Effects of breed, sex, and age on the variation and ability of fecal near-infrared reflectance spectra to predict the composition of goat diets[1,2]

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ABSTRACT: The effects of breed, sex, and age of goats on fecal near-infrared reflectance spectroscopy-predicted percentage juniper in the diet were investigated, as were spectral differences in feces from goats differing in estimated genetic merit for juniper consumption. Eleven goats from each breed, sex, and age combination, representing 2 breeds (Angora and meat-type), 3 sex classifications (female, intact male, and castrated male), and 2 age categories [adult and kid (less than 12 mo of age)] were fed complete, pelleted rations containing 0 or 14% juniper. After 7 d on the same diet, fecal samples were collected for 3 d, and the spectra from the 3 replicate samples were averaged. Fecal samples were assigned to calibration or validation data sets. In a second experiment, Angora and meat goats with high or low estimated genetic merit for juniper consumption were fed the same diet to determine the effect of consumer group on fecal spectra. Feces were scanned in the 1,100- to 2,500-nm range with a scanning reflectance monochromator. Fecal spectra were analyzed for the difference in spectral characteristics and for differences in predicted juniper in the diet using internal and independent calibration equations. Internal calibration had a high precision (R^2 = 0.94), but the precision of independent validations (r^2 = 0.56) was low. Spectral differences were affected by diet, sex, breed, and age ($P < 0.04$). However, diet was the largest source of variation in spectral differences. Predicted percentage of juniper in the diet also showed that diet was the largest source of variation, accounting for 95% of the variation in predictions from internal calibrations and 51% of the variation in independent validations. Predictions from independent calibrations readily detected differences ($P < 0.001$) in the percentage of juniper in the 2 diets, and the predicted differences were similar to the actual differences. Predicted juniper in the diet was also affected by sex. Feces from goats from different juniper consumer groups fed a common diet were spectrally different, and the difference may have resulted from a greater intake by high- compared with low-juniper-consuming goats. Fecal near-infrared reflectance spectroscopy predictions of botanical composition of diets should be considered an interval scale of measurement.

Key words: diet composition, feces, goat, juniper, near-infrared reflectance spectroscopy

J. Anim. Sci. 2007. 85:518–526
doi:10.2527/jas.2006-202

INTRODUCTION

Selective grazing by livestock is an important factor affecting botanical composition and productivity of grazing lands. Historically, grazing was considered to have a negative impact on grazing lands, which led to the call for removal of livestock for the purpose of resource conservation. However, the role of grazing livestock, particularly goats, for improving ecological condition has recently been elucidated (Perevolotsky and Seligman, 1998). The unique dietary habits of goats make them potentially important biological agents for the management of invasive plant species such as juniper (*Juniperus* spp.). To maximize the potential for improving livestock as a rangeland improvement tool, a rapid method of determining botanical composition of the diet is necessary (Walker and Hodkinson, 1999).

[1]This research was supported by Research Grant Award No. IS-3555-04 from BARD, The United States - Israel Binational Agricultural Research and Development Fund and Texas Food and Fibers Commission Project No. b-04-05-02.
[2]The authors thank B. S. Engdahl for superior technical support.
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Received April 3, 2006.
Accepted September 3, 2006.

Fecal near-infrared reflectance spectroscopy (**NIRS**) can predict chemical (Lyons and Stuth, 1992) and botanical (Walker et al., 1998) composition of diets. Walker et al. (2002) showed that NIRS predictions of dietary sagebrush from independent calibration equations, although precise, were of low accuracy. Fecal NIRS has been used to identify sex in cattle (Tolleson et al., 2000), sheep (Godfrey et al., 2001), and several species of wildlife (Tolleson et al., 2005). In addition to sex, NIRS was used to categorize age in white-tailed deer (Osborn et al., 2002), and species in red and fallow deer (Tolleson et al., 2005). The ability of NIRS to distinguish sex and age are a potential cause for concern because sex and age may become sources of bias in predictions of botanical composition.

The purpose of this study was to investigate sources of NIRS variation in fecal material from goats that differed in age, sex, breed, or estimated genetic preference for juniper. Effects of age, sex, and breed were also tested for their effects on the accuracy and precision of fecal NIRS-predicted juniper in the diet.

MATERIALS AND METHODS

All procedures involving animals were approved by the Texas A & M University Institutional Agricultural Animal Care and Use Committee under protocol 2003–129.

Experiment 1

Eleven goats in each of the following breed, sex, and age groups were used in this experiment: female Angora (34 ± 4 kg of BW), intact male Angora (56 ± 11 kg of BW), castrated male Angora (39 ± 5 kg of BW), female meat goats (44 ± 5 kg of BW), intact male meat-type goats (Boer × Spanish; 59 ± 8 kg of BW), castrated male meat goats (51 ± 10 kg of BW), Angora female kids (11 ± 2 kg of BW), and Angora male kids (12 ± 2 kg of BW), for a total of 88 goats. Two complete, mixed, pelleted rations containing 0 or 14% juniper were fed to goats of differing breed, sex, and age. Fourteen percent juniper was used because it was within the range of differences (i.e., 10 to 15%) we desired to detect. The study was conducted in August 2004 at the Texas A & M University Agricultural Research and Extension Center in San Angelo, TX (31° 32′ 55″ N, 100° 30′ 31″ W).

Juniper for the 14% juniper ration was collected in the mornings during June 2004 at the Texas A&M University Sonora Research Station (30° 15′ 56″ N, 100° 33′ 50″ W) located in the western Edwards Plateau resource region. Juniper branches were harvested and the leaves were hand-stripped, dried at 38°C for 48 h, and ground using a hammermill with a 2.5-mm screen. Dried and ground leaves were added to a commercial, complete, mixed goat ration to create a ration that contained 14% juniper. Ingredients were not adjusted to equalize the nutrient content of the 0 and 14% diets (Table 1). The rations were pelleted to prevent the goats from ingredient sorting while they were on the 14% juniper diet.

Table 1. Nutrient composition[1] (DM basis) of 0 and 14% juniper diets fed in Exp. 1

	Diet	
Nutrient, %	0% Juniper	14% Juniper
CP	15.2	13.2
ADF	27.0	30.8
NDF	36.1	44.5

[1]Determined by Forage Testing Laboratory, Dairy One Inc., Ithaca, NY.

Goats were assigned to 4 groups [kids (both sexes), adult females, adult males, and adult castrated males] and were given ad libitum access to the 0% juniper diet for a 10-d preconditioning period to familiarize them with the diet and experimental procedures. Unconsumed pellets were removed, and fresh pellets were fed once daily at 0800. After the preconditioning period, all goats were sequentially fed, first the 0% and then the 14% juniper diets for 10 d each using the same protocol described for the preconditioning period. On the last 3 d of each feeding period, fecal samples were collected at 1000. A 20-mm wide and 200-mm long rectal speculum (Minitube Australia, Pty., Ltd., Sebastopol, Victoria, Australia) was used to ensure adequate sample size (i.e., approximately 20 fecal pellets).

Experiment 2

This study analyzed spectral differences of feces from goats categorized as high- or low-juniper consumers while they were on a common diet devoid of juniper. The experiment was conducted on female Angora (29 ± 6 kg of BW) and meat-type (i.e., Boer × Spanish, 34 ± 13 kg of BW) goats when they were in the drylot for breeding. Classification of goats was based on genetic merit for percentage juniper in the diet, which was estimated using a separate animal model for each breed. The Angora predictions used 778 records from 577 goats and the meat goat predictions used 239 records from 176 goats. Percentage juniper in the diet of goats for calculating genetic merit was estimated using NIRS predictions of fecal samples collected when they were free-grazing on juniper-infested pastures.

This study used animals with the greatest and lowest estimated genetic merit for juniper consumption from each breed. For Angora, there were 25 high (estimated genetic merit = +1.86 ± 0.91%) and 25 low (estimated genetic merit = –1.64 ± 0.75%) goats; and for meat goats, 20 high (estimated genetic merit = +4.6 ± 1.7%) and 22 low (estimated genetic merit = –5.1 ± 1.5%) goats were sampled. The Angora goats were selected from a flock of 420 goats, and the meat goats were selected from a flock of 190 Boer × Spanish nanny goats.

Angora goats received a medicated (Alpharma Inc., Fort Lee, NJ), commercial, show-goat feed (labeled 17% CP, minimum; 3% crude fat, minimum; and 18% crude fiber, maximum) at a rate of 900 g·goat^{-1}·d^{-1} on an as-fed basis, with ad libitum access to coastal bermuda hay. Meat goats received medicated (Deccox) alfalfa pellets (labeled 17% CP, 2 Mcal/kg of DE), at the rate of 900 g·goat^{-1}·d^{-1} on an as-fed basis. Goats were grouped by breed and juniper consumption category and fed as a group. In November 2005, fecal samples were collected from each goat using a rectal speculum.

Laboratory Analysis

Fecal samples were ground in a cyclone mill to pass through a 1-mm screen, dried in a forced-air oven (50°C for 12 h), and conditioned for 24 h in an environment with constant temperature and humidity (21°C, 65%). Approximately 4 g of ground, conditioned samples were packed in quarter-cup sample cells with a near-infrared, transparent, quartz cover glass (Foss, 2000). Cells were scanned 32 times using a scanning reflectance monochromator (model 6500, NIR Systems Inc., Silver Springs, MD). Reflected energy (log [1/R], where R = reflectance) was measured and averaged over the 32 scans and recorded at 2-nm intervals from 1,100 to 2,500 nm.

NIRS Calibration Equation Development

Before calibration, reflected energy data were transformed using multiplicative scatter correction (Geladi et al., 1985) and a 2,8,8,1 math treatment using WINISI II software (ISI, 1999), in which the first number is the order of the derivative, the second number is the gap (number of data points over which the derivative is calculated), and the third and fourth numbers are the smooth (number of data points in a moving average and the number of nanometers over which the second smoothing is applied, respectively). Data pretreatment has the effects of removing nonlinearity caused by light scatter, correcting for baseline drift, and enhancing absorption peaks (Williams, 1987). Modified, partial, least squares regression (Martens and Naes, 1987) was used to develop calibration equations with stored NIRS spectra from fecal samples as the independent variables and percentage of juniper fed in the diets as the dependent reference data.

Two modified, partial, least squares calibration equations using the ISI procedures were used to determine if breed, sex, or age affected predicted juniper in the diet. The calibration equations were based on an internal calibration using a subsample of observations from Exp. 1 and an independent calibration using previously conducted feeding studies with goats (Whitworth, 2002). Before allocating samples to the internal calibration data set, spectra from the 3 replicate fecal samples in Exp. 1 were averaged, and 4 observations (2% of samples) that did not have at least 2 daily replicates or were spectral outliers [Mahalanobis distance < 4 (Mahalanobis, 1936); ISI, 1999] were eliminated. The remaining samples were randomly allocated to an internal calibration set or a validation set. Four or 5 goats from each breed, sex, and age group were randomly allocated to the calibration data set, and the remaining 6 goats from each group were used for validation and for testing the fecal NIRS, predicted percentage of dietary juniper for treatment effects. The independent calibration data were from feeding studies conducted in 1999 and 2002 that used diets with known percentages of juniper and a variety of background forages (Whitworth, 2002), plus feces collected in 2003 and 2004 from goats grazing natural rangeland areas from which all juniper had been removed; i.e., diets with 0% juniper.

Statistical Analysis

The quality of the calibration equations was assessed using the R^2 (multiple coefficient of determination) and standard error of cross validation (**SECV**). Precision and accuracy of predictions for samples not included in the calibration data were assessed by examining the r^2 (simple coefficient of determination of NIRS predicted values and reference values), standard error of prediction (**SEP**), and the slope of the line between actual and predicted values (Naes et al., 2002). Differences in predicted percentage of juniper in the diets between internal and independent calibrations were tested using a paired *t*-test. Standard deviations were compared using an *F*-test.

For Exp. 1, the effects of diet (0 or 14% juniper), breed (Angora or meat), and sex (female, intact male, or castrated male) were investigated using data only from adult animals because there were no meat-goat kids. Likewise, the effects of diet (0, 14), age (adult, kid), and sex (female, intact male) were investigated using only the Angora goat data. Analysis of variance was used to test for differences in main effects using a split-plot design with diet as the within-animal factor. The error term for between-animal effects was animal within (breed × sex) or animal within (age × sex), and the residual was the error term for diet. Omega-squared (ω^2; Hays, 1963) was calculated to measure the effect size of the model components. Two dependent variables, namely principal component (**PC**) scores and predicted juniper, were analyzed with the ANOVA. The first 5 PC scores (Morrison, 1976), which in all cases accounted for a minimum of 95% of the variation in the original spectra, were used to determine spectral difference among treatments.

Principal component analysis is a variable-reduction technique, in which a new variable is calculated as a linear combination of the original data, with the following restrictions: 1) the first PC represents the maximum variability possible in the original data; and 2) each succeeding PC accounts for the maximum remaining variability possible and is not correlated with previous PC.

Table 2. Comparison of calibration and validation statistics for modified, partial, least squares equations used to predict the percentage of juniper consumption in the 2004 breed, sex, and age feeding trial

Calibration data	Calibration statistics			Validation statistics[1]				
	n	R^2	SECV,[2] %	r^2	Slope	SEP,[3] %	Mean, %	SD, %
Internal[4]	76	0.96	2.0	0.94	0.96	1.7	7.2	7.0
Independent[5]	138	0.88	6.4	0.56	1.04	10.3	15.0	9.8

[1]Validation data consisted of diet and fecal data from the 2004 trial. Validation samples had a mean = 7% and a SD = 7%, and were not used to develop the internal calibration equation.
[2]SECV = Standard error of cross validation.
[3]SEP = Standard error of prediction.
[4]Internal modified, partial, least squares calibration equations were developed by randomly selecting 4 or 5 animals in each breed, sex, and age combination from the 2004 feeding trial.
[5]Independent, modified, partial, least squares calibration equations were developed using data from feeding trials conducted in 1999 and 2002 and from goats grazing pastures with no juniper.

Fecal NIRS-predicted juniper in the diet from the internal and independent calibrations was analyzed to test for bias resulting from treatment effects. Principal component scores were calculated separately for Angora and meat goats from the spectral data in Exp. 2, and the scores for the first 5 PC were tested for differences between high-and low-consumer groups using a separate 1-way ANOVA for Angora and meat goats. Wavelengths that differed between treatments were identified with a 1-way ANOVA of reflected energy for each wavelength, and the mean differences between groups were enhanced using a 2,8,8,1 math treatment to identify important absorption bands (Williams and Norris, 1987).

RESULTS

Experiment 1

Calibration and validation statistics for internal and independent calibration equations are shown in Table 2. Both data sets produced acceptable calibrations, although the internal calibration was better than the independent calibration presumably because the former represented samples from a single study, whereas the independent calibration had samples from multiple sources. As expected, the internal calibration validated well and validation statistics were essentially the same as calibration statistics. However, validation statistics for the independent calibration showed that predictions from this equation lacked precision ($r^2 = 0.56$) and accuracy as indicated by the fact that the mean (15%) of the predicted samples was over twice as large ($P < 0.001$) as the actual mean (7%). However, the slope (1.04) was close to unity indicating the error was similar at the 2 levels of juniper in the diet (Figure 1).

The first 5 PC scores (PC1 to PC5) for adult Angora and meat goats (Table 3) accounted for about 98% of the variation in the original data. Analysis of the effect of diet showed that it differed ($P < 0.001$) on PC1, which accounted for 71% of the variation in the original data set. Sex was the second most important factor affecting fecal spectral characteristics as shown by differences in PC2, PC3, and PC4 ($P < 0.03$), which accounted for a total of 26% of the variation in the original data. The breed effect was only significant for PC5 that accounted for 1% of variation.

Analysis of the first 5 PC scores for the adult and kid Angora goat data showed that PC1 accounted for 82% of the variation in the original data set and differed between the 2 diets ($P < 0.001$). Age was the second most important factor affecting fecal spectral characteristics as shown by differences in PC2 and PC4 ($P < 0.016$) and accounted for 11% of the variation of the spectral data. The sex effect was only significant for PC3 ($P = 0.04$).

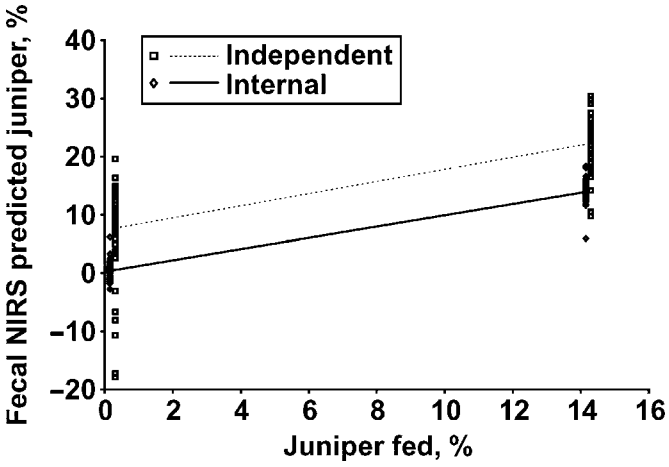

Figure 1. Effect of source of data for the fecal near-infrared reflectance spectroscopy (NIRS) calibration equation on predicted percentage of juniper in the diet. Validation spectra were from a feeding trial in which goats of different breed, sex, and age classes were fed a complete pelleted ration containing 0 or 14% juniper. An internal calibration was based on a subset of these samples that were not used for validation. An independent calibration was based on feeding studies conducted in 1999 and 2002, and feces from goats grazing pastures with no juniper.

Table 3. Effect of treatments on principal component[1] (PC) scores calculated from reflectance data of feces from goats on the feeding trial

Item	PC1	PC2	PC3	PC4	PC5
Adult breed and sex comparison	Percentage of variation				
Individual PC score	71.0	19.3	4.3	2.1	1.0
	Probability of a greater *F*				
Diet	0.000	0.097	0.560	0.004	0.380
Breed	0.313	0.843	0.950	0.656	0.001
Sex	0.819	0.000	0.000	0.029	0.604
Angora age and sex comparison	Percentage of variation				
Individual PC score	81.6	8.9	4.2	2.6	1.0
	Probability of a greater *F*				
Diet	0.000	0.227	0.360	0.485	0.885
Age	0.262	0.033	0.229	0.001	0.252
Sex	0.267	0.505	0.038	0.592	0.498

[1]Principal component is a variable-reduction technique in which a new variable is calculated as a linear combination of the original data, with the following restrictions: 1) the first principal component represents the maximum variability possible in the original data; and 2) each succeeding principal component accounts for the maximum remaining variability possible and is orthogonal with previous principal components.

Analysis of fecal NIRS-predicted percentage of juniper in the diet from the internal and independent equations (Table 4) showed that predictions from the independent equation were about twice the actual percentage of juniper fed. Despite the upward bias for the independent equation, differences were readily detected ($P < 0.001$), and the difference between the 2 diets was close to the actual differences (i.e., 13 and 18 percentage units for adult and Angora, respectively). As expected, predictions for the internal equation were near the actual values. For the internal equation the effect of diet accounted for 95% of the variance (i.e., ω^2) compared with only about 50% for the independent equation. Most of the remaining variation in the analysis of variance for the independent predictions was in the error. This is consistent with the low validation r^2 for the independent equation.

The effect of sex for the adult data was significant for the internal ($P = 0.05$) and independent ($P = 0.004$) equations (Table 4). The effect of sex was relatively less important compared with the diet effect ($\omega^2 = 0$ and 9% for internal and independent predictions, respectively). In the Angora data, sex was significant ($P = 0.003$; $\omega^2 = 7\%$) only for the independent predictions. The actual differences between sexes were about 1 percentage unit for the internal equation but 7 percentage units for the independent equations. The effects of breed and age as predicted by both equations were not different.

Treatment differences in SD (Table 4) provide insight into limitations of the calibration equations. The SD of internal predictions was similar to the actual SD, which was 0 for percentage juniper in the diet and 7 for other effects. Standard deviations of independent predictions of percentage juniper in the diet for the adult and Angora data sets were about 75% greater ($P < 0.08$) for the 0% juniper feces than for the 14% juniper feces. Comparison in the adult data set of the standard deviations of Angora to meat goat and female to male or castrated male also showed over a 70% greater SD for Angora and nanny compared with the other classifications ($P = 0.04$). Furthermore, on average, SD for predictions in the Angora data set were greater than ones from the adult data set. We assume that the larger SD for Angoras and nanny goats was because independent calibrations were developed primarily with fecal spectra from meat goat wethers.

Experiment 2

Comparison of spectral differences between high- and low-juniper consumer groups showed relatively minor differences for the meat goats but larger differences for the Angora goats. Meat goat PC scores differed ($P < 0.02$) for only the third and fourth PC, which together accounted for a total of about 15% of the variation in the original spectra (Table 5). Angoras differed ($P < 0.02$) on PC1, PC2, and PC5, which accounted for a total of about 83% of the variation in the original spectra. The greater difference in the Angora goats may reflect that hay was provided ad libitum, and the disparity is a reflection of differences in proportion of hay in the diets, total intake, or both.

Figure 2 shows the reflected energy (log [1/R]) values (A) and the difference (B) between the 2 groups for the average of the high and low groups within Angora and meat goats from Exp. 2. Absorption was greater ($P < 0.001$) for the Angora compared with meat goats, and similar to the analysis of the PC scores, the differences between high and low consumers were greater ($P < 0.001$) for the Angora than the meat goats. Analysis of reflected energy at individual wavelengths indicated that 65% of the wavelengths differed ($P < 0.05$) for Angora goats compared with only 20% for meat goats. Absorbance for high consuming Angora goats was gen-

Table 4. Internal and independent fecal NIRS-predicted mean percentage of juniper (SD), probability of a difference, and omega squared (ω^2)[1] in the diet of goats fed a complete pelleted ration containing 0 or 14% juniper, as affected by diet, age, breed and sex

Item	Internal[2]	Independent[3]
Breed and sex comparison		
% Juniper in diet	$P = 0.000$ $\omega^2 = 95.5\%$	$P = 0.000$ $\omega^2 = 51\%$
0	0.5 (1.5)	9.3 (7.7)
14	14.1 (1.5)	22.4 (4.6)
Breed	$P = 0.365$ $\omega^2 = 0\%$	$P = 0.492$ $\omega^2 = 0\%$
Angora	7.5 (7.4)	15.3 (11.4)
Meat	7.2 (6.6)	16.4 (6.3)
Sex	$P = 0.0480$ $\omega^2 = 0\%$	$P = 0.004$ $\omega^2 = 9\%$
Billy	6.8[a] (6.9)	18.4[a] (6.2)
Wether	7.8[b] (7.7)	17.2[a] (7.5)
Nanny	7.3[a,b] (6.4)	11.9[b] (11.8)
Angora age and sex comparison		
% Juniper in diet	$P = 0.000$ $\omega^2 = 95\%$	$P = 0.000$ $\omega^2 = 57\%$
0	0.1 (1.2)	4.2 (9.9)
14	13.6 (1.9)	22.3 (5.5)
Age	$P = 0.583$ $\omega^2 = 0\%$	$P = 0.383$ $\omega^2 = 1\%$
Adult	7.0 (6.9)	14.1 (12.9)
Kid	6.7 (7.2)	12.3 (11.4)
Sex	$P = 0.494$ $\omega^2 = 0\%$	$P = 0.003$ $\omega^2 = 7\%$
Intact male	7.0 (7.2)	16.5 (10.0)
Female	6.7 (6.9)	10.0 (13.3)

[a,b]Within a column, means of sex group without a common superscript differ ($P < 0.05$).

[1]$\omega^2 = [SS_{effect} - (df_{effect} \times MS_{error})]/(MS_{error} + SS_{total})$; ω^2 is an estimate of the dependent variance accounted for by the independent variable in the population for a fixed effects model.

[2]Internal, modified, partial, least squares calibration equations were developed by randomly selecting 4 or 5 animals in each breed, sex, and age combination from the 2004 feeding trial.

[3]Independent, modified, partial, least squares calibration equations were developed using data from feeding trials conducted in 1999 and 2002, and from goats grazing pastures with no juniper.

erally greater than for low consumers with this difference being significant for wavelengths greater than 1,646 nm (Figure 2B). In contrast, absorbance for high consuming meat goats was generally lower than for low consumers, and the only significant differences were that low consumers had greater ($P < 0.05$) absorption for wavelengths less than 1,382 nm. The second derivative of the difference between high and low consumers identified similar difference peaks for Angora and meat goats at 1,734, 2,308 and 2,352 nm, which are associated with absorbance by protein, protein, and cellulose, respectively.

Table 5. Effect of high- vs. low- juniper consumer groups for Angora and meat goats on principal component[1] (PC) scores calculated separately for each breed from fecal NIRS reflectance data

	Angora goats		Meat goats	
Principal component	Percentage of variation	Probability	Percentage of variation	Probability
PC1	55	0.001	56	0.755
PC2	26	0.016	22	0.958
PC3	7	0.283	12	0.023
PC4	5	0.284	3	0.006
PC5	3	0.002	2	0.928

[1]Principal component is a variable-reduction technique in which a new variable is calculated as a linear combination of the original data, with the following restrictions: 1) the first principal component represents the maximum variability possible in the original data; and 2) each succeeding principal component accounts for the maximum remaining variability possible and is orthogonal with previous principal components.

DISCUSSION

The low r^2 between fecal NIRS-predicted juniper and actual juniper in the diet for the independent calibration equation contrasts with previous comparisons of independent calibrations (Walker et al., 2002) that showed independent calibrations were precise enough to provide useful predictions. In the current study, the external validation represented a "worst case scenario" where calibration data developed from fecal spectra from forage-based diets, were used to predict concentrate-based diets. Although the independent validation r^2 was less than the recommended minimum (Williams, 2001), diet effects were readily detected ($P < 0.001$).

Analysis of PC scores indicated that similar to previous research (Tolleson et al., 2000, 2005; Godfrey et al., 2001) feces from animals differing in sex were spectrally different. In this study, the spectral differences can only be attributed to actual sex effects or to differences of intake between the sexes because animals were fed the same pelleted ration. Animals in other studies were free grazing, and the potential for actual dietary differences as the reason for spectral differences in their feces

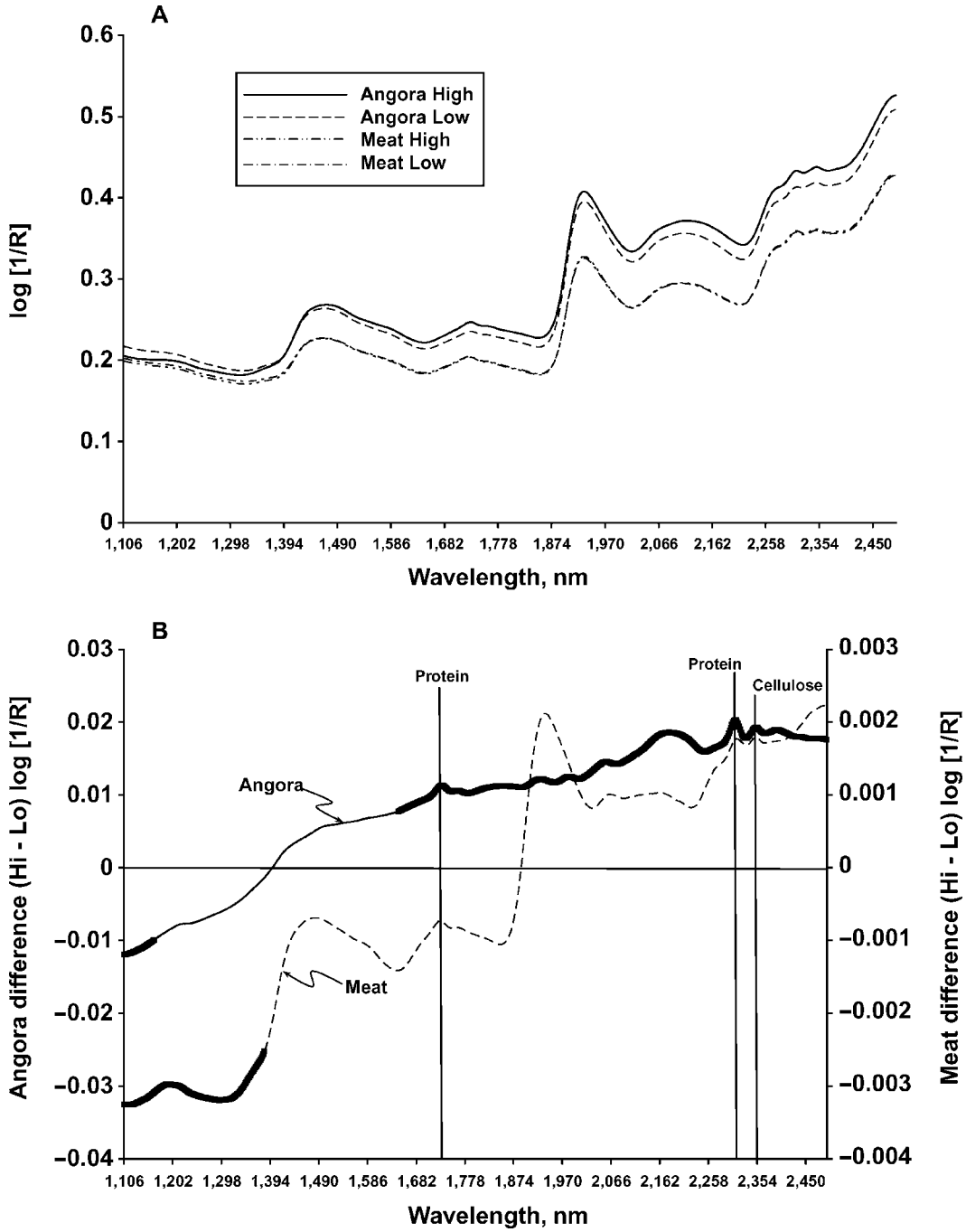

Figure 2. Effect of breed and estimated genetic potential (i.e., high vs. low) for juniper consumption on spectral characteristics of feces from goats on the same diet devoid of juniper. Actual reflected energy (log [1/R]) values are shown in A. The difference (i.e., high minus low) between consumer groups (B) is plotted on different y-axes because differences for Angora compared to meat goats were about 10 times as large. Thickened areas of the lines in (B) indicate portions of the spectra for which the difference between consumer groups was different ($P < 0.05$). Vertical lines in (B) identify areas for which derivatization indicated the largest difference between groups.

cannot be excluded. The spectral differences as a result of sex affected the predicted juniper in the diets primarily when predictions were based on the independent equations.

In the Angora data set, PC3, PC4, and PC5 differed among age groups compared with only PC3 differing between sexes. This indicates that for this data set, age resulted in greater spectral differences than sex. However, in contrast to sex, age did not cause a difference in predicted juniper in the diet, which indicates that age affected areas of the spectra that were relatively unimportant in the predictive equation, whereas spectral areas that differed due to sex were also relevant for predicting percentage of juniper in the diet.

The effect of sex for the independent equation indicated that fecal NIRS should not be used to make comparisons between different sexes.

In contrast to the rather poor validation results for the independent validation (Table 2), the ANOVA of these predictions showed that the diet differences could be detected and the difference between the 2 diets in predicted percentage of juniper was close to the actual difference (Table 4). This finding is consistent with the conclusion of Walker et al. (2002) that fecal NIRS predictions of botanical composition of the diet from independent calibration equations represent an interval scale of measurement. An interval scale of measurement means that treatments can be ranked and the differences between treatments have meaning and are equal across the range of measurements, but there is not a true zero point. Thus, it is appropriate to say that the difference between treatment A and C is twice as large as the difference between treatment A and B. But it would not be appropriate to say that treatment A is twice as large as treatment C. Finally, the SD for predicted percentage of juniper in the diet from independent calibration equations indicated that when feces came from animals that differed in breed and sex compared with the animals used for calibration, variation of predictions also increases. If ratio scale data are considered necessary, then observation and hand-simulated diets can be used to ensure that calibration and prediction samples are from the same population (Landau et al., 2005).

The greater spectral differences between high and low consuming groups of Angora compared with meat goats probably reflects that hay was provided ad libitum, and the differences represented differences in proportion of hay, intake of the diets, or both. This hypothesis is further supported by the greater ($P < 0.001$) reflected energy values for Angora, which were fed ad libitum, compared with meat goats that were restricted to 900 $g \cdot goat^{-1} \cdot d^{-1}$ (Figure 2A). Furthermore, second derivative math treatment of the difference spectra (Figure 2B) identified important peaks at 1,734, 2,308 and 2,352 nm, which correspond to absorption bands for protein, protein, and cellulose, respectively (Williams and Norris, 1987). Fecal nitrogen is positively related to intake (Holloway et al., 1981; Wofford et al., 1985; Leite and Stuth, 1990). Furthermore, as intake rate of goats increases, so does rate of passage (Castle, 1956), which could result in more cellulose leaving the rumen undigested and thus account for the greater ($P < 0.001$) concentration of cellulose in the feces. These spectral differences may indicate that high juniper consuming goats have a greater appetite than low consumers. This hypothesis is further supported by preliminary evidence that high consuming Angora goats are heavier and produced more fiber compared with low consumer groups (Taylor et al., 2005). The increased appetite could result in high juniper consumers ingesting more of the readily available, but chemically defended, juniper to meet this demand. However, differences in fecal spectra between the 2 groups should not be considered definitive but rather indicate potentially productive directions for future research.

Recommended procedures for using NIRS suggest that 5% of samples be analyzed using standard laboratory procedures to monitor the accuracy of NIRS calibration equations. However, reference samples are normally not available to validate calibration equations developed for fecal NIRS. Therefore, it is imperative to understand the limits of fecal NIRS calibrations and factors that can affect their precision and accuracy. This study demonstrated that even in what would be considered a worst-case scenario with an independent calibration equation, fecal NIRS predictions of dietary botanical composition can be considered interval scale data and true treatment differences can be detected. However, it also showed that nontreatment factors such as sex could bias predictions and comparisons. Spectral differences between groups of goats with different genetic merit for juniper consumption may indicate physiological reasons for this difference. Fecal NIRS can aid in the management of free-grazing livestock being used as ecological enhancement agents.

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