Near Infrared Spectroscopy of Sheep Feces for Predicting Botanical Composition of Diets

J.W. Walker, S.D. McCoy, K.L. Launchbaugh, and M.J. Fraker

ABSTRACT

The lack of simple and affordable techniques for estimating the dietary composition (both chemical and botanical) of free-grazing ruminants is a major limitation to grazing management. The objectives of this study were to expand the use of near infrared spectroscopy (NIRS) of fecal samples for predicting the percentage of mountain big sagebrush (*Artemisia tridentata* Nutt. ssp. *vaseyana* (Rydb) Beetle) in the diet, investigate the ability of NIRS to simultaneously predict multiple botanical species or categories, and to quantify the limitations of using NIRS of fecal samples to predict diet composition. This research was conducted at the USDA, U.S. Sheep Experiment Station located in Dubois, Ida. Fecal materials from three feeding trials with known levels of sagebrush were used to develop NIRS fecal calibration equations and to validate these equations. The 1996 trial varied both level of sagebrush and levels of alfalfa and grass hay. The 1998 trial compared frozen to air-dried sagebrush. The Wyoming trial was a metabolism study using frozen sagebrush. Trials used different levels of sagebrush varying from 0 to as much as 30% of the diet in increments of 4 to 10 percentage points. All data sets had acceptable calibrations for percentage of sagebrush in the diet. Coefficients of determination ranged from 0.91 to 0.95 with a standard error of calibration (SEC) of about 2 percentage points or about 15% of the mean level of sagebrush in the diets fed. The validation statistics for independent data sets were generally acceptable, with some exceptions. Validation for internal data sets, i.e., when validation data were a subset of samples used for calibration, were excellent with an R^2 exceeding 0.90, slopes of 1.0 and coefficient of variation from 11 to 22%. The average H statistic far exceeded the recommended value of 3 for all external validation data sets (i.e., data sets that were not a subset of the same trial from which the calibration was developed). However, a small average H did not ensure that the bias and slope did not exceed acceptable limits. This use of NIRS of fecal samples to predict botanical composition of diets made research possible that could not have been conducted with other procedures.

Sheep and Goat, Wool and Mohair CPR 2000:81-94

Introduction

The lack of simple and affordable techniques for estimating the dietary composition (both chemical and botanical) of free-grazing ruminants is a major limitation to grazing management. Near infrared spectroscopy (NIRS) of fecal samples to predict the percentage of leafy spurge (*Euphorbia esula* L.) in the diets of sheep and goats is an alternative to more costly procedures such as microhistological analysis (Walker et al., 1998).

The objective of this study was to expand the use of NIRS of fecal samples for predicting the percentage of mountain big sagebrush (*Artemisia tridentata* Nutt. ssp. *vaseyana* (Rydb) Beetle) in the diet and as a methodology for estimating the phenotypic expression of sagebrush consumption for estimation of the heritability of preference for sagebrush (Snowder et al., 2000). We also investigated the ability of NIRS to simultaneously predict multiple botanical species or categories. Finally, we tried to quantify the limitations of using NIRS of fecal samples to predict diet composition in the case typical of free grazing animals where samples are not available for validating suspected spectral outliers.

Methods

This research was conducted at the USDA, U.S. Sheep Experiment Station located in Dubois, Ida. Fecal materials from three feeding trials with sheep fed known levels of sagebrush were used to develop NIRS fecal calibration equations and to validate these equations. A feeding trial was conducted in 1996 to develop a NIRS calibration equation. A feeding trial in 1998 was conducted to validate the 1996 trial and determine if the intake of sagebrush was affected by air-drying compared to storing samples frozen before feeding. Fecal samples from a trial conducted in Wyoming to determine the effect of increasing levels of sagebrush on intake and digestibility were analyzed to validate and compare results from the 1996 and 1998 feeding trials.

1996 Feeding Trial

The 1996 feeding trial consisted of feeding varying levels of mountain big sagebrush, early bloom alfalfa hay (*Medicago sativa* L.) and a grass hay that was composed of smooth brome (*Bromus inermis* Leyss.) and timothy (*Phleum pratense* L.). The sagebrush was collected in late September 1996 and air-dried in the shade (maximum daily temperature 70° to 85° F). Diets were mixed to contain 0, 4, 8, 12, 16 and 24% sagebrush with a base diet of alfalfa/grass hays in the following proportions: 0:100, 20:80, 40:60, 60:40, 80:20 and 100:0 for a total of 36 different diets. Molasses was added to the diets (5% DM) during the mixing process to cause feed to adhere together and reduce sorting when fed.

The diets were fed to 36 mature white-faced western range ewes housed in individual pens. Diets were fed at 0800 and 1600 h and refusals were removed at 0800 h. The trial

consisted of a 6-d adaptation period and 4-d collection period. Fecal samples were collected from the floor of the pens at1600 and 0800 h, and dried in a forced air oven at 140 °F.

1998 Feeding Trial

The 1998 feeding trial consisted of feeding varying levels of mountain big sagebrush that was stored either air-dried or frozen before the study (Fraker 1999). The sagebrush for this trial was current season's growth of vegetative stems (no flowering stalks) collected the last week of August 1997. Half of the sagebrush was air dried (maximum temperature 85° to 90°F) and stored in plastic bags, and half was frozen promptly after collection and stored in a freezer. Diets were mixed to contain 0, 8, 16 and 24% of either dry or frozen sagebrush with a base diet of 1:1 alfalfa/grass (grass was a mixture or smooth brome and timothy hay) for a total of 7 different diets. Molasses was added to the diets (5% DM) during the mixing process to cause feed to adhere together and reduce sorting.

An intake and digestion trial was conducted in January 1998 using 6 to 7-month-old white-faced crossbred lambs. The animals were penned individually and 5 animals were assigned to each diet for a total of 35 animals (34 wethers and 1 ewe). Animals were fitted with fecal bags to determine total fecal production. Feed was offered in excess from 0800 to 1100 h and from 1300 to 1600 h. Every 30 min, while feed was offered, feed bunks were checked and more feed was added if needed. This procedure limited the potential for sorting and minimized the amount of refusal. The trial consisted of a 5-d adaptation period and a 6-d collection period. Fecal bags were emptied twice daily, and a sub-sample of feces was collected. Afternoon samples were composited with collections made the following morning and dried in a forced air oven at 140°F.

Wyoming Feeding Trial

Fecal samples from a feeding trial conducted by Ngugi et al. (1995) and kindly provided by Jeff Powell, University of Wyoming, were used to validate a calibration equation based on the combined 1996 and 1998 trials. This trial used 16 Rambouillet wether lambs (60 to 90 lb body weight) fed diets containing mixtures of hand-harvested current year's growth of mountain big sagebrush leaves and native grass hay. Sagebrush leaves were harvested in September from the western edge of the Medicine Bow Range, Carbon County, Wyo., and stored in sealed plastic bags in a freezer until fed. Four diets in the following proportions of grass hay:sagebrush: 100:0, 90:10, 80:20, and 70:30 were fed. Four animals received each diet. The trial consisted of a 9-d adjustment period followed by a 6-d collection period when all urine and feces were collected. Fecal samples were composited by animal for the 6-d collection period and ground through a 1-mm screen of a Wiley mill. Samples were reground through a cyclone mill before being scanned to collect NIRS reflectance. Details of this trial were presented by Ngugi et al. (1995).

Equation Development

All fecal samples were ground in a cyclone mill to pass a 1-mm screen, packed into sample cells with a near-infrared transparent quartz cover glass, and scanned 32 times using a NIR Systems, Inc. (Silver Spring, Md.) model 6500, scanning reflectance monochromator. Reflected energy (log 1/R) was measured, averaged over the 32 scans and recorded at 2-nm intervals from 400 to 2,500 nm.

Calibration equation development was done using stored NIRS spectra from fecal samples as the independent variables and percent sagebrush, grass or alfalfa fed in the diets as the dependent reference data in the 1996, 1998 and Wyoming trial diets. Before calibration, each spectrum was transformed with a (1,8,8) derivative. The first number in parenthesis is the order of the derivative the second number is the gap (number of data points), and the third number is the smooth (number of data points). Scatter correction was done with the standard normal variance and detrend procedure. Prediction equations were developed using stepwise regression. A maximum of five wavelengths were selected with entry criteria based on maximizing R^2.

Data Analysis

Data from the 1996 and 1998 trials were individually examined to identify and remove outlier samples. Separate calibration equations and outlier eliminations were done for the 1996 and 1998 trials. Samples, which were contained in the calibration data set, that had an H larger than 3 or a residual "t" statistic greater than 3 were eliminated. This resulted in the elimination of 6 and 10 samples from the 1996 and 1998 data sets, respectively, or approximately 5% of the samples from each of the data sets.

Calibration equations were evaluated for usefulness based on validation statistics for unrelated samples. Calibration refers to the development of a multiple regression equation using the reflected energy (log 1/R) of the near infrared spectra as independent variables to predict the botanical component (i.e., dependent variable or reference value). Validation refers to the ability of the calibration equation to predict the reference value of a sample that was not part of the data set used in the development of the calibration equation. Validation samples are referred to as internal if they were a subset of a uniform group of samples used to develop a calibration equation and external if they were from a set of samples from a different trial or treatment. The statistics that were evaluated included standard error of prediction (SEP), coefficient of determination, slope and bias calculated from the predicted and actual values for percent composition of the diet. Six pairs of calibration and validation data sets were evaluated for the ability to predict percentage of sagebrush in the diet.

1. The 1996 and 1998 data sets were combined and divided into calibration and validation data sets. Calibrations for the 1996 data were based on samples collected on d 1, 2, and 4 of the 4-d collection period and d 1, 3, and 5 for the 6-d collection period in 1998. Validation data sets were composed of samples collected on the

remaining days so that the calibration data set contained about 72% of the samples and the validation set contained 28% of the samples (96&98 Internal).

2. Combining all of the 1996 and 1998 data for the calibration data set and validating these data with the Wyoming data (96&98 → Wyoming).
3. Using the 1996 samples for calibration and the 1998 samples for validation (1996 → 1998).
4. Using the 1998 samples for calibration and the 1996 samples for validation (1998 → 1996).
5. Using the 1998 diets from dry sagebrush to predict the 1998 frozen sagebrush diets (DRY → FROZEN).
6. Using the 1998 diets from frozen sagebrush to predict the 1998 dry sagebrush diets (FROZEN → DRY).

The only samples that were appropriate to evaluate the ability of NIRS of fecal samples to predict multiple botanical components in the diet were the 1996 samples. For this analysis the samples from d 1, 2 and 4 were used for calibration and d 3 was used for validation.

Results and Discussion

All data sets had acceptable calibrations for percentage of sagebrush in the diet (Table 1). Coefficients of determination ranged from 0.91 to 0.95 with a SEC of about 2 percentage points or about 15% of the mean level of sagebrush in the diets fed. The validation statistics for independent data sets were generally acceptable, with some exceptions. As expected, the validation of combined 1996 and 1998 samples (96&98 Internal) with an equation based on a subset of these samples resulted in one of the best calibrations. The Wyoming validation had a high coefficient of determination, but the slope of 3.1 indicated that the range of the predicted values was about one-third the range of the actual samples and the bias of 11.1 showed that the predicted values averaged 11 percentage points less than the actual values (Fig. 1).

The low coefficient of determination (0.68) for the validation of the 1998 frozen samples with the calibration equation calculated from the 1998 dry samples (DRY → FROZEN) was caused primarily by predicted values for percentage of sagebrush in the diet that were lower in the 24% sagebrush diets (Fig. 2). This may have been caused by a lower intake by animals fed this high level of FROZEN sagebrush (Fraker, 1999). In contrast, the Wyoming data validated well (R^2 = 0.89, SEP = 13.7) even though there was a much greater decrease of intake with increasing levels of sagebrush (Ngugi et al., 1995) compared to the 1998 trial. Validation statistics for the calibration equation developed from the 1998 frozen samples to predict the 1998 dry samples were much improved. Presumably, the stepwise regression procedure of the 1998 frozen samples

identified wavelengths that were not affected by reduced intake when higher percentage levels of dry sagebrush were fed.

The use of calibration equations developed from either of the two different trials to predict the other trial (96 → 98 or 98 → 96) resulted in somewhat similar validation statistics. Both calibrations had coefficients of determination of 0.83, but the slope and bias for the predicted 1998 samples indicated that the range of the predicted values and the mean of these values were less than actual values (Fig. 3). In contrast, the predicted values for 1996 samples had a range and mean that was greater than the actual values (Fig 3). The higher SEP (9.9 percentage points) for the 1998 validation statistics compared to the 1996 samples (4.6 percentage points) was a result of the higher bias in the former set of samples.

Internal validation of the 96&98 data set provided a satisfactory average H statistic (1.0) and a perfect slope (1.0) proving that NIRS can be a highly reliable tool for estimating botanical composition of diets if the predicted samples are from the same population as the calibration samples. However, for all external validation data sets, i.e., data sets that were not a subset of the same trial from which the calibration was developed (Table 1), the average H statistic far exceeded the recommended value of 3. A small average H did not ensure that the bias and slope did not exceed acceptable limits (Shenk et al., 1989) as in the 1998 feeding trial DRY → FROZEN or FROZEN → DRY calibration and validation test. Based on the suggested limits of Shenk et al. (1989), these equations would not be applicable beyond similar samples generated in the same feeding trial from which calibrations were developed as in the 96&98 internal calibration validation data sets.

Calibration and internal validation statistics for the prediction of sagebrush, alfalfa, and grass from fecal samples from the 1996 trial (Table 2) show that NIRS of fecal samples could predict an array of dietary components (Fig. 4). The SEP was greater in the Alfalfa and Grass components, but this was related to the larger mean for these components. The CV for these different components ranged from 11 to 20%. For comparison, Walker et al. (1998) showed a CV of around 10% for NIRS predictions of leafy spurge in the diet, and Coleman et al. (1995) reported a CV of around 16% for percent digestibility of diets.

Conclusions

The results of this study show that NIRS of fecal samples is a useful tool for predicting botanical composition of diets of free-ranging ruminants when the calibration fecal samples are a sub-sample of the population of fecal samples whose near infrared spectra are being used to predict the diet composition. We also believe this is a useful but less accurate procedure when calibration equations are based on fecal samples from a different population than the one being predicted. These results indicate that the use of NIRS of fecal samples to predict botanical composition of diets should be limited to

instances where relative differences between treatments or animals grazing similar pastures are a sufficient level of measurement.

We recently used equations developed from these data sets to estimate the amount of sagebrush in diets of almost 2,000 free-ranging sheep. This was done to estimate the heritability of preference for sagebrush (Snowder et al., 2000a,b). Whereas the accuracy of the predictions is unverifiable, the results were in line with what we anticipated in that animals that consumed more sagebrush in September also consumed more in October. Cook and Harris (1968) also reported that overall sagebrush consumption increased as the season progressed. However, the mean level of sagebrush in the diets was higher than we would have anticipated, indicating a probable bias in the data. Despite this potential shortcoming a study of that magnitude would not have been possible with any other available technology.

Table 1. Calibration and validation statistics for fecal NIRS equations used to predict percentage of sagebrush in the diets of sheep

	Calibration Statistics				Validation Statistics						
Data Sets	n	R^2	Mean[a]	SEC[b]	n	R^2	SEP[c]	Slope[d]	SEP(C)[e]	Bias[f]	Average H[g]
96 & 98 Internal	240	0.92	12	2.3	97	0.90	2.7	1.0	2.7	1.4	1.0
96 & 98 Wyoming	337	0.91	12	2.4	20	0.89	13.7	3.1	8.3*	11.1*	10.2*
1996 → 1998	137	0.95	10	1.8	200	0.83	9.9	1.7	4.4*	8.8*	8.1*
1998 → 1996	200	0.93	13	2.2	137	0.83	4.6	0.7	4.5*	-0.8	5.2*
DRY → FROZEN	106	0.94	13	2.0	94	0.68	7.6	1.2	4.6*	6.0*	1.6
FROZEN → DRY	94	0.95	13	1.8	106	0.89	3.1	1.1	2.9*	1.2*	1.6

[a]Mean = mean percentage of sagebrush in calibration diets.
[b]SEC = standard error of calibration.
[c]SEP = standard error of prediction.
[d]Slope = slope of the line between reference and predicted values.
[e]SEC(C) = standard error of prediction corrected for bias.
[f]Bias = systematic error (i.e., intercept).
[g]Average H = standardized Mahalanobis distance.
*Exceeded value recommend by Shenk et al. 1989.

Table 2. 1996 feeding trial calibration and validation statistics for fecal NIRS equations used to predict sagebrush, alfalfa, and grass in the diets of sheep

	Calibration Samples				Validation Samples							
	n	R^2	Mean[a]	SEC[b]	n	R^2	SEP[c]	Slope[d]	SEC(C)[e]	Bias[f]	Average H[g]	CV[h]
Sagebrush	106	0.95	11	1.8	31	0.93	2.1	1.0	2.1	-0.1	1.1	0.20
Alfalfa	102	0.97	44	5.1	30	0.98	5.1	1.0	4.5	2.4	1.0	0.11
Grass	102	0.96	45	5.9	30	0.96	6.2	1.0	6.2	0.9	1.1	0.14

[a]Mean = mean percentage of sagebrush in calibration diets.
[b]SEC = standard error of calibration.
[c]SEP = standard error of prediction.
[d]Slope = slope of the line between reference and predicted values.
[e]SEC(C) = standard error of prediction corrected for bias.
[f]Bias = systematic error (i.e., intercept).
[g]Average H = standardized Mahalanobis distance.
[h]CV = residual coefficient of variation calculated by dividing SEP by the reference mean × 100.

Figure 1. Actual percent mountain big sagebrush fed vs predicted percent using NIRS prediction equations based on fecal spectra. Data represent an internal validation of the combined 1996 and 1998 feeding trials subset into calibration and validation data sets (•), or an external validation using the entire 1996 and 1998 data to develop an equation to predict samples from a Wyoming feeding trial (+).

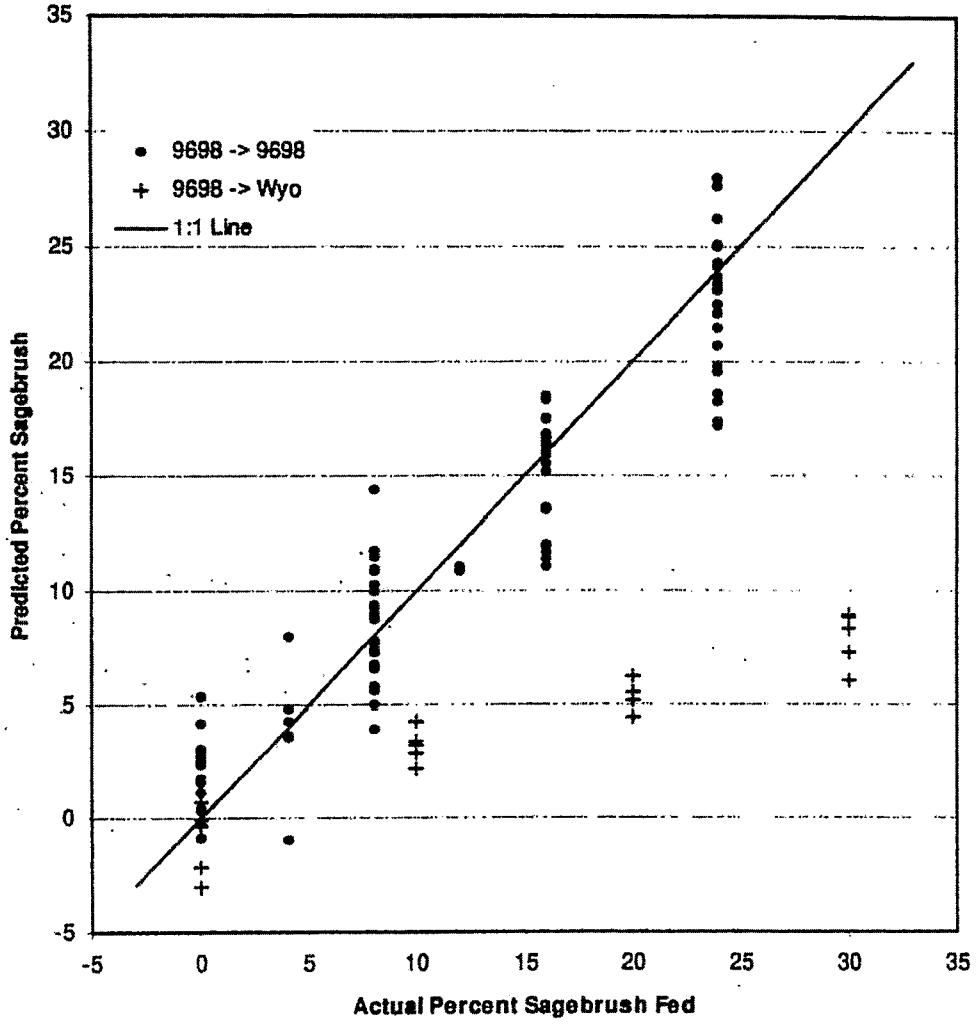


Figure 2. Actual percent mountain big sagebrush fed vs NIRS prediction equations using fecal spectra. Data represent equations developed with the 1998 DRY samples to predict the 1998 FROZEN samples (•), or the 1998 FROZEN samples to predict the 1998 DRY samples (+).

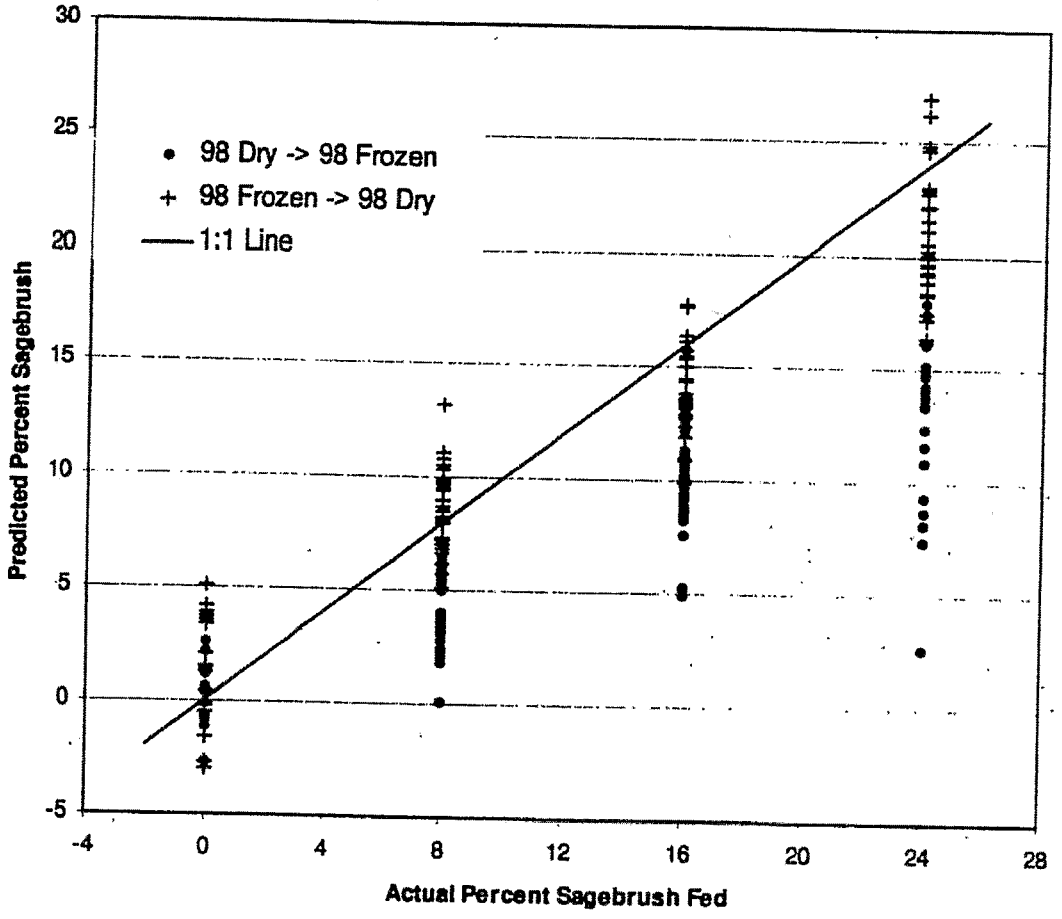


Figure 3. Actual percent mountain big sagebrush fed vs NIRS prediction equations using fecal spectra. Data represent equations developed from the 1998 feeding trial to predict the 1996 feeding trial samples (•), or from the 1998 feeding trial to predict the 1996 feeding trial samples (+).

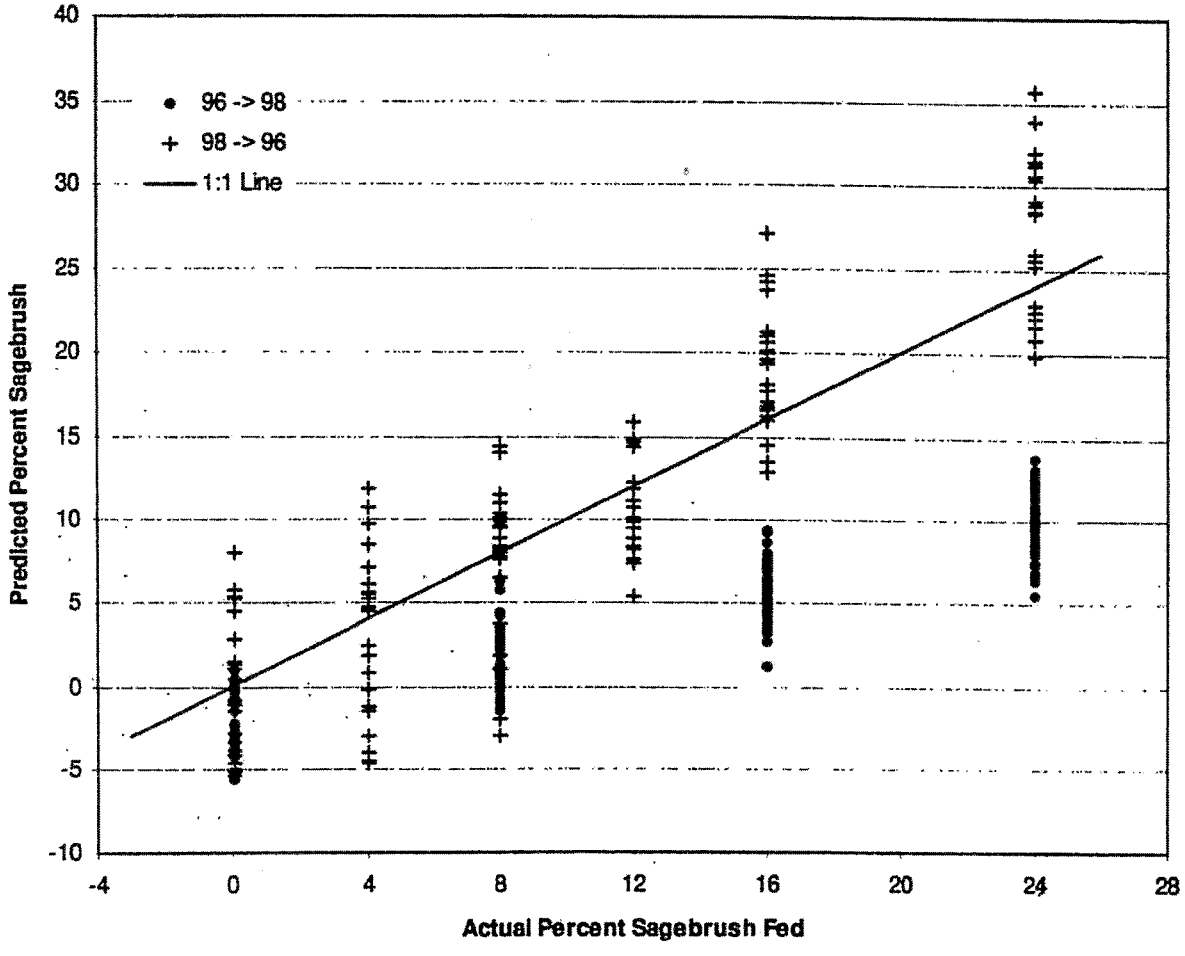
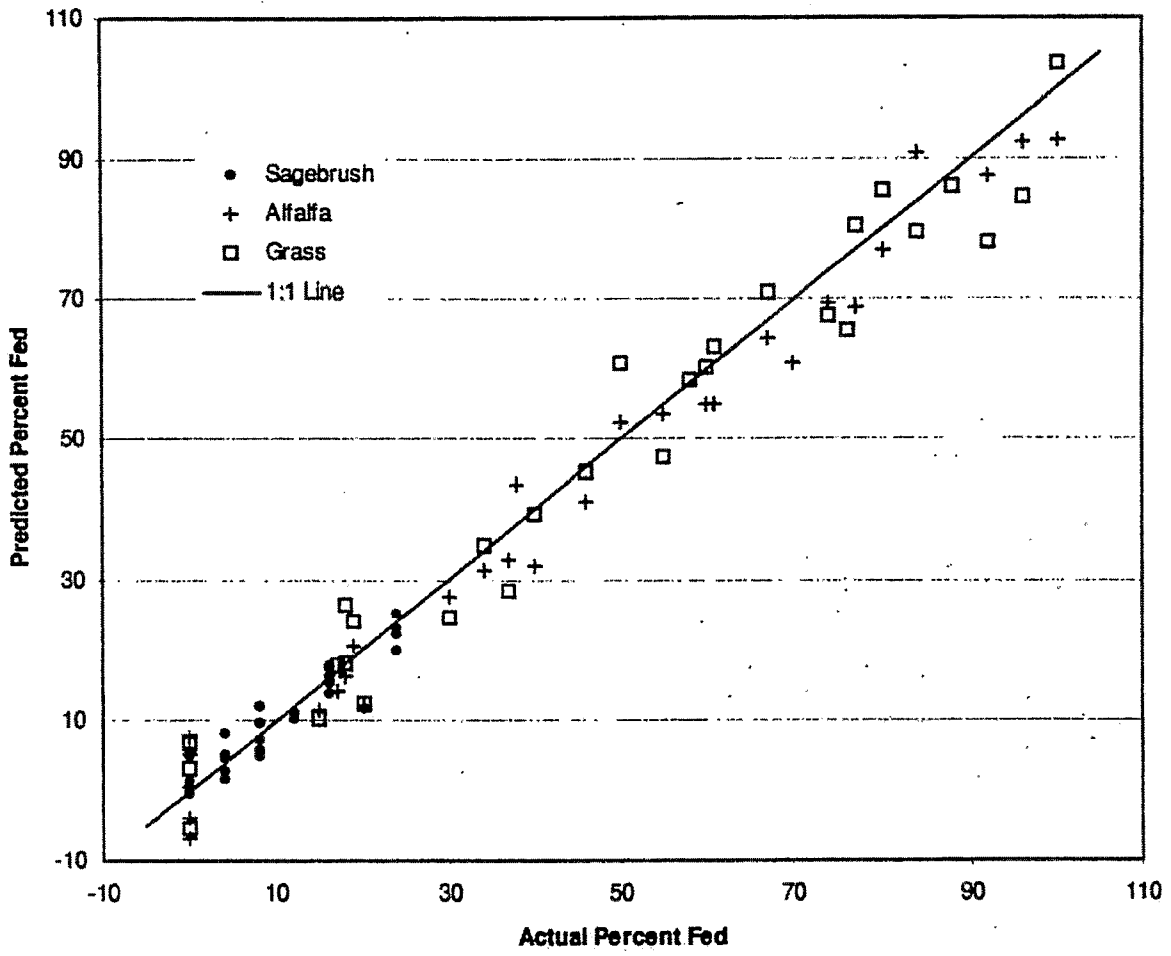


Figure 4. Internal validation of composition of diets for three botanical components (sagebrush, alfalfa and grass) using NIRS prediction equations based on spectra of feces from animals fed the diets.



Literature Cited

Coleman, S.W., J.W. Stuth, and J.W. Holloway. 1995. Prediction of intake by near-infrared spectroscopic analysis of fecal samples. p. 145-155. *In:* F.N. Owens, D. Gill, K. Lusby, and T. McCollum (Ed.) Symp: Intake in Feedlot Cattle. Oklahoma Exp. Sta. P-942.

Cook, C.W., and L.E. Harris. 1968. Nutritive value of seasonal ranges. Utah State Univ. Agric. Exp. Sta. Bull. 472. 55pp.

Fraker, M.J. 1999. Examination of digestive and behavioral mechanisms to explain individual variation in sagebrush consumption by sheep. M.S. Thesis, Univ. Idaho, Moscow.

Ngugi, R.K., F.C. Hinds, and J. Powell. 1995. Mountain big sagebrush browse decreases dry matter intake, digestibility, and nutritive quality of sheep diets. J. Range Manage. 48:487-492.

Shenk, J.S., M.O. Westerhaus, and S.M. Abrams. 1989. Supplement 2. Protocol for NIRS calibration: Monitoring analysis results and recalibration. pp 104-110. *In:* G.C. Maren, J.S. Shenk, and F.E. Barton II (Ed.). Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality. USDA Agric. Handbook No. 643.

Snowder, G.D., J.W. Walker, K.L. Launchbaugh, and L.D. Van Vleck. 2000a. Genetic and phenotypic parameters for dietary selection of mountain big sagebrush (*Artemisia tridentata* spp. *vaseyana*) in Rambouillet sheep. J. Anim. Sci. (in press)

Snowder, G.D., J.W. Walker, K.L. Launchbaugh, and L.D. Van Vleck. 2000b. Heritability of dietary selection of mountain big sagebrush (*Artemisia tridentata* spp. *vaseyana*) in Rambouillet sheep. Texas Agric. Exp. Sta. Sheep and Goat, Wool and Mohair.

Walker, J.W., D.H. Clark, and S.D. McCoy. 1998. Fecal NIRS for predicting percent leafy spurge in diets. J. Range Manage. 51:450-455.