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Estimation of qualitative parameters in *Coronilla varia* and *Medicago sativa* using chemical methods and Near-infrared reflectance (NIR)

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ABSTRACT: Improvement of the traits related to forage quality including Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and CP in forage species has a significant impact in increasing livestock production. In breeding programs that the number of samples is occasionally high, the use of chemical methods is time-consuming and costly. For this reason, NIR technology has been introduced as a rapid and accurate method in estimating chemical composition of agricultural products. This research was aimed to investigate the possibility of using this technology in estimation of NDF, ADF, and CP in *Coronilla varia*. A total of 45 samples of each species were selected during different phenological growth stages and from different vegetation types. CP was measured by Kjeldahl method and *Coronilla varia* method was applied to measure NDF and ADF. For NIR calibration, samples were divided into two categories so that a part was considered for calibration and the rest for evaluating the accuracy of NIR in estimating the samples. All samples were irradiated by NIR wavelengths and the best regression equation was fitted between the chemical method and NIR based on statistical methods. According to the results, it could be concluded that NIR can be used as a rapid, accurate, and reliable method with enough potential to assess the quality traits in breeding programs.

Keywords: *Coronilla varia*, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Crude Protein (CP), Near Infrared Reflectance Spectroscopy (NIRS).

INTRODUCTION

Knowledge on the nutritional value of plant species helps range managers and ranchers to balance between available food and livestock requirement to maximize livestock performance. Supplying livestock requirement is necessary in terms of energy, protein, minerals, and vita-mins and it is possible when forage quality is studied in terms of chemical and physical compounds (Arzani, 2002).

Several indicators are measured to determine forage quality including crude protein, crude fat, NDF, ADF, DMD, ME, WSC, nitrogen-free extract, crude fiber, lignin, minerals (phosphorus, potassium, calcium, etc.), relative nutritional value, and so forth. Among the mentioned fac-tors, those must be considered that firstly less time and cost are spent measuring them and secondly provide a good estimation of forage quality. In recent years, NIR technology has developed and the measurement of agricultural and livestock products is possible with this system. Nowadays, quality traits of forage species are measured using this technology. This method is appropriate for selective programs in which plant breeders face a number of dif-ferent plant populations and rapid and inexpensive methods are required to measure the traits. The percentage of crude protein and the content of ADF and NDF are important quality traits in improving the nutritional value of forage species including *Coronilla varia*. Several me-thods have been introduced for quality traits. For example, currently, two chemical methods including the Kjeldahl and LECO nitrogen analyzer are used to measure the percentage of crude protein.

In both methods, CP is calculated according to the formula (N × 6.25) with the difference that the speed and accuracy of LECO is higher compared to Kjeldahl.

The method of Van Soest (1963) and the device of Fibertic 2010 are used to measure ADF. In addition, NDF is measured by the above method, with the difference that acid solution (ADS) and neutral solution (NDS) are used to measure ADF and NDF, respectively. NIR method is based on near-infrared absorption and reflection in the wavelengths of 700 - 2500 nm. In this method, radiation is emitted on samples and reflected energy (R) from samples is calculated according to log1/R. The device is calibrated based on multiple linear regressions (MLR) between the energy reflected from the object and chemical data. NIR measurement accuracy depends on calibration method. Therefore, the chemical methods must be accurate and standardized and forage samples should also have sufficient range for traits. Therefore, it would be better to collect the samples from different growth stages and different sites (Beerepoot and Angew, 1997). NIR spectrometry has been used since 1970 to analyze the factors, including the percentage of protein, NDF%, and the percentage of digestibility in cereals and forage species (Norris *et al*., 1994, Deaville *et al*., 2000). Norris *et al*., (1976) estimated the standard error prediction to be 0.95, 3.1, 2.5, 2.1, and 3.5% for the percentage of crude pro-tein, NDF, lignin, and digestibility, respectively. In a similar study, conducted on legumes and grasses by Garcia *et al*., (2006), NIR calibration revealed relatively high correlation coefficients and low standard error for ADF, CP, DM, and DMD. Gatius *et al*., (2004) applied the NIR in the estimation of crude protein (CP) and a calibration was performed for 27 samples with three growth stages (vegetative, flowering, and seeding). The raw content of the samples was measured by a comprehensive model, for all growth stages, and a unique model for the seeding stage. In the same results, Parnell and White (1983) showed NIR performance as an appropriate alternative to determining the crude protein of different forage species. Jafari (2001) evaluated the possibility of using NIR in estimation of digestibility and crude protein content in forage grasses and introduced the NIR method as a new, fast, accurate and efficient technology in measuring the forage quality of range species. The same results also have been reported by Ahmadi (2003), who compared laboratory and NIR methods for measuring the forage quality of a few rangeland species in different phenological stages. Charehsaz *et al*., (2012) evaluated the performance of NIR method in estimation of crude protein, digestibility, ADF, and total ash. They reported SEC values of 0.15-1.09, 0.83-3.94, 0.52-4.96, and 0.21-0.86 and SEP values of 0.13-0.75, 84.62-3.34, 0.31-3, and 0.17-0.61 for the mentioned traits, respectively. Correlation coefficient between the experimental data and the results of NIR prediction for all traits, particularly CP, was very high (<95%). Arzani *et al*., (2007) studied the forage quality of rangeland species in three provinces of Semnan, Markazi, and Lorestan to calculate daily animal unit requirement. According to the results, the changes of parameters were estimated to be 8.8, 9.5, and 9 for CP; 40, 41, and 42% for ADF; 54, 54, and 52% for DMD, and 7.2, 7.09, and 6.86 MJ per kg dry matter. The purpose of this study was to investigate the possibility of using NIR technology to estimate the NDF, ADF, and CP in *Coronilla varia* and *Medicago sativa* as forage species.

MATERIALS AND METHODS

A. Study area

Golestan National Park is a mountainous region, located in the Far East of Northern forests of Iran.

Geographically, this park is located between latitudes 37° 16' 34" and 37° 31' 00" N and longitudes 55° 43' 00" and 56° 17' 45" E, between the cities of Gonbad Kavous and Bojnord.

Golestan National Park is located 55 km from east Gonbad Kavous and 115 km from west Bojnord.

This Park is in the jurisdiction of the three provinces of Khorasan, Golestan and Semnan, but in terms of protection authority and responsibility, it is under monitoring of the General De-partment of Environment of Golestan province.

B. Methodology

In the present study, sampling was conducted randomly with three replications. In other words, a total of 45 samples of each species were selected at different growth stages from different vegetation types. Samples were dried in an oven at a temperature of 70°C for 48 hours; then, they were ground and kept in closed containers. A part of samples was considered for laboratory studies and the remaining for calibration and measurement of traits by near-infrared method.

Chemical methods: Measurement of the percentage of crude protein (CP): The percentage of crude protein was obtained through calculating nitrogen % of samples. Kjeldahl method was used for this pur-pose. Finally, with regard to the volume of acid consumed in the titration and according to the following equation, nitrogen% of samples was obtained and crude protein was calculated through multiplying the percentage of nitrogen by 6.25 (McDonald, 1996):

(5/0)% N= A × N ×1.4/

In this equation: A is the volume of acid and N is the normality of acid.

Measurement of ADF: (extraction with acid detergent)

Van Soest method (1963) and Fibertec equipment were used to measure the ADF of samples. One gram of the sample was poured in the container of the device called Crucible, and one hundred ml of acid detergent solution (ADS) was added to each sample. After laboratory procedures, the samples were weighed again and the weight difference before and after placement in the furnace showed the ADF.

Measurement of NDF: (Extraction with neutral detergent)

Measurement method is similar to ADF, with the difference that here, neutral detergent solu-tion (NDS) was used instead of acid detergent solution (ADS). After exiting the containers from the oven, the samples were weighed and subtracted from the empty container. Finally, multiplying this value by 100, gives the percentage of NDF.

Calibration and measurement by NIR

To provide an efficient calibration, the samples should have the minimum and maximum of the trait with a normal distribution. For this reason, it was attempted to collect the samples from different growth stages. After chemical measurements, samples were scanned by NIR-Percon Inframatic 8620, having 20 optical filters.

Calibration of the device was performed by using multiple linear regression (MLR) and SE-SAME software (Branand Lubbe, 1996). To determine the best calibration, the combinations of three, four, five and six of 20 wavelengths were used. For each trait, the best calibration was selected based on standard error of calibration (SEC), the multiple correlation coefficients (R), F test, and statistical parameters of t and H (Bran and Lubbe, 1996). In the present study, the method introduced by Westrhaus (1988) was used for calibration. In this method, statistical parameters of t and H were used for removal of suspicious and wrong samples and the best calibration model was selected on the basis of low standard error estimation (SEP), high correlation coefficient between the chemical and NIR data, the slope of the regression line close to 1, and low deviation of NIR data from chemical method.

RESULTS

Ranges, means, and standard deviations of the measured samples of *Coronilla varia* and Me-dicago sativa by chemical methods for CP, ADF, and NDF are presented in Table 1 and Table 2, respectively. According to the method of sample collection, the range and diversity of traits is to the extent that it covers minimum and maximum range reported in the literature.

Table 1: Summary of laboratory results of qualitative traits in *Coronilla varia.*

Qualitative traits	Minimum	Maximum	Average	Standard deviation
ADF	11.51	32.43	22.83	**6.82**
NDF	21.70	50.47	29.25	**8.08**
CP	18.87	26.68	20.70	**3.51**

Table 2: Summary of laboratory results of qualitative traits in *Medicago sativa.*

Qualitative characters	Minimum	Maximum	Average	Standard deviation
ADF	18.23	36.29	25.06	**5.29**
NDF	23.12	46.14	30.84	**7.87**
CP	13.89	28.87	22.44	**5.49**

Table 3: Calibration results and NIR assessment to measure the quality traits in *Coronilla varia.*

	Calibration				Evaluation		
Qualitative traits	Number of wavelengths	Number of	Coefficient of determination	Standard error	Number of samples	Coefficient of determination	Standard error
NDF	5	30	0.93	1.66	15	0.92	1.18
ADF	5	30	0.98	1.15	15	0.91	1.14
CP	4	30	0.99	1.18	15	0.99	1.15

The statistical parameters including standard error of calibration, multiple correlation coefficient, standard error estimation, and simple correlation coefficient are shown in Tables 3 and 4 for *Coronilla varia* and *Medicago sativa*, respectively. The regression equation between chemical data and estimated data by the NIR and its parameters including coefficient of determination, the slope of the regression line, and the deviations between the observed and estimated data for the studied traits are presented in Figures 3 to 8. Changes in the studied traits in three growth stages including vegetative growth, flowering and seeding of *Coronilla varia* and *Medicago sativa* are presented in Figures 1 and 2, respectively. The results showed that vegetative growth stage had the highest percentage of crude protein and this percentage declined with aging plant. Therefore, if plant is harvested late, plant protein will not meet required animal protein. In general, the highest and lowest crude protein was recorded for vegetative growth and seeding stages, respectively. Contrary, the lowest ADF for both species was recorded in vegetative growth stage, indicating maximum digestibility. Comparison of phenological stages showed that the minimum and maximum percentage of crude fiber was obtained in vegetative growth stage and seeding stage, respectively.

Table 4: Calibration results and NIR assessment to measure the quality traits in *Medicago sativa.*

	Calibration				Evaluation		
Qualitative trait	**Number of wavelengths**	**Number of samples**	**Coefficient of determination R2**	**Standard error SEC**	**Number of samples**	**Coefficient of determination R2**	**Standard error SEC**
NDF	5	30	0.93	1.88	15	0.92	**2.18**
ADF	5	30	0.98	1.22	15	0.91	**1.21**
CP	4	30	0.99	1.12	15	0.99	**1.10**

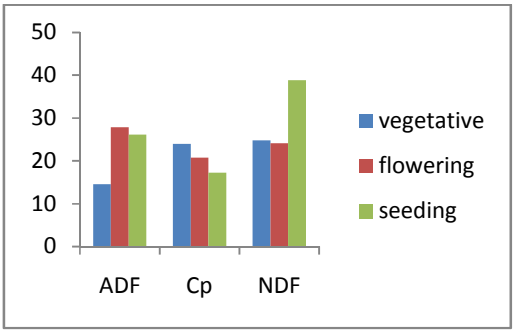

Fig. 1. The changes trend of the studied traits at different growth stages of *Coronilla varia.*

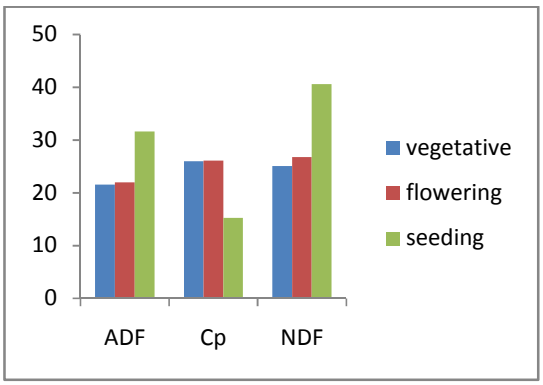

Fig. 2. The changes trend of the studied traits at different growth stages of *Medcago sativa.*

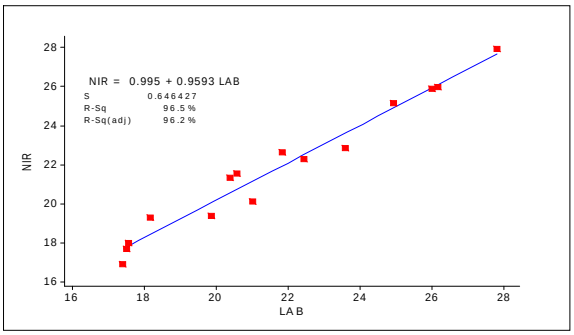

Fig. 3. Regression equation between laboratory and NIR methods for measuring crude protein in *Coronilla varia.*

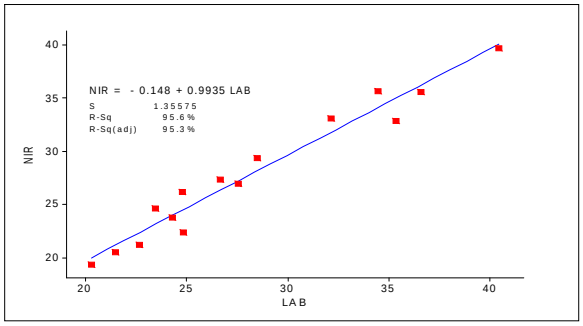

Fig. 4. Regression equation between laboratory and NIR methods for measuring NDF in *Coronilla varia.*

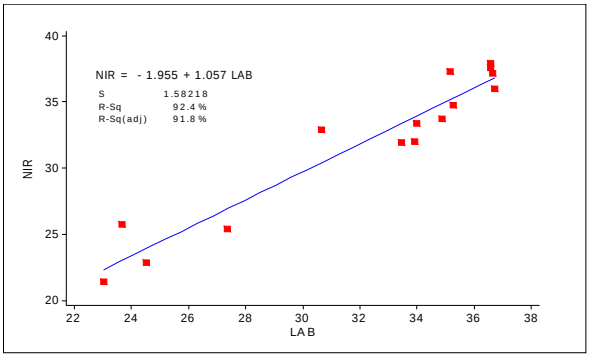

Fig. 5. Regression equation between laboratory and NIR methods for measuring *ADF* in *Coronilla varia.*

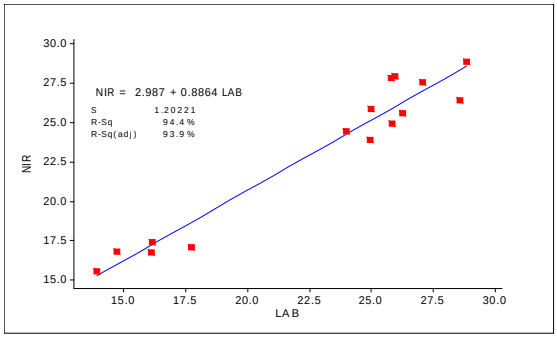

Fig. 6. Regression equation between laboratory and NIR methods for measuring crude protein in *Medicago sativa.*

According to the results, a close relationship was found between the observed and estimated results for NDF with a correlation coefficient of 0.93 and standard error of estimation of 1.66 and 1.88 for *Coronilla varia* and *Medicago sativa*, respectively (Tables 3, 4 and Figs. 4, 7). Calibration equation for ADF is so that it could estimate a series of 45 samples, different from the calibration series with a correlation coefficient of 0.98 and standard error of estimation of 1.15 and 1.22, for *Coronilla varia* and *Medicago sativa*, respectively (Tables 3, 4 and Figs. 5, 8). Calibration equation fitted for crude protein showed more accuracy compared to other two traits as it could estimate a series of 45 samples with a correlation coefficient of 0.99 and standard error of estimation of 1.18 and 1.12, for *Coronilla varia* and *Medicago sativa*, respectively (Tables 3, 4 and Figs. 3, 6).

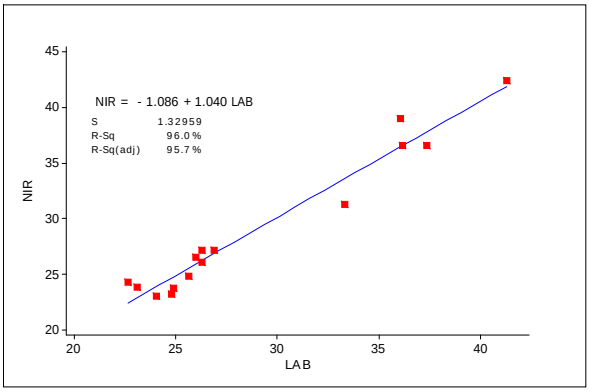

Fig. 7. Regression equation between laboratory and NIR methods for measuring NDF in *Medicago sativa.*

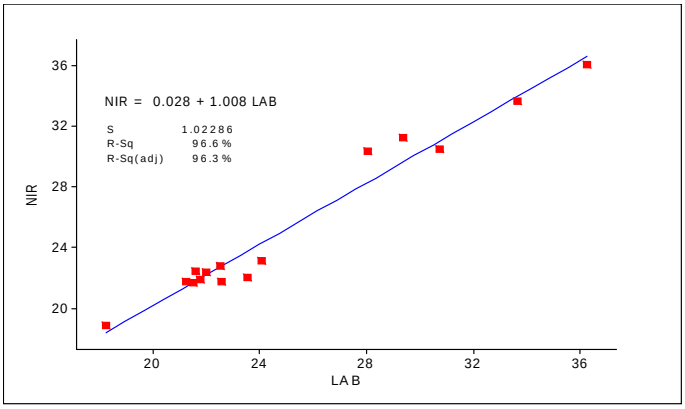

Fig. 8. Regression equation between laboratory and NIR methods for measuring *ADF* in *Medicago sativa.*

DISCUSSION AND CONCLUSION

According to the comparison of standard error between two replications of chemical method (Table 1) and NIR (Table 2) and also regression equation between the results obtained from chemical and NIR methods, NIR could be used as an accurate alternative to measure quality traits and chemicals in breeding programs, animal science and range management. The results of the calibration equation for CP, ADF and NDF are similar to the results reported in various literature. Ahmadi (2003) in a research on several species reported a higher coefficient of determination (R2 = 0.98) and a stronger regression equation for CP compared to ADF and NDF which is fully consistent with the results of current study with higher coefficient of determination (R2 = 0.99), compared to other traits. Charehsaz *et al*., (2012) investigated the CP, DMD, ash, and ADF of several species and reported a coefficient of determination of 0.93 for crude protein, greater than that of the coefficient of determination of other traits. The same result was also obtained in the present study. Also, the obtained correlation coefficient between the experimental (laboratory) results and NIR for crude protein was stronger than that of other studied traits in abovementioned research. Our results are also in agreement with the results obtained by Garcia and Cozzolina (2006), who predicted the chemical composition of forage species through NIR calibration model.

In their research, regression relationship between NIR and laboratory data of legumes and grasses for CP was higher than other traits which corresponded with the results of present study. Nie *et al*., (2007) studied the forage quality parameters of alfalfa including CP, ADL, ADF, NDF, Ash, and DMD and reported a correlation coefficient of 0.96-0.99. Similarly, in this study, a correlation coefficient of 0.93-0.99 was obtained. Fontencli *et al*., (2004) predicted the chemical compositions of Cynodon dactylon by NIR, and R2 was reported to be 0.98 and 0.99 for CP and ADF, respectively.

Locher *et al*., (2005) studied legumes in intercropping of grass and legume by improving the NIR calibrations and reached a coefficient of determination greater than 99% as a much higher R2 was obtained in grasses, separating them from legumes. Generally, the results of relationship between NIR data and the values obtained from the chemical analysis of samples in the laboratory showed that the best correlation was found between the two data sets for CP and a higher coefficient of determination was obtained. Several researchers have reported the efficacy of NIR for CP. Parnell and White (1983), Posseth (1985), Robert *et al*., (1986), O'Keeffe *et al*., (1987), Winch and major (1981), Shenk *et al*., (1981), and Jafari (2001) reported standard error of prediction of 0.8, 0.38, 63.99, 0.0, 0.92, 0.90, and 0.68 for CP of perennial grasses. Colelho *et al*., (1987), Norris *et al*., (1976) and Martin and Linn (1968) reported the coefficients of determination of 0.98, 0.91, and 0.96 for ADF. Generally, according to the reported results and the results of current study, it can be concluded that the near-infrared spectrometey could be used as a new and advanced technology as well as a fast and efficient alternative to evaluate the quality indicators and chemical composition of forage species with less measurement error and no need for chemical preparations. It had better plant species be harvested with high replications from different growth stages, so that more reliable and accurate calibrations could be conducted when using near-infrared spectrometry and closer comparisons could be done between the two methods with reference methods. Undoubtedly, such calibrations could be applied with high confidence for future research.

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