# Potential of near-infrared reflectance spectroscopy (NIRS) to predict nutrient composition of *Bromus tomentellus*

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**Abstract.** Near-infrared reflectance spectroscopy (NIRS) was used to analyse 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 predict 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 indicate that NIRS have a potential to be used to predict the N, ADF, and the estimated DMD and ME content of forage samples.

**Keywords:** Range management, animal nutrition, *Bromus tomentellus*, near-infrared reflectance spectroscopy (NIRS).

## Introduction

One of the main objectives of range management is livestock production, which depends to a great extent on the nutritive value of available forage (Stoddart *et al.* 1975). 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. 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 and 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 and Westerhaus 1994). NIRS technology is based on major organic chemical components of a sample having near infrared absorption properties in the region 700-2500 nm allowing the rapid prediction of the nutritive value of feeds and forages (Garrido 1997). Several authors have tested NIR to estimate Forage Nutrient Content (Starks *et al.* 2004; Andrés *et al.* 2005; 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.

## 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 data from chemical analysis provided by Arzani *et al.* (2011) was used to compare NIR results with laboratory methods [Nitrogen was measured using the Kjeldahl 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 = 0.17DM D% - 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 ranged 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. The resulting calibration equations between the chemical reference values and the NIRS data were evaluated based on the coefficient of determination in

**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 |

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

**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 |

Note: 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. 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 |

Note: 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.

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).

## Results

The descriptive statistics (mean, range and standard deviation) of the chemical parameters in the calibration and validation sets are shown in Table 1. In both calibration 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.

Tables 2 and 3 show the calibration and validation statistics for each of the chemical constituents analysed. The R2 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). 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.

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.

## Conclusion

The results from this study suggested that *Bromus tomentellus* samples might be analysed by NIRS spectroscopy 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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