Full Length Article:

Potential of Near-Infrared Reflectance Spectroscopy (NIRS) to Predict Nutrient Composition of *Bromus tomentellus*

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Abstract. Determination of forage quality of available species is one of the fundamental factors for the management of rangelands. Near-Infrared Reflectance Spectroscopy (NIRS) was used to analysis the Nitrogen (N), Acid Detergent Fiber (ADF), Dry Matter Digestibility (DMD) and Metabolizable Energy (ME) content of three phenological stages (vegetative, flowering and seeding) of Bromus tomentellus samples in grazing pastures of Iran. The sample set consisted of 40 samples for calibration and 23 samples for validation was used to prediction N, ADF, DMD and ME, separately. The samples were measured by reflectance NIR in a 950-1650 nm range. Calibration models between chemical data and NIR spectra were produced using the method of Partial Least Squares (PLS). The coefficients of determination (R^2) and standard error of cross validation (SECV) were 0.94 (SECV: 0.208%), 0.98 (SECV: 1.76%), 0.98 (SECV: 1.97%), and 0.97 (SECV: 0.34%) for N, ADF, DMD and ME, respectively. The results obtained from this study indicated that NIRS had a potentiality to be used in predict the N, ADF, and the estimated DMD and ME of forage samples content.

Key words: Range management, Animal nutrition, N, ADF, DMD, ME, *Bromus tomentellus*, Near-Iinfrared Reflectance Spectroscopy (NIRS).

Introduction

One of the main objectives of range management is livestock production, which depends on great extent on the nutritive value of available forage (Stoddart *et al.*, 1975). Ganskopp & Bohnert (2003) proposed that livestock and wildlife managers must be aware of the nutritional dynamics of forages to sustain satisfactory growth and reproduction of their animals, and assure fair value of the pasture. Valentine (1990) believed that the nutrient balance of animals, whether grazing or penfed is dependent upon four basic factors including the animal's nutrient requirements, nutrient content of the feedstuffs consumed and the amount consumed. Knowledge of generalized nutrient trends in the forage plants available to grazing animals will assist in achieving their most timely utilization and help predict nutrients decencies, and suggest supply maintain need. Knowledge of nutritional quality of the forage for maintaining animal health requires forage quality analysis and monitoring for proper feed rationing development (Calderon *et al.*, 2009). The *Bromus tomentellus* is a stable species with cold season grazing value and cluster biological form. It is a palatable species which is consumed by all classes of livestock, particularly sheep. Forage will vary greatly in chemical composition and nutritional value (Murray, 1986). Traditionally wet chemical analyses have been used to characterize forages, and to predict their nutritive value. These are time-consuming, costly and in some cases hazardous chemicals are involved (Kokaly & Clark, 1999; Graeff *et al.,* 2001; Li *et al.,* 2006). Forage analysis with NIRS was first reported in 1976 (Norris *et al.,* 1976). Near Infrared Reflectance (NIR) has become widely recognized as a valuable tool in the accurate determination of the chemical composition of a wide range of forages (Murray, 1993; Shenk & Westerhaus, 1994). NIRS technology is based on the fact that the major organic chemical components of a sample have near infrared absorption properties in the region 700-2500 nm allowing the instantaneous prediction of the nutritive value of feeds and forages (Garrido, 1997). This technique requires consistent sample handling and a calibration method based in multivariate analysis, which converts spectral (NIR absorption) into laboratory reference method information (Alomar *et al.*, 1999; Deaville & Flinn, 2000; Cozzolino *et al.*, 2000, 2003; Roberts *et al.*, 2004). Several authors have tested NIR to estimate Forage Nutrient Content (Starks *et al.*, 2004; Andrés *et al.*, 2005; Scholtz *et al.*, 2009; Charehsaz *et al.*, 2010).

The objective of this study was to assess the potential of the NIRS technique to predict the N, ADF, DMD and ME contents of *Bromus tomentellus* species.

Materials and Methods

A total of 63 samples of *Bromus tomentellus* were collected at 5 localities (sites) grazing pasture of Iran [West Azarbaijan, East Azarbaijan, Ardabil, Zanjan and Isfahan]. Samples were collected from three phenological stages (vegetative, flowering and seeding stages) with three replications. The range of mean annual precipitation in above regions is 326.2-590 mm and its climates located in cold semi arid with moderate summers and cold winters. The soil texture of these regions was combination of moderate to semi deep and was sandy and clay-loam texture.

The data of chemical analysis provided by Arzani *et al.* (2011) was used to compare NIR results with laboratory methods [Nitrogen was measured using the Kjeldhal technique (AOAC, 1995), Acid Detergent Fiber (ADF) was measured using the procedure described by Van Soest (1963). Dry matter digestibility was estimated using the

formula

$$DMD\% = 83.58 - (0.824\,ADF\% + 2.626\,N\%)$$

suggested by Oddy *et al.* (1983) and metabolizable energy was also predicted using the equation $ME = [0.17\,DMD\% - 2]$ suggested by SCA (1990)].

Samples were ground to pass through a 2 mm sieve size and 5 grams of each sample were scanned by NIRS. The scanning range from 950-1650 nm (DA 7200 Perten instruments, Sweden) and the spectra were recorded as log (1/R) at 2 nm intervals. Before scanning the samples pre-dried at 60°C overnight in an oven to standardize moisture conditions. Samples were scanned twice in duplicate repacking.

Spectral data was exported into the Unscrambler (CAMO AS, version 9.5, Norway) software for multivariate analysis. Principal Component Analysis (PCA) was performed before Partial Least Squares (PLS) regression models were developed. PCA is a mathematical procedure for resolving sets of data into orthogonal components whose linear combinations approximate the original data to any desired degree of accuracy (Naes *et al.*, 2002). Since the PLS method is a soft-modelling method (Wold *et al.*, 2001; Naes *et al.*, 2002), therefore calibrations models were developed using PLS regression. The optimum number of terms in the PLS calibration models were determined by full cross validation and defined by the PRESS (prediction residual error sum of squares) function in order to avoid over fitting of the models (Naes *et al., 2002*). The resulting calibration equations between the chemical reference values and the NIRS data were evaluated based on the coefficient of determination in calibration (R^2_{cal}) and the Standard Error of Cross Validation (SECV). Another measure of the models is the Residual Prediction Deviation (RPD) which is the ratio of Standard Deviation (SD) to the standard error of cross validation (SECV). This is particularly useful in comparing the prediction abilities between alternative models (Lomborg *et al.*, 2009). An RPD value greater than three is considered adequate for analytical purposes in most of the NIRS applications for agricultural products (Williams, 2001; Fearn, 2002), whereas a value of 2.5 for the RPD may be regarded as a lower limit for robust NIRS calibrations in quantitative analysis (Williams, 2001). The NIRS calibration models were tested using the validation set. The standard error of prediction (SEP), bias and slope were used to evaluate the calibrations developed.

Results and discussion

The descriptive statistics (mean, range and standard deviation) of the chemical parameters in the calibration and validation sets are showed in (Table 1). In both calibrations and validation sets a wide range in variation in chemical composition was observed due to the different stages (phenological or harvest times) of *Bromus tomentellus* samples (vegetative, flowering and seeding) collected. This variation or range in chemical composition was considered adequate to test the feasibility of developing NIR calibrations for the chemical parameters analysed.

Table 1. Descriptive statistics of *Bromus tomentellus* samples used to develop the NIRS calibration (% DM basis)

Variable	N	Mean	SD	Range
Calibration samples (40)				
N%	40	1.36	0.87	0.36-3.88
ADF%	40	47.34	7.86	30.4-61.84
DMD%	40	48.13	8.58	33.49-68.72
ME%	40	6.19	1.45	3.69-9.68
Validation samples (23)				
N%	23	1.58	1.00	0.37-4.17
ADF%	23	42.38	6.72	33.07-58.08
DMD%	23	52.81	7.72	38.29-67.12
ME%	23	6.98	1.31	4.24-9.41

N: number of samples; N: Nitrogen; ADF: Acid Detergent Fiber; DMD: Dry Matter Digestibility; ME: Metabolizable Energy; SD: Standard Deviation

Fig. 1 shows the score plot of the first two principal components of the *Bromus tomentellus* samples analysed NIRS. The first PC accounts obtained the maximum possible variance of the data (Osborne *et al.*, 1993). The second PC is orthogonal to the first PC, uncorrelated with it must explained the largest part of the residual variance unexplained by the first component (Ventura & Papini, 1997). The first two principal components (PCs) explain 91% of the variation in the spectra of the samples analysed (PC1 = 83% and PC2 = 8%). The separation observed between samples is related to the time of sampling harvest.
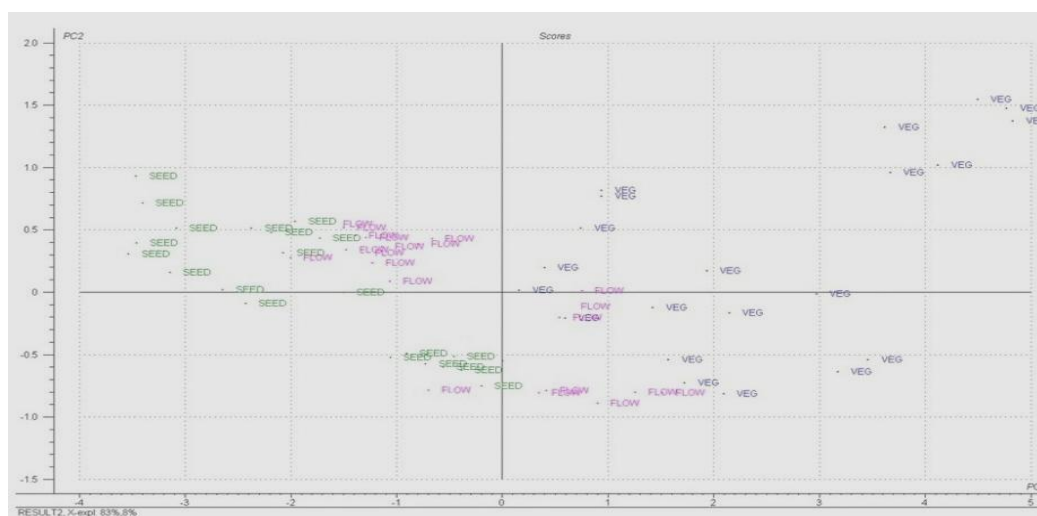

Fig. 1. Score plot of the two principal components in *Bromus tomentellus* marked according to phenological stages (Vegetative=VEG, Flowering=FLOW and Seeding=SEED)

Tables 2 and 3 show the calibration and validation statistics for each of the chemical constituents analysed. The R^2 and SECV were for DMD 0.98 (SECV: 1.97 %), for N 0.94 (SECV: 0.208 %), for ADF 0.98 (SECV: 1.76 %) and for ME 0.97 (SECV: 0.34 %). According to the report by Stchur *et al.* (2002), if the SEC and SECV values approximately be similar values, then the calibration model is probably a valid one. In this study according to the Table (2), the SEC and SECV values obtained for the parameters analysed were the same values, approximately. Williams (2003) stated that a value for R^2 between 0.66 and 0.81 indicates approximate quantitative predictions, whereas, a value for R^2

between 0.82 and 0.90 reveals good prediction. Calibration models having a value for R^2 above 0.91 are considered to be excellent. In our research the values of R^2 for all parameters analysed were above 0.91. The RPD for the NIRS calibration for the evaluated parameters demonstrated how well the calibration models performed in predicting the reference data. If a product shows a narrow range in composition, or if the error in estimation is large compared with the spread (as SD) in composition, then the regression method finds increasing difficulty in finding stable NIRS calibrations. Sinnaeve *et al.* (1994) reported that a ratio >3.0 is indicative of a calibration which should perform well for quantitative purposes whilst a ratio >2.5 and <3.0 indicates a calibration that can be used for screening purposes. A value <2.5 indicates that a calibration is of only limited value. The RPD values obtained in calibration for the chemical parameters analysed were 4.35, 4.46, 4.35 and 4.26 for N, ADF, DMD and ME respectively. The RPD values indicated that the PLS calibrations developed can be used on routine analysis. Similar results were reported by other authors when forage samples were analysed using NIRS (Shenk & Westerhaus, 1993; Muray, 1993; Garrido, 1997; Alomar *et al.*, 1999; Deaville & Flinn, 2000; Roberts *et al.*, 2004).

Table 2. Near infrared reflectance calibration statistics for whole *Bromus tomentellus* samples variables

Variable	N	Mean	SEC	SECV	R^2	1-VR	RPD
N%	40	1.33	0.19	0.20	0.94	0.75	4.35
ADF%	40	43.75	1.49	1.76	0.98	0.95	4.46
DMD%	40	51.00	1.44	1.97	0.98	0.95	4.35
ME%	40	6.80	0.3	0.34	0.97	0.95	4.26

N: number of samples in calibration; SD: Standard Deviation; SEC: Standard Error of Calibration; SECV: Standard Error of Cross Validation; R^2: Coefficient of Determination for Calibration; 1-VR: Coefficient of Determination for Cross Validation; RPD: SD/SECV

Table 3 shows the NIRS validation statistics. The R^2 and SEP were for DMD 0.96 (SEP: 2.48), for N 0.93 (SEP: 0.36), for ADF 0.97 (SEP: 2.45) and for ME 0.97 (SEP: 0.55). The predictive accuracy for the NIR models was considered intermediate as judged by the RPD values obtained. The RPD values obtained in validation for the chemical parameters analysed were 2.71, 2.73, 3.45 and 2.38 for N, ADF, DMD and ME respectively.

Table 3. Validation statistics for whole *Bromus tomentellus* samples variables

Variable	N	SEP	Bias	R^2	Slope	Offset	RPD
N%	23	0.36	-0.001	0.93	0.88	0.13	2.71
ADF%	23	2.45	-0.015	0.97	0.95	2.24	2.73
DMD%	23	2.48	-0.017	0.96	0.93	3.23	3.45
ME%	23	0.55	-0.002	0.97	0.94	0.36	2.38

N: number of samples in validation; SEP: Standard Error of Prediction; Bias: average between reference and NIRS values; Slope: Slope of reference vs. NIRS; Offset = the point where a regression line crosses the ordinate (y-axis); RPD: Standard Deviation/SEP

The results from this study suggested that *Bromus tomentellus* samples might be analysed by NIRS spectroscopy in order to determine N, DMD, ME and ADF. However, the prediction accuracy obtained (RPD values in validation) is less than desirable for analytical purposes. Differences in the calibration statistic were observed when samples were split into calibration and validation sets. Differences in the prediction performance of the NIRS method (see Table 3) developed imply that the calibration models might be sensitive to the range of sample types (harvest or phenological stages) used to develop calibration models. Therefore, samples from more years or harvest need to be included in the calibration data in order to increase the robustness of the NIRS models for routine analysis. Further work will be carried out in order to assess the robustness of the NIRS calibrations models and to incorporate more chemical parameters.

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