East Africa region accounts for more than 45% of the total donkey population of the continent. However, in many countries the potential of donkeys as draft animals has not been fully utilized (Pearson and Quassat 2000) largely because of poor nutrition (Aganga et al. 2000). Widespread nutritional constraints are caused mostly by a lack of forages or supplementary feed, and inadequate management of the resources (Muvirimi and Ellis-Jones 1999).

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Management of grazing animals generally requires knowledge of the quality and quantity of nutrients that an animal can obtain from forage. However, a rapid reliable method of determining the diet quality of grazing equines, particularly donkeys, has been lacking. Prior research has focused on estimating forage quality using various analytical methods including chemical procedures (Clark et al. 1995), in vitro (Coleman and Moore 2003), in situ (Adesogan et al. 1998), and marker-based in vivo techniques (Van Soest 1982). Under a free-grazing situation, however, analysis of clipped forage samples only provides quality estimates of plant components that the animal could potentially select. Estimation of diet quality via hand plucking plant species and parts has generally been of limited use due to selectivity of free-ranging animals. As an alternative method, visual appraisal of body condition has also been used to monitor the nutritional status of donkeys (Pearson and Quassat 2000), but body condition reflects only past nutrition (Lyons 1990; Stuth et al. 1999).

Fecal-near infrared reflectance spectroscopy (fecal-NIRS) has the potential for predicting diet quality of free-grazing animals (Lyons and Stuth 1992; Leite and Stuth 1995). Prior fecal-NIRS studies have focused on ruminants, and have successfully been used as routine methods for predicting the diet quality of free-grazing cattle, sheep, goats, deer, and elk (Lyons and Stuth 1992; Leite and Stuth 1995; Ossiya 1999; Awuma 2003; Keating 2005; Showers et al. 2006; Li et al. 2007). However, research involving free-grazing equines, which are hindgut fermenters, is lacking. The objective of this study was to determine the ability of fecal-NIRS to predict dietary crude protein (CP) and digestible organic matter (DOM) in domestic donkeys. We hypothesized that analysis of fecal material via near infrared reflectance spectroscopy would characterize the diet quality of equines (hindgut fermenters).

## METHODS

Paired calibration samples for relating diet chemistry: fecal spectrum (D:F) were generated from two independent studies (feeding trials) conducted in Texas (United States) and Kenya. The same protocol generally was used at both sites. Differences between sites will be noted in the following description of procedures.

### US Study Site

The US feeding trial was conducted at the Texas A&M University Horse Center in College Station, Texas (lat 30°37'N, long 96°21'W). College Station has a mean annual precipitation of 940 mm and varies from 780 to 1100 mm; mean temperature ranges from 10°C in January to 30°C in July (US Department of Commerce 1990, cited in Leite and Stuth 1995). The feeding trial was conducted for 11 wk, between December 2002 and February 2003.

### Kenya Study Site

The Kenya feeding trial was conducted at the Naivasha Research Center, in the facilities of the Kenya Agricultural Research Institute (KARI). The Naivasha Research Center is located at an altitude of 1936 m, lat 0°40'S and long 36°26'E,

and has a mean annual precipitation of 657 mm (KARI 2004). The trial was conducted for 5 wk during November and December 2003.

### Experimental Animal Management

In the US feeding trial, 10 mature female donkeys (five nonpregnant and five pregnant) ranging from 2 to 6 yr of age, and mean initial body weight (BW) of  $196.8 \pm 51.9$  kg were used. In the Kenya trial, 10 mature female nonpregnant donkeys were used. Prior to the initiation of the trial, donkeys were subjected to standard quarantine procedures, dewormed, and vaccinated against West Nile virus, Venezuelan eastern western encephalomyelitis, and tetanus. Donkeys were also subjected to a pregnancy test using ultrasound by a trained practitioner. Following the standard quarantine, donkeys were placed in dry lot at the Equine Nutrition facility housed in 3 × 4 m individual stalls, and offered coastal bermudagrass hay (*Cynodon dactylon* L.) for 2 consecutive wk. All experimental procedures and facilities were designed in such way as to fulfill the requirements of an approved animal use protocol by the University's Institutional Animal Care and Use Committee.

### Diet Preparation

For the US and Kenya feeding trials, 13 and 12 feed types (forage and crop residues) were used to create a total of 100 and 40 unique diets, respectively. At both sites, each feed type was analyzed for CP ( $N \times 6.25$ ) using micro-Kjeldahl procedures (Association of Analytical Chemists [AOAC] 1995) before blending into unique diets. Forages included were tropical as well as temperate grasses, forbs, and browse varying from 3.3 to 21.4% CP. In the US feeding trial, the most frequently used ingredients included alfalfa (*Medicago sativa* L.), bermudagrass (*Cynodon dactylon* L.), little bluestem (*Schizachyrium scoparium* Michx.), and peanut hay (*Arachis fabaceae* L.). In the Kenya feeding trial, wheat straw (*Triticum aestivum* L.), barley straw (*Hordeum vulgare* L.), oat hay (*Avena sativa* L.), maize-stover (*Zea mays* L.), and alfalfa (*Medicago sativa* L.) were the predominant ingredients.

### Feeding Trials

In vivo feeding trials were conducted for 11 consecutive weeks at the US site and for 5 consecutive weeks at the Kenya site. The first week (week 0) was designated as an adjustment period during which experimental animals were housed in individual stalls and fed the same diet. Following this period, fecal and diet sample collections for calibration were made for 10 wk in the US trial, and for 4 wk in the Kenya trial. Feed types were changed each week. Given the fact that hindgut fermenters such as donkeys have short digesta transit time, i.e., less than 40 h (Izraely et al. 1989; Pearson et al. 2001), they need at most 4 d to balance their intake, clear out previously undigested diets, and balance their fecal output (Dr G. Potter, personal communication, June 2002). Thus a 7-d in vivo feeding trial consisted of a 4-d adaptation period followed by 3 d of sample collection.

Donkeys were fed twice per day at 12-h intervals (0700 hours and 1900 hours) and had free access to diets between successive feedings. The daily diet allowance for each donkey was determined as 2% BW (as fed) as recommended by

LaCasha et al. (1999). When necessary, adjustments were made based on the previous week's intake level. CP content of diets provided to donkeys was low in first trial (week 1), but gradually increased in succeeding trials to avoid any negative effect on feed intake and inaccuracy in data collection. Accordingly, during trial 1, all donkeys received diets varying from 5% to 5.9% CP and then gradually increased through trial 9. In trial 9, all donkeys received diets with highest CP ranging from 18% to 19.4%. This range of dietary CP content did not match exactly the 4% to 20% CP range commonly seen in diets of free-ranging herbivores; therefore, all donkeys received diets with CP ranging from 4% to 5% in trial 10. No adverse reaction to the low CP concentration was noted when animals were fed these diets. Donkeys had free access to trace mineral blocks (12% Ca and 12% P with high trace mineral concentration), and fresh water throughout the experimental period. In addition, donkeys were turned out from their stalls daily and allowed 20 min of exercise in a larger pen.

Diets were thoroughly hand mixed, and samples taken before weighing for each feeding trial. Each diet was measured in a single pan-balance to the nearest gram and diet offered ( $\text{kg} \cdot \text{d}^{-1}$ ) was recorded for each animal. Orts (refusals) were collected from the trough and floor twice daily (0700 hours and 1900 hours) and weighed. Samples of diets and Orts were stored in paper bags at room temperature for chemical analysis.

Fecal sampling was repeated for each diet for 3 consecutive days (fifth, sixth, and seventh days). Throughout the collection period, 24-h surveillance procedures were used in order to minimize any inaccuracy in sample collection from coprophagy and/or from other possible contaminations (e.g., urine). Feces were collected immediately following each defecation event off the floor using a hand brush and scoop as the event occurred, and weighed at 4-h intervals. Each collected fecal ball was crumbled, thoroughly mixed, and a 5% (wet weight) representative sample was placed in a sealable plastic bag and stored at  $-4^{\circ}\text{C}$  until further processing.

### Chemical Analysis

For each donkey, the 3-d composite diet samples and 3-d ort samples were thoroughly mixed and dried in a forced-air oven at  $60^{\circ}\text{C}$  for 48 h. Samples were ground using a Thomas Mill to pass a 2-mm screen (Lyons and Stuth 1992), packed in paper bags, and stored at room temperature until used for chemical analysis. Each diet and ort sample was analyzed for dry matter (DM), organic matter (OM), and CP, and the corresponding fecal sample was analyzed for DM and OM. Both DM and ash were assayed using the standard methods of AOAC (1995). The OM was determined by ignition of each subsample in a muffle furnace at  $500^{\circ}\text{C}$  for 4.5 h. The *in vivo* DM digestibility (DMD) across animal by diet was derived using the model described by Osuji et al. (1993):

$$\text{DMD} = (a - b - c) \times 100 / (a - b) \quad [1]$$

where  $a$  = total DM offered ( $\text{kg} \cdot \text{d}^{-1}$ ),  $b$  = total DM refusal ( $\text{kg} \cdot \text{d}^{-1}$ ), and  $c$  = total fecal DM ( $\text{kg} \cdot \text{d}^{-1}$ ). The DOM was computed as grams of OM digested for every gram of DM ingested as described by Leite and Stuth (1995). Dietary and ort N was determined on DM basis by the micro-Kjedahl method

(AOAC 1995) and then converted to an estimate of CP. When CP concentration of ort was different from that of original diet offered, the CP values of the latter were subjected to correction to get an ort-adjusted whole-diet CP value. These were derived using the Hach Company (1987) model

$$y = (a - [b \times d/c] / [1 - d/c]) \quad [2]$$

where  $y$  = ort corrected whole-diet CP (%),  $a$  = CP(%) of diet offered,  $b$  = CP(%) of ort,  $c$  = weight of DM of diet (kg), and  $d$  = weight of DM of ort (kg).

In the Kenya trial, analysis for CP and DM content of the diet samples was conducted as described above. Analyses necessary to arrive at DOM estimates of diet and fecal samples were not completed before some of the samples were misplaced and lost. Because of this unfortunate occurrence, we are reporting only on the CP equation from the Kenya trial.

### Fecal Processing and Spectra Collection

Fecal samples ( $N = 100$ ) were scanned in the Grazingland Animal Nutrition Laboratory (GANLAB) at Texas A&M University for the US experiment and at KARI in Kenya for the Kenya samples ( $N = 40$ ). Each fecal composite was pooled and dried in a forced-air oven at  $60^{\circ}\text{C}$  for 48 h. Following drying, each sample was ground using a Cyclotec 1093 Sample Mill (FOSS Tecator, Eden Prairie, MN) to pass a 1-mm screen and saved in paper coin envelope for storage. After grinding, each sample was redried in a forced-air oven at  $60^{\circ}\text{C}$  overnight (12 h) to eliminate any recaptured moisture and directly placed in desiccators for 1 h to cool to ambient temperature (Lyons et al. 1995). A subsample of approximately 0.75 g was packed in a small ring cup (40-mm diameter) and scanned as dry ground powder in reflectance mode (between 400 and 2500 nm) using a Pacific Scientific (Neotec) model 6500 monochromator. Reflectance data were stored as the logarithm of reciprocal of reflectance ( $1/R$ ) at every 2-nm interval (Shenk et al. 2001). Inrasoft International software 1.5 versions (Win ISI Port Matilda, PA) was used for spectral data collection and processing, and calibration development. Near infrared spectroscopy analysis of fecal samples from the Kenya trial was conducted in KARI using the standard procedures described above on a FOSS 5000 that was calibrated against the NIR spectrophotometer used in the US experiment.

### Spectral Pretreatment and Calibration Equation Development

Fecal spectra were corrected for scattering using standard normal variat (SNV) and detrending as described by Murray and Williams (1987). Mathematical transformation and smoothing functions of the spectra were carried out to improve the predictive models. A number of possible combinations of derivative (1, 2), gap (4, 8, 12), and smoothing (1) treatments of the spectra were compared. Critical H (10) and critical T (2.5) were used to identify outlier samples (Workman 1992). The number of outlier elimination passes was set at two times (i.e., the program attempted to remove outliers two times before completing the calibration). For calibration, the modified partial least square regression (MPLS) was used as recommended by several authors (Gordon et al. 1998; Ruano-Romos et al. 1999).

**Table 1.** Calibration results for crude protein and digestible organic matter equations from the US, Kenya, and US+Kenya calibration datasets.<sup>1</sup>

Diet constituent	<i>N</i>	Mean	SD	SEC	<i>R</i>	SECV	Bias	Math	SECV/SD	$\lambda$
US crude protein	96	11	4.3	0.77	0.97	1.19	-0.72	2	0.28	1600
Kenya crude protein	39	8.6	2.8	0.88	0.9	1.03	-0.62	1	0.39	2332
US+Kenya crude protein	101	10.2	4.2	0.97	0.95	1.25	-0.75	2	0.4	1604
US digestible organic matter	93	45.4	4.1	2.58	0.6	2.8	-1.68	2	NA	1732

<sup>1</sup>*N* indicates number of samples; SEC, standard error of calibration; *R*<sup>2</sup>, coefficient of determination; SECV, standard error of cross validation; Math, derivative;  $\lambda$ , selected dominant wavelength (nm); and NA, not applicable.

### Selection of Calibration Equations

In selecting the best calibration equations, the standard error of calibration (SEC), the coefficient of determination in calibration (*R*<sup>2</sup>) and the standard error of cross validation (SECV) were used. The SECV/SD and SECV/mean ratio were also determined to evaluate the performance of the calibrations (Cazzolino and Moron 2004). Calibration equations were selected based on the lowest SEC and SECV and highest *R*<sup>2</sup>. The standard error of prediction (SEP), slope, and bias were also considered during equation validation (Shenk et al. 1992; Westerhaus et al. 2004). Major wavelength was selected for each equation based on the magnitude of the coefficient of wavelength and examined for its biological significance (Murray and Williams 1987). In selecting wavelength, we did not use F values because the MPLS model, which was used for calibration, does not compute the F values for wavelengths.

### NIRS Equation Validation

To validate the performance of the US+Kenya CP calibration, a subset of 25% of the combined US and Kenya diet:fecal (D:F) set was selected using the SELECT program of Win ISI 1.5 version software. Equation validation of the Kenya CP calibration was conducted using the US D:F sets. The US calibrations were validated using the Kenya D:F sets. Validation of the selected CP and DOM calibrations was performed both with and without the critical T outlier samples.

## RESULTS AND DISCUSSION

For the US and Kenya trials, a total of 140 diet samples with matching fecal samples were generated. Crude protein content of ort-adjusted diets from the US trial ranged from 4.1% to 19.4% (10.9% ± 4.3 SD) and CP of diets from the Kenya trial ranged from 3.8% to 14.0% (8.7% ± 2.9 SD). The DOM content of diets from the US trial ranged from 12.1% to 61.9% (44.9% ± 6.1 SD).

### Spectrum Data

Scanning of fecal samples resulted in NIRS spectra over the range from 1100 to 2498 nm, yielding a spectrum of 700 data points. The Mahalanobis distance (*H* = 8) of each spectrum, with respect to the population average spectrum, indicated that of the total fecal sample sets, about 97% and 98%, were less than 8 *H*, for the US and Kenya fecal samples, respectively. These results indicated that there was great uniformity of wavelength distribution of the fecal spectrum (in terms of particle size and moisture) compared to the population mean. In addition, inclusion of the Kenya dataset into the US dataset did not

change the overall spectra distribution as indicated by the low number of H outlier samples. These results indicated that the majority of the samples were within the range of the same population of spectra, which is required criteria for calibration.

### US CP Equation

The US CP equation was developed using 96% of the D:F pair calibration sets. Diets used in this calibration varied from 4.1% to 19.4% CP (within the CP range for the whole diet samples). Generally, NIRS equations are considered acceptable when they have *R*<sup>2</sup> > 0.80, and SEC values less than twice standard error of laboratory (SEL) value. The SEC and *R*<sup>2</sup> for the US CP equation were excellent, and superior to the above standards (Table 1). Also, cross validation was used to test the predictive ability of the calibration equation. This process involves removing a certain number of samples (e.g., 10%) during the calibration procedure, and predicting these with a calibration created from the remaining samples (e.g., 90%; Li et al. 2007) and measured in terms of SECV value. The cross validation of the US CP calibration resulted in a SECV value slightly higher than the SEC for the equation.

### Kenya CP Equation

This equation was developed with second derivative and incorporated 97.5% of the total calibration set (Table 1). The equation had excellent SEC and *R*<sup>2</sup>, comparable to the US equation reported herein. This equation had an SECV value slightly higher than the SEC value reported for the same equation.

### US+Kenya CP Equation

The combined sample set was divided into two subsamples of 105 and 35 D:F pairs, and used for calibration and validation, respectively. Inclusion of the Kenya sample set into the US sample set expanded the range of the dietary CP (from 3.8% to 19.4%). In developing the selected equation, 5% of the calibration samples were identified as outliers. The resultant SEC and corresponding *R*<sup>2</sup> for the US+Kenya CP equation were excellent. The SECV for this equation was slightly higher than the computed SEC for the same equation.

Calibration statistics for CP showed that the US CP equation had the lowest SEC value compared to the Kenya and US+Kenya CP equations (Table 1). The highest SEC value was observed for the US+Kenya CP equation and an intermediate value for the Kenya equation. The relatively high SEC value for the US+Kenya equation might be attributed to multiple laboratory errors associated with reference wet chemistry (Showers et al. 2006), and/or the large number of

**Table 2.** Validation results for the US, Kenya, and US+Kenya crude protein and US digestible organic matter equations using independent validation sets both with and without eliminating critical T (2.5) outlier samples.<sup>1</sup>

Diet constituent	<i>N</i>	SEP	<i>R</i> <sup>2</sup>	SEPC	Bias	Slope
US crude protein						
with outliers	40	2.73	0.69	1.64	2.2	0.89
without outliers	32	2.05	0.79	1.27	1.62	0.98
Kenya crude protein						
with outliers	100	2.91	0.66	2.78	0.92	0.76
without outliers	79	1.88	0.85	1.77	0.64	0.86
US+Kenya crude protein						
with outliers	35	1.79	0.82	1.74	0.41	0.84
without outliers	34	1.56	0.87	1.52	-0.56	0.81
US digestible organic matter						
with outliers	40	15.6	0.2	11.63	-10.5	2
without outliers	15	4.79	0.73	4.81	-1.14	2.1

<sup>1</sup>*N* indicates number of samples used in validation, SEP, standard error of prediction; *R*<sup>2</sup>, coefficient of simple correlation; and SEPC, standard error of prediction bias corrected.

diets mixed from different forage species and locations (Li et al. 2007), which was designed to achieve diversity.

Results of calibration also indicated that in all three CP equations, less than 5% of the samples were eliminated as T outliers, which is within acceptable limits for CP calibration (Hruschka 1987). Incorporating the majority of the calibration set indicated that the calibration equation had covered the full range of attribute values of samples, while maintaining acceptable accuracy. The SEC values for the three CP equations were less than twice the laboratory standard error set for CP (SEL = 0.5), indicating acceptable limits for NIRS calibration equations (Hruschka 1987). In addition, the SEC values were comparable to those reported by other authors (Lyons and Stuth 1992; Lyons et al. 1995; Purnomoadi et al. 1996; Showers et al. 2006). In terms of *R*<sup>2</sup>, the three equations had values above 0.90, indicating excellent calibration, and higher than those reported in other studies (Purnomoadi et al. 1996; Boval et al. 2004; Showers et al. 2006; Li et al. 2007).

Calibration results also showed subtle variation in SECV values among CP equations (Table 1). Although the US CP equation had the highest *R*<sup>2</sup> and lowest SEC values, it had SECV slightly higher than Kenya CP equations. This relatively high SECV value for the US equation might be explained partly by high within-animal variation. A standard error of 0.3 was obtained for CP due to individual animal variation, which is in accord with those reported by Stuth et al. (2003). Despite this disparity, the SECV represents approximately 10.8%, 13.0%, and 16.0% of the error of the mean CP concentration of the reference diets, respectively, for the US, Kenya, and US+Kenya calibration sets.

Recently, Cazzolino and Moron (2004) suggested that in addition to the standard statistical parameters (SEC, SECV, and *R*<sup>2</sup>) used in evaluating the performance of calibration equations, the SECV/SD ratio is a good indicator of how well the calibration equations perform for a constituent such as CP. Results from the present study showed that SECV/SD ratio were 0.28, 0.39, and 0.40 for the US, Kenya, and US+Kenya CP equations, respectively. These results indicated that the US CP equation was more stable than the Kenya and US+Kenya CP equations; i.e., the US equation is a better predictor when applied to diets outside the population. Dominant wavelengths

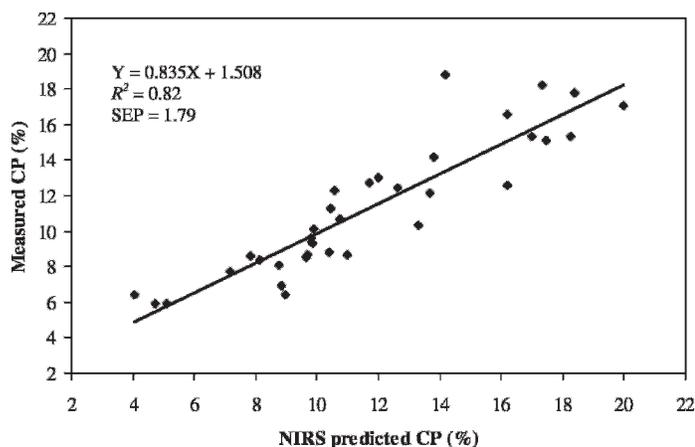
selected for each of the three CP equations (Table 1) were associated with protein and protein fraction materials, including amino acids and amines, and were in accord with wavelengths reported in other studies for ruminant. Murray and Williams (1987) further discuss specific information about the biological association of wavelengths. In general, based on the various predefined calibration parameters used to evaluate the predictive ability of CP equations, all three equations were deemed successfully developed and could be considered as a tool for characterizing diet quality once they are validated with independent sample sets.

### Validation of US CP Equation

Validation of the US equation was performed both with and without samples that had T values greater than 2.5 (Table 2). In both cases, SEP and *R*<sup>2</sup> values were much inferior to the SEC and *R*<sup>2</sup> values obtained for US CP calibration. These results indicate that the US equation showed moderate success in predicting Kenya dietary CP, particularly when the equation was applied to a validation set including T outlier samples. However, prediction error was considerably improved when the outliers were removed. The removal of about 20% from the Kenya sample set indicated the presence of a potential cross-laboratory error. This disparity was further confirmed by the presence of large bias values (1.62–2.2). However, the predictive ability of the US CP equation was still acceptable as indicated by the low prediction error related to the overall mean dietary CP.

### Validation of Kenya CP Equation

When the Kenya CP equation was applied to the US sample sets the resultant SEP was higher than the SEC obtained in calibration (Table 2). These results indicated that the Kenya CP equation was less successful in predicting CP of US diets. However, the *R*<sup>2</sup> indicated that two-thirds of the variation in the US CP value was expressed by the Kenya CP equation, indicative of a good relationship between fecal-NIRS and wet chemistry of diets. Improvements in terms of SEP and *R*<sup>2</sup> were also observed when the Kenya CP equation was applied to the US sample set after removal T outliers. These results indicated that the presence of errors associated with reference values can



**Figure 1.** Relationship between NIRS predicted and measured CP for the US + Kenya equation using a subsample of the combined dataset as independent validation set.

inflate the error associated with NIRS prediction equations, and better performance could be obtained if the equation were applied to validation sets without outlier samples. However, application of the CP equation to samples both with and without outliers resulted in bias values of less than 1%. These bias values indicated that the Kenya CP equation systematically underpredicted the CP content of diets obtained from the US trial, probably a result of differences in analysis technique between the two laboratories.

#### Validation of US + Kenya CP Equation

The validation set was comprised of 78% and 22% from the US and Kenya sample sets, respectively, and varied from 5.9% to 18.8% CP (within the range in the calibration set; Table 2). The  $R^2$  was relatively high, indicating the fecal-NIRS equation was successful in predicting the CP concentration of independent diets. As depicted in the Figure 1, there was a strong association between chemically measured (reference) and NIRS-predicted dietary CP. However, the SEP between measured and the NIRS-predicted CP was still high compared with the required criteria for protein. Considerable improvements were observed in terms of SEP and  $R^2$  when the CP equation was applied to sample sets after the removal of one outlier sample. Despite this improvement, the SEP was still high especially when compared with the SEC, indicating an effect of laboratory error on the predictive ability of the equation. However, when compared to previous results, the present SEP was considerably lower than those reported by Ossiya (1999) in ruminant animals.

The bias value underpredicted the validation sets by a mean of only 0.41, suggesting that the largest part of the error was due to random variation. In addition, linear comparison of predicted vs. measured CP demonstrated that there was an accurate fit for the average CP values (11.65 vs. 11.24). Thus, as evidenced from validation results, the CP equations (especially the US equation) showed acceptable performance in predicting independent samples and can be important tools for monitoring the dietary CP of forages consumed by donkeys with acceptable criteria for CP.

#### DOM Equation

Although the  $R^2$  value for the DOM equation was less than the acceptable standard, it still indicated that the majority of the variation in digestibility was explained by the equation (Table 1). Interestingly, in relation to the SEC value obtained for the DOM equation, the low  $R^2$  was unexpected and it could be attributed mainly to the uneven distribution of DOM values within the calibration set. The range of DOM was 12% to 62% in the calibration set, but only three of the 93 diet samples had digestibility below 30%, whereas more than 50 of the samples had digestibility above 50%. This discrepancy could contribute to the poor relationship between measured diet digestibility and fecal spectra.

The SEC value for the DOM was lower than twice the laboratory SE of 1.68 and 1.57, respectively, reported by Lyons and Stuth (1992) and Leite and Stuth (1995) in GANLAB, indicating that no significant amount of error was introduced due to laboratory or experimental procedures in the US trial. When compared to previous reports for ruminants, the present DOM equation showed an intermediate accuracy. The SEC was lower than those reported by Ossiya (1999) and Awuma (2003), comparable to those reported by Coates (1998) and Gibbs et al. (2002), and higher than those reported by other authors (Lyons and Stuth 1992; Li et al. 2007). It is noteworthy, however, that earlier reported equations were derived from either in vitro or in situ digestion trials that have relatively less source of error (Coates 1998, 2000). In vivo estimates of digestibility derived from total fecal collection are subject to a wide range of variations due to both animal and diet factors (Van Soest 1994). Animal variation could contribute to an increase in SEC and a decrease in  $R^2$  values (Boval et al. 2004). We were able to determine the individual animal variation by feeding donkeys the same diet (bermudagrass hay with 9% CP) during the adaptation week. The DOM of the diet was predicted using the selected equation. The SE for predicted DOM was 0.30 units, which is high value for equines but similar to ruminants (Stuth et al. 2003; Li et al. 2007). Another possible reason for the relatively high SEC value for the DOM equation could be the heterogeneous nature of the diets used in the US feeding trial. The accuracy of the calibration equations can be influenced by the composition of diets used in calibration because NIRS measures some associated effects of diets (e.g., metabolites and microbial composition; Boval et al. 2004). Although there are a number of possible sources of error associated with diet and animal, compared to the overall mean digestibility of the diets, the observed error (SEC) for DOM equation is still in acceptable level. This was further evidenced by the minimum SECv to population mean ratio (6.3%) observed for the equation.

#### Validation of DOM Equation

Predicting the digestibility of Kenya diets using the US equation was not satisfactory. The relationship between NIRS predicted and measured digestibility was poor as indicated by high values for SEP and bias, low values for  $R^2$ , and slope (Table 2). The presence of bias values greater than 10% indicated that the equation overpredicted the DOM concentration of the Kenya diets. When the same equation was applied after eliminating 65% of the original validation set as outlier samples, both SEP

and  $R^2$  were improved. Removal of major portion from the validation set, however, does not necessarily mean that the equation is not useful, particularly when the diverse nature of diets (both in calibration and validation) is considered. Instead, elimination of outliers was a reflection of elimination of poor field and/or laboratory techniques in the Kenya trial. As mentioned earlier, various animal and environmental variations could be contributing factors to the poor predictive performance of the equation. In this case, additional research might be required to determine if fecal-NIRS can attain a better level of accuracy for predicting digestibility in equines.

## MANAGEMENT IMPLICATIONS

We were able to calibrate and then validate first generation fecal-NIRS (CP and DOM) equations for donkeys. Both calibration and validation results indicated that CP equations had precisions equivalent to that of conventional wet chemistry methods, and were comparable to other fecal-NIRS equations reported for ruminants. Although reasonable results in some aspects of the calibration performances (e.g., SEC) were observed, the overall predictive performance of the DOM equation was less than the predefined standards.

Many resource managers and researchers still rely on direct chemical analysis of plant materials to assess forage quality, and animal nutritional status. We demonstrated that indirect prediction of diet quality via fecal-NIRS equations can determine the nutritional status of grazing donkeys with a reasonable error. In addition, NIRS has proven to be a cost-effective, fast, and, more importantly, a safe-to-operate technique. Also, we believe that quantitative data on diet quality obtained via fecal-NIRS can considerably enhance users' capacity to generate real-time information required for research and nutritional advice.

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