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Original Research

Comparison of Near Infrared Reflectance Spectroscopy and Raman Spectroscopy for Predicting Botanical Composition of Cattle Diets☆

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A B S T R A C T

Diet selection is an important driver of ecosystem structure and function that is difficult to measure. New spectroscopic instruments are available for evaluating their applicability to ecological field studies. The objective of this study was to compare near-infrared reflectance spectroscopy (NIRS) to Raman spectroscopy of fecal samples for predicting the percentage of honey mesquite *(Prosopis glandulosa)* in the diet of ruminally fistulated cattle fed three different hay diets and compare them for their ability to discriminate among the three base diets. Spectra were collected from feces from a feeding trial with mesquite fed at 0%, 1%, 3%, and 5% of the diet and base hay diets of timothy hay *(Phleum pratense)*, Sudan hay *(Sorghum sudanense)*, or a 50:50 combination of Bermudagrass hay *(Cynodon dactylon)* and beardless wheat hay *(Triticum aestivum)*. NIRS and Raman spectra were used for partial least squares regression calibrations with the timothy and Sudan hays and validated with the Bermudagrass/beardless wheat hay diets. NIRS spectra provided useful calibrations ($r^2 = 0.88$, slope = 1.03, intercept = 1.88, root mean square error = 2.09, bias = 1.95, ratio of performance to deviation = 2.6), but Raman spectra did not. Stepwise discriminant analysis was used to select wavenumbers for discriminating among the hays. Fifteen of 350 possible wavenumbers for NIRS spectra and 29 of 300 possible wavenumbers for Raman spectra met the $P \leq 0.05$ entry and staying criteria. Canonical discriminant analysis using these wavenumbers resulted in 100% correct classification for all three base diets, and the Raman spectra provided greater separation than NIRS spectra. Discrimination using Raman spectra was primarily associated with wavenumbers associated with undigestible constituents of the diet (lignin). In contrast, discrimination using fecal NIRS (f.NIRS) spectra was primarily associated with wavenumbers associated with digestible constituents in the diet (protein, starch, and lipid). We believe that Raman spectroscopy deserves further investigation as a quantitative technique in ecological field studies.

Introduction

Honey mesquite (*Prosopis glandulosa* Torr.) is an invasive shrub that is common in the southwestern United States and northern Mexico. The leaves of honey mesquite have forage quality characteristics similar to moderate quality hay, with crude protein levels of 12 − 20% and neutral detergent fiber levels of 35 − 40% (Baptista and Launchbaugh, 2001; Mayagoitia González, 2015). Mesquite leaves are available during late spring and early summer before monsoon rains when grasses are typically dormant and low quality. However, mesquite leaves contain secondary compounds, including alkaloids and phenolic compounds (Achakzai et al., 2009; Witmore, 2009), which limit intake by livestock. Animals that can consume small amounts of mesquite may be more adapted to southwestern rangelands than animals that avoid mesquite. Development of a method to cost effectively determine the amount of mesquite in cattle diets would facilitate selection of animals more adapted for rangelands in the southwestern United States.

Near-infrared reflectance spectroscopy (NIRS) has a long history as a spectroscopic technique with useful applications in agriculture (Williams and Norris, 2001) and ecology (Foley et al., 1998). In contrast,

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Table 1
Dry matter (DM), crude protein, neutral detergent fiber (NDF), and acid detergent fiber (ADF) of the honey mesquite leaves, beardless wheat hay, Bermuda grass hay, Sudan grass hay, and timothy hay expressed on a DM basis

Diet item	DM, %	Crude protein, %	NDF, %	ADF, %
Honey mesquite leaves	96.27	16.39	40.10	33.21
Bermudagrass beardless wheat hay	95.93	7.75	62.66	42.53
Sudan grass hay	95.23	5.19	58.86	41.29
Timothy hay	95.31	12.02	57.18	36.46

Raman spectroscopy has not been as widely applied because of early difficulties with sample degradation and fluorescence; however, many of these problems have been overcome (Yang and Ying, 2011) and portable field instruments are now available. NIR spectra originate from absorption of light by vibrating and rotating molecules, and Raman spectra originate from scattering of light by vibrating and rotating molecules. NIRS detects vibrations when the electrical dipole moment changes, while Raman spectroscopy identifies vibrations caused by electrical polarizability changes. NIR spectra are characterized by broad, often overlapping peaks that are matrix dependent and affected by moisture content. In contrast, Raman spectra have narrow, highly resolved peaks that are not affected by matrix or moisture. Another advantage is that mononuclear diatomic molecules (O_2, N_2 etc.) are Raman active but do not absorb in the NIR range. However, an electrically unsymmetrical bond may be NIR active and Raman inactive or both NIR and Raman active (Anderson, 1973; Colthup et al., 1990). Disadvantages of Raman are weak signal-to-noise ratio, sample heating, and sample fluorescence. Because Raman is a weak process, it requires high power and high sample purity. Consequently, Raman is most commonly used for homogenous samples and NIRS is often used for heterogeneous samples. Both methods have advantages and limitations but can be used as complementary methods. For example, studies on bond angles, bond lengths, and other structural information require Raman data in addition to NIR analysis (Yadav, 2005).

Previous studies using diet/fecal pairs and NIRS (f.NIRS) to predict diet composition have used dried and ground feces (Walker et al., 1998, 2002). Because Raman spectroscopy has the potential to be effective without processing of feces and portable instruments are available, the objective of this study was to compare f.NIRS with Raman for predicting the percentage of mesquite in cow feces and the discrimination of base diets by the two techniques.

Methods

Feeding Trial

This research was conducted at New Mexico State University Campus Livestock, Education, and Research Center (Las Cruces, New Mexico) during November and December 2013. Fecal material was obtained from a feeding trial where known amounts of honey mesquite leaves were introduced intraruminally into six ruminally fistulated cows. Cows were mature Hereford × Angus crosses with an average weight of 568 kg. Honey mesquite leaves were harvested from the Chihuahuan Desert Rangeland Research Center located 35 km north of Las Cruces, New Mexico during July 2013. Leaves were harvested by hand and allowed to air dry. During a 14-d pretrial period, animals were fed beardless wheat hay (*Triticum aestivum* L.) ad libitum. Following the pretrial, two animals were randomly assigned to one of three base diets: timothy hay (*Phleum pratense* L.), a C3 perennial; Sudan hay (*Sorghum sudanense* [Piper] Stapf), a C4 annual; or a 50:50 combination of Bermudagrass hay (*Cynodon dactylon* [L.] *Pers.*), a C4 perennial, and beardless wheat hay, a C3 annual (BBW). Base rations (hay) were fed, and refusals were collected and weighed daily. The base hay rations were fed for four periods of increasing levels of mesquite. The initial period was 9 d, in which no mesquite was fed and base diet was fed at 2% of body weight. The subsequent periods were 7 d each. For the second period, the base diet was fed at 1.9% of body weight and 1% of the diet was mesquite leaves. For the third period, the base diet was fed at 1.7% of body weight and 3% of the diet was mesquite leaves. For the fourth period, the base diet was fed at 1.5% of body weight and 5% of the diet was mesquite leaves. Each morning at approximately 0800 hr during the final three periods, air-dried mesquite leaves that were ground to an approximate length of 1 cm were introduced through a rumen cannula. Fecal samples were collected at the same time on the final 2 days of each period. Previous research (Walker et al., 2010) showed that NIR-determined percentage juniper in the diet of goats did not change after the third day when percentage juniper in the goat diet increased from 0% to 10%. Mesquite leaves and the base rations

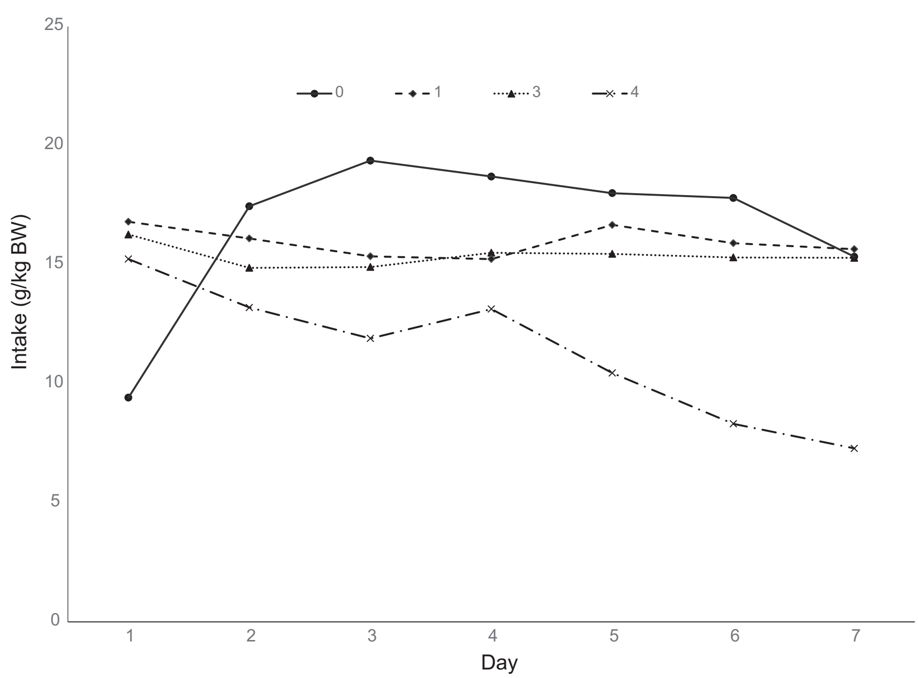

Figure 1. Hay intake (g/kg BW) averaged across three different hays as affected by mesquite as a percentage of total intake placed in the rumen.