

Faecal near infrared reflectance spectroscopy to predict diet quality for sheep

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Received 4 August 2004; received in revised form 26 October 2005; accepted 26 October 2005

Available online 6 January 2006

Abstract

Faecal near infrared reflectance spectroscopy (NIRS) is a non-invasive method to determine diet quality in herbivores, but has not been reported for sheep (*Ovis aries*) in US. A diet reference chemistry:faecal near infrared spectra calibration ($n=78$) was developed to determine if faecal NIRS can predict diet quality of forage-fed sheep. In 2002 ($n=15$) and 2003 ($n=20$) mature ewes (55 ± 2.4 kg) were fed individual diets for 7 days. Diets ranged from 4.3 to 23.5% crude protein (CP) and 52.4 to 75.8% digestible organic matter (DOM) and were composed of various grass, forb and browse components. Daily intake was recorded. Faecal samples were collected on days 6 and 7. CP was determined by micro-Kjeldahl and DOM by an in vivo corrected in sacco technique. Partial least squares (PLS) and stepwise regression (SWR) techniques were used to develop predictive equations. Calibration results for percent dietary CP were: SWR, $R^2=0.93$, SE calibration (SEC)=1.27 and PLS, $R^2=0.95$, SEC=1.08. Calibration results for DOM were: SWR, $R^2=0.78$, SEC=1.58 and PLS, $R^2=0.80$, SEC=1.51. Equation validation was accomplished by cross validation, predicting an independent validation set, and by predicting day 7 samples with a day 6 derived equation within this study. Validation results indicate acceptable predictive ability. To determine the effect of individual animal variation on faecal NIRS predictions, five ewes were fed different forages in two, 7-day trials. Predicted percent CP and DOM from both trials indicate minimal effect on NIR predicted diet quality due to individual animal variation. Diet quality of forage-fed sheep can be accomplished by faecal NIRS.

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Keywords: Sheep; Near infrared reflectance spectroscopy; Faeces; Protein; Digestibility

1. Introduction

Near infrared reflectance spectroscopy (NIRS) of faeces has been successfully used to predict diet quality of livestock (*Bos* spp.: Boval et al., 2004; Coates,

1998; Lyons and Stuth, 1992; *Capra hircus*: Landau et al., 2004; Leite and Stuth, 1995). This technique has been applied to sheep (*Ovis aries*) in East Africa (Awuma, 2003; Ossiya, 1999) and Europe (Krachounov et al., 2000), but has not been applied to this species in North America. North American derived faecal NIRS equations for cattle have been used successfully in African cattle (Dyke, 1999) but it is not known whether the converse is also true. Cattle-derived faecal NIRS diet quality equations have been applied to

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sheep, but with undetermined accuracy (Stuth, personal communication).

Sheep, like cattle are domestic ruminants classified as grass and roughage eaters (Hofmann, 1988) but possess different anatomical and physiological characteristics and may consume proportionally more dicots on a given sward than do cattle (Holechek et al., 2001). This difference could result in different faecal physico-chemical characteristics and thus, NIR spectra, than cattle. Mixed-species grazing systems using sheep and cattle have been developed as a means of exploiting these dietary differences and improving pasture utilization (Holechek et al., 2001). With these differences in mind, it seems a logical assumption that to effectively monitor the nutrition of grazing animals with NIRS, species-specific diet quality calibrations are necessary. The objective of the study described herein was to determine the efficacy of faecal NIRS calibrations for predicting dietary CP and DOM in forage-fed U.S. sheep.

2. Materials and methods

2.1. Study area

The study was conducted at the small animal feeding facility, Animal Science Teaching, Research, and Extension Center of Texas A&M University, College Station (30.6° N, 96.3° W). All procedures were within standards as approved by the University Laboratory Animal Care Committee.

2.2. Experimental scheme

Two feeding trials were designed to obtain diet reference chemistry: faecal near infrared spectrum (D:F) pairs for use in developing NIRS predictive equations (Table 1). In Experiment 1 (2002), 15 animals were used in three consecutive feeding periods (i.e. fed three different experimental diets) and in Experiment 2 (2003), 20 animals were used in two consecutive feeding periods. Diets consisted of grass hays (C₃ and C₄ species), forbs, and browse species collected from across the western U.S. and were also used in a concurrent feeding study

with elk (*Cervus elaphus*). For a detailed description of diet components, see Keating (2004). Prior to mixing, forages were air dried and chopped to approximately 2.5 cm length. Eighty-five diets were prepared and fed but only 78 D:F pairs were successfully created in the two trials. Several animals were removed from the study for reasons of health or refusal to consume a particular diet. Mature non-pregnant crossbred ewes (55 ± 2.4 kg) of both fine and coarse wool breeds were used in both trials. Each ewe was housed in a 4 m² concrete floored pen with ad libitum water. All animals were fed an adaptation diet (~7% CP) for 7 days prior to being individually fed a unique experimental diet for 7 days. Diets were initially offered at 2% BW (as fed) and were split into two feedings, morning and evening. Daily intake was monitored and feed offered was adjusted to minimize orts. Diet samples and orts were collected on days 5–7. On day 5, each pen was cleaned to facilitate faecal sampling. Faecal samples were collected on days 6 and 7, and were frozen (–20 °C) until processed for NIRS.

2.3. Chemical analysis

Diet samples and orts were dried in a forced-air oven at 60° for 48 h and ground to pass a 2 mm screen. DOM was determined by in sacco procedures corrected to in vivo standards (Awuma, 2003). Briefly, samples were subjected to 48 h in situ rumen fermentation, using the Ankom filter bag technique (Komarek et al., 1994), followed by 1 h neutral detergent fiber (NDF) analysis (Van Soest and Wine, 1967) using an Ankom fiber analyzer. The 48 h in situ fermentation replaced the in vitro fermentation of Tilley and Terry (1963). The hay standards with known in vivo organic matter digestibility (Hunt et al., 1995) were alfalfa (*Medicago sativa*, 76.26%), kleingrass (*Panicum coloratum*, 64.98%), and wheat (*Triticum aestivum*, 54.81%). Diet and orts were analyzed for CP by micro-Kjeldahl procedures (AOAC, 1984). The ort-corrected dietary CP and DOM values were averaged across days within animal for use as a reference value in NIRS equation development.

Frozen faecal samples were thawed at room temperature (~20 °C), dried at 60° in a forced air oven

Table 1
Diet quality characteristics for sheep fecal NIRS calibrations

Experiment	Animals, <i>N</i>	Diets, <i>N</i>	Diet constituent	Range (%)	Mean (%)
1	15	45	CP	4.30–23.50	10.09
			DOM	52.38–75.78	67.74
2	20	40	CP	5.60–21.60	13.08
			DOM	52.84–66.47	61.02

for 12 h and ground in a laboratory mill to pass a 1 mm screen. Ground samples were then subjected to the same drying regimen to stabilize moisture content. Before scanning, samples were placed in a desiccator for 1 h to equilibrate with ambient temperature. Faecal spectra (1108–2498 nm) were obtained on a Foss 6500[®] monochromator with spinning drawer attachment.

2.4. NIRS equation development

For comparison, calibration equations were developed using multiple stepwise (SWR, Hruschka, 1987) and modified partial least squares regression procedures (PLS, Martens and Naes, 1987) in Win ISI[®] v 1.50 software. For each regression method and constituent, 24 iterations varying in derivative, gap, smooth, and segment were performed (see review by Dryden, 2003). The “best” predictive equation was then selected from this set. Equation selection involves consideration of several factors including: (1) standard error of calibration (SEC), (2) laboratory standard error for the reference method (SEL), (3) coefficient of determination (R^2), (4) equation wavelength F -statistics, and (5) standard error of cross validation (SECV).

Outlier selection was determined on two different characteristics: (1) spectral outliers, i.e. those samples with spectra greater than eight Mahalonobis distance (GH) units from the centroid spectrum in the calibration set and (2) reference method outliers, i.e. those samples having predicted values outside the 95% confidence interval based on observed reference values of similar spectra in the calibration set.

2.5. NIRS equation validation

To validate the calibrations, independent D:F sets (CP only) were obtained from two groups of mature crossbred ewes in South Dakota. In Experiment 3, similar to the calibration procedures, 10 ewes were fed 10 different rations in a 7-day feeding trial. Faecal samples were collected on day 7. Diet samples were a composite of days 6 and 7. Each ration was a mixture of prairie hay and either 0, 5, 10, 20, or 40% of two different grass/forb mixtures, hand harvested from weed infested farm fields. In Experiment 4, six ewes were fed six different diets in two, 4-day digestibility trials. Thus, in each trial, two ewes received a given diet. All diets were combinations of alfalfa, crested wheatgrass (*Agropyron cristatum*), and prairie hay. Each trial was preceded by a 2-week adaptation period. Diet samples were a composite of all 4 days. Faecal samples were collected on day 4.

Additionally, calibration equations from Experiments 1 and 2 were ultimately developed with day 6 faecal samples; day 7 faecal samples then served as a within study validation set (CP and DOM). Validation of the selected CP and DOM equations was performed by simple linear regression.

The contribution of individual animal variation to measurement error was determined using five mature crossbred ewes in Texas (Experiment 5). These ewes, not used in the calibration, were fed a bermudagrass (*Cynodon dactylon*) hay/wheat hay mixture (Trial 1), and alfalfa (Trial 2), ad libitum in 7-day feeding trials. Again, diet quality for individual faecal samples was predicted with calibration equations developed in Experiments 1 and 2. Means and standard errors were calculated for each group of samples.

3. Results and discussion

3.1. Crude protein

Table 2 contains equation performance statistics for the selected SWR and PLS equations from the current study. To be considered acceptable, NIRS equations should have an $R^2 > 0.80$, and SEC no larger than approximately $1.5\text{--}2 \times \text{SEL}$. Both CP equations have excellent R^2 , similar to previous studies (0.98, Boval et al., 2004; 0.94, Coates, 1998; 0.98, Landau et al., 2004; 0.94, Leite and Stuth, 1995; 0.92, Lyons and Stuth, 1992). The SEC for CP are similar to those reported for goats (1.12) by Leite and Stuth (1995), but higher than that reported (0.4) by Landau et al. (2004). The current SEC values are also higher than those observed for cattle (0.33, Boval et al., 2004; 0.83, Coates, 1998; 0.89, Lyons and Stuth, 1992). Keating (2004) attributed the higher SEC he observed for elk than for cattle CP equations to diets containing relatively large (>15%) proportions of browse. Many of these same browse species were used to formulate rations in

Table 2
Calibration results for fecal NIRS sheep diet quality equations

Regression method	Diet constituent	SEL ^a	SEC ^b	R^2	SECV ^c
PLS ^d	CP	0.50	1.08	0.95	1.51
	DOM	1.44	1.51	0.80	2.06
SWR ^e	CP	0.50	1.27	0.93	1.35
	DOM	1.44	1.58	0.78	1.65

^a Standard error of laboratory reference method.

^b Standard error of calibration.

^c Standard error of cross validation.

^d Partial least squares regression.

^e Stepwise regression.

this study, although in smaller proportions; a fact which could have influenced the SEC in the current CP equations. No samples were identified as outliers (spectral or reference method) in the SWR calibration for CP; three were identified as reference method outliers in the PLS calibration.

3.2. Digestible organic matter

The DOM equations yielded acceptable (PLS, 0.80), or just less than acceptable (SWR, 0.78) R^2 values (Table 2). Lyons and Stuth (1992) (0.80) reported similar values for cattle. Leite and Stuth (1995) observed higher values (0.93) for goats. Boval et al. (2004) reported $R^2 = 0.98$ for OMD in cattle, while Landau et al. (2004) observed $R^2 = 0.72$ for DDM in goats. The PLS equation had a SEC value (1.51) very close to that of the SEL (1.44). This SEC is similar to that reported (1.66) by Lyons and Stuth (1992) for cattle, but lower than that observed (2.02) by Leite and Stuth (1995) in goats. Krachounov et al. (2000) in sheep and Coates (1998) in cattle reported SEC values of 2.26 and 2.20 for DDM and OMD respectively. For comparison, the SWR DOM equation had a SEC = 1.58. No outliers were selected for DOM by the PLS calibration and only one sample was determined to be a reference method outlier in the SWR calibration.

3.3. NIRS equation validation

Cross validation is often employed when an independent validation set is unavailable or when removal of samples from a calibration set results in too few samples for effective equation development. Briefly, this process involves removing a certain number of samples during the calibration procedure, e.g. 25%, and predict-

ing these with the remaining 75%. This step is then repeated until all have served as validation samples. The combined standard error for each of these steps is the SECV. The SECV values reported here (Table 2) are similar to the SEC for each equation and within the acceptable limits compared to SEL as described above. This indicates acceptable predictive ability for these equations when applied to samples exhibiting spectra within eight GH units from the mean. In direct NIRS calibrations (spectrum collection and reference method performed on the same material), a GH value of 3 is usually employed, however the results of Walker et al. (2000) suggest that larger GH values can be used for faecal NIRS predictions of dietary constituents (indirect). This results in fewer samples being considered as spectral outliers, and thus greater spectral diversity in the calibration.

Results of using the selected CP equations to predict diet quality from faecal samples in Experiments 3 and 4 are listed in Table 3. In Experiment 4, R^2 values for both PLS and SWR were greater than 0.80 and slope values were near 1.0. The predicted mean CP for PLS was not different ($P > 0.10$) than the mean reference method CP. The SWR mean predicted CP was greater ($P < 0.03$) than the reference value, and is reflected in both a high bias and SEP. There was only one sample identified as an outlier (reference method) by the SWR equation in this validation set; none were identified by the PLS equation. Removal of this sample had a minor affect; R^2 and SEP improved to 0.97 and 3.33, respectively. In Experiment 3, the range of values was small (7.4–10.5), with 6 of the 10 samples between 7 and 8% CP. As a result, R^2 and slope values were also small (<0.6 and 0.8, respectively). The bias and SEP values in this group, however, indicate predictive accuracy to within ~1.0% unit for CP (95% CI). Mean values for predicted and reference CP were

Table 3
Results of sheep fecal NIRS equation validations: South Dakota and Texas feeding trials

Location	Experiment	N	Equation type	Diet constituent	Observed mean	S.D.	Predicted mean	S.D.	R^2	SEP ^a	Bias	Slope
South Dakota	3	10	PLS	CP	8.03	0.98	7.48	1.01	0.55	0.88	0.55	0.72
			SWR	CP	8.03	0.98	7.74	0.99	0.22	1.01	0.29	0.47
	4	12	PLS	CP	11.18	4.09	11.97	3.45	0.80	1.92	-0.79	1.06
			SWR	CP	11.18*	4.09	14.91*	3.58	0.95	3.85	-3.73	1.11
Texas	1 and 2	78	PLS	CP	12.16	4.97	12.04	4.78	0.81	2.17	0.11	0.94
			SWR	CP	12.16	4.97	12.45	4.65	0.89	1.65	0.29	1.01
	1 and 2	78	PLS	DOM	63.66	3.36	63.09	3.17	0.66	2.09	0.58	0.86
			SWR	DOM	63.66	3.36	63.40	3.29	0.67	1.98	0.26	0.84

^a Standard error of prediction.

* $P < 0.03$.

Table 4
Effect of individual animal variation on sheep fecal NIRS diet quality predictions

Experiment	Forage	N	CP mean	CP minimum	CP maximum	S.E.	DOM mean	DOM minimum	DOM maximum	S.E.
5	Bermuda/Wheat	5	11.2	10.3	12.3	0.47	62.2	61.4	62.8	0.29
	Alfalfa	5	21.1	20.3	22.5	0.40	65.6	64.4	66.8	0.43

not different ($P > 0.01$). There were no outliers identified in this group of samples.

Adding samples from Experiments 3 and 4 to those from the calibration in 1 and 2 resulted in CP equations with $R^2 = 0.95$ and $SECV = 1.30$ for PLS and, $R^2 = 0.92$ and $SECV = 1.31$ for SWR. Removing a random 10% of this combined calibration set and using them for validation resulted in CP equations with $R^2 = 0.96$ and $SECV = 1.18$ for PLS and, $R^2 = 0.93$ and $SECV = 1.24$ for SWR. Prediction of the 10% validation set resulted in $R^2 = 0.96$ and 0.93 , $SEP = 1.18$ and 1.56 , and slope = 0.98 and 0.97 , for PLS and SWR, respectively. Addition of Experiment 3 and 4 samples to those derived in Experiments 1 and 2, improved the predictive ability of the original calibration.

Results of using CP equations (Experiment 1 and 2) developed from day 6 samples to predict day 7 samples are illustrated in Table 3. As one might expect from the calibration results, CP validations yielded better prediction statistics than those for DOM. Values for R^2 were larger, bias was less, and slope closer to 1.0 in the CP versus DOM predictions, respectively. Interestingly, values for SEP were similar between the two constituents for both PLS and SWR and mean predicted versus reference values were not different ($P < 0.10$) for PLS nor SWR. Removal of five, or three, reference method CP outliers improved R^2 and SEP to 0.91 and 1.51, and 0.91 and 1.46, for PLS and SWR, respectively. Similarly, one versus two reference method DOM outliers were removed from PLS and SWR validations, respectively; improving R^2 and SEP to 0.70 and 1.91, and 0.74 and 1.76.

3.4. PLS versus SWR

Earlier faecal NIRS equations were most often developed using SWR; either this was the method of choice at the time the work was carried out (e.g. Lyons and Stuth, 1992; Leite and Stuth, 1995), or gave better results than PLS in later studies (Showers, 1997). PLS has been employed in more recent studies (Boval et al., 2004; Landau et al., 2004). Our results indicate no clear advantage to either method in this study.

3.5. Individual animal variation

The range of predicted values in Experiment 5 was approximately 2.0 units for both CP and DOM across both forage types (Table 4). The SE for predicted CP was 0.44%; for predicted DOM the SE was 0.36%. The individual animal variation observed here is small compared to overall prediction error. Recall that SECV for CP and DOM equations were approximately 1.40 and 1.75%, respectively. Thus, individual animal variation, as observed in this study, would only be approximately 25–50% of the observed diet quality prediction error.

4. Conclusions

As has been observed for other domestic and wild ruminants, faecal NIRS can be used to determine diet quality in sheep. The predictive equations developed here will improve diet quality monitoring capabilities for North American sheep producers. Consistent with the conclusions of others who have reported first generation faecal NIRS equations however, the current calibration set should be expanded to include greater variation in forage diets (temporal and spatial). Faecal NIRS not only provides a method to monitor and improve diet quality of grazing animals, it also allows scientists and resource managers to use grazing animals as a means of monitoring grazing land health.

Acknowledgments

The authors would like to thank Trey Dittmar, Kris Banik and Allison Groves for help conducting the feeding trials; Dave Schmidt for forage collection; Jennifer Kramer and Kathy Black for manuscript preparation.

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