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The Estimation of Qualitative Parameters of three Species of Grasses using Technology (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 three species of grasses. 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 Van Soest 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: forage value, crude protein, Acid Detergent Fiber, Neutral Detergent Fiber, 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 lives-tock requirement is necessary in terms of energy, protein, minerals, and vitamins 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 factors, 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 different 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 *Agropyron cristatom*, *Dactylis glomerata* and *Festuca ovina*. Several methods have been introduced for quality traits. For exam-ple, 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 protein, 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 ca-libration 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 con-tent 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 rangel-and 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 *Agropyron cristatom*, *Dactylis glomerata* and *Festuca ovina* 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 Department 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 70oC 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 ca-libration and measurement of traits by near-infrared method.

C. 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 purpose. 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):

$\%N = A \times N \times 1.4/(5/0)$

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 con-tainer 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 solution (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 SESAME 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.

DISCUSSION AND CONCLUSION

Table 4 lists the range, mean, and standard deviation of the raw protein, ADF, and NDF characteristics that were measured using chemical methods. Considering the method used for collecting the samples, the range and the variety of the characteristics are at levels that cover the minimum and the maximum reported in references. The statistical parameters, which include the standard error of calibration, the multiple correlation coefficient, the standard error of estimation, and the simple correlation coefficient, are shown in Table 5. Furthermore, the regression equation between the chemical data and the data estimated by NIR and its parameters such as the coefficient of determination, the slope of the regression line and the deviations between the observed and estimated data for the studied characteristics are presented in Diagrams 1 to 3. Table 5 and Diagram 2 show there is a close relationship between the observed and estimated results for the NDF characteristic with the correlation coefficient of 0.93 and standard error of estimation of 1.88.

Table 1: The results of the calibration equation for the NDF characteristic on other samples.

Source	**R2**	**SEP**	**SEC**
Martin and Linn (1986) For silage	0.62	-	3.4
Flinn and Murray (1986)	0.92	1.98	-
Berardo (1997) In *Trifolium repens*	-	4.7	3.7
Shenk *et al* (1981) In Wheat	0.97	2.27	1.91

Table 2: Colelho *et al.* (1987), Norris *et al.* (1976), and Lin and Martin (1986) presented results for ADF.

	R2	**SEC**	**The number of wavelengths needed for the evaluation equation**
Martin and Linn (1986)	0.98	1.76	
Colelho *et al*	0.91	1.43	7
Norris *et al*	0.96		

Table 3: Standard error of calibration and the standard error of estimation for Crude protein.

	Calibration		**Evaluation**	
Source	**SEC**		**SEP**	
Winch and major(1981)	0.11	0.96	0.21	0.92
O Keeffe *et al* (1987)	0.58	0.97	0.63	0.95
Posselt(1985)	0.25	0.98	0.38	0.94
Parnell and white (1983)	0.70	0.99	0.80	0.96
Robert *et al* (1986)	0.75	0.98	0.99	0.98
Shenk *et al* (1981)	0.62	0.98	0.90	-
Jaafari (1996)	0.51	0.99	0.90	-

Table 4: The average index of forage quality in different plant species.

RFV	ME (MJ)	DMI (%)	DMD (%)	NDF (%)	ADF (%)	CP (%)	Qualitative indicator species
172.91	8.90	3.38	64.10	37.59	31.41	15.25	***Agropyron.cristatom***
163.96	8.82	3.22	63.64	39.81	31.93	15.18	***Dactylis. glomerata***
177.74	8.97	3.47	64.56	36.59	30.60	14.73	***Festuca.ovina***
199.31	47.39	3.64	67.88	34.81	27.86	17.66	**Average**

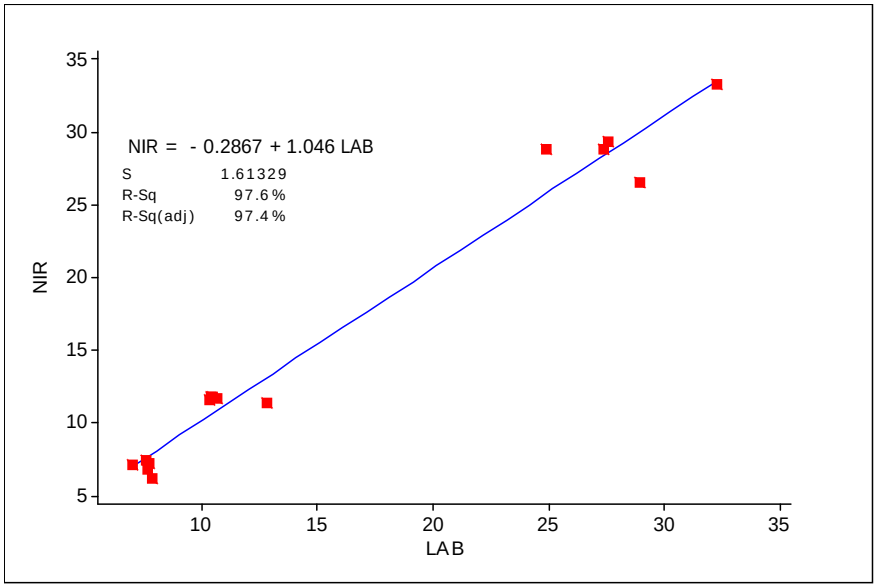

Fig. 1. Regression equation between laboratory and NIR methods for measuring crude protein in *Agropyron cristatom.*

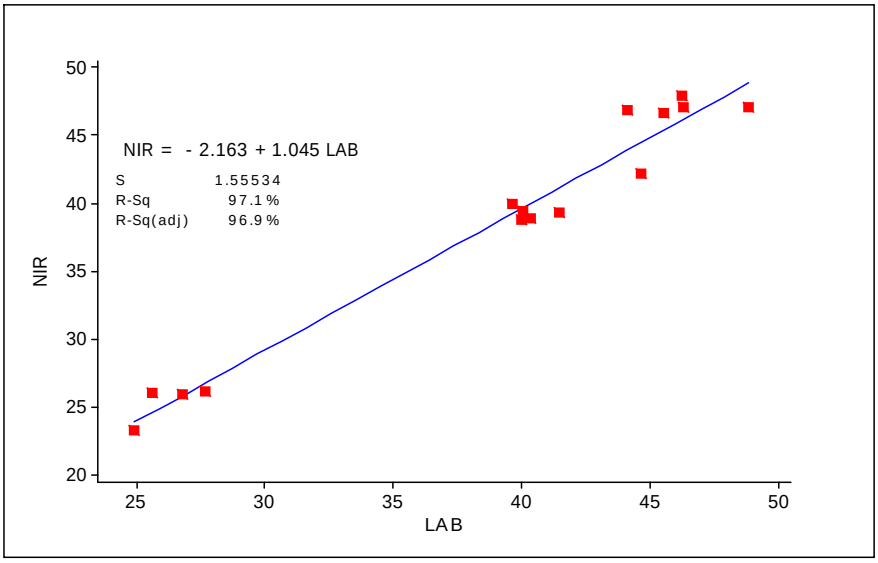

Fig. 2. Regression equation between laboratory and NIR methods for measuring NDF in *Agropyron cristatom.*

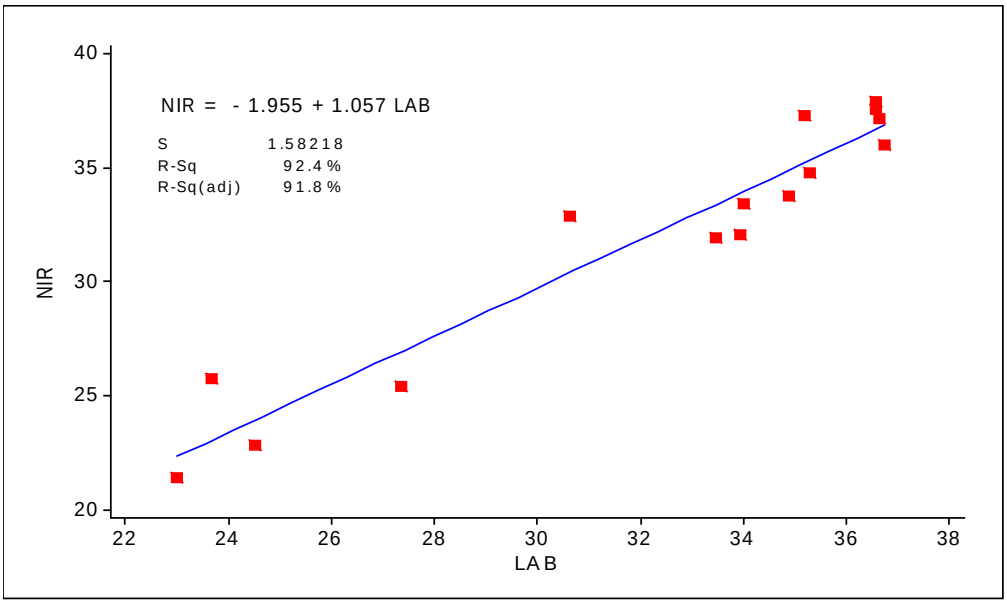

Fig. 3. Regression equation between laboratory and NIR methods for measuring ADF in *Agropyron cristatom.*

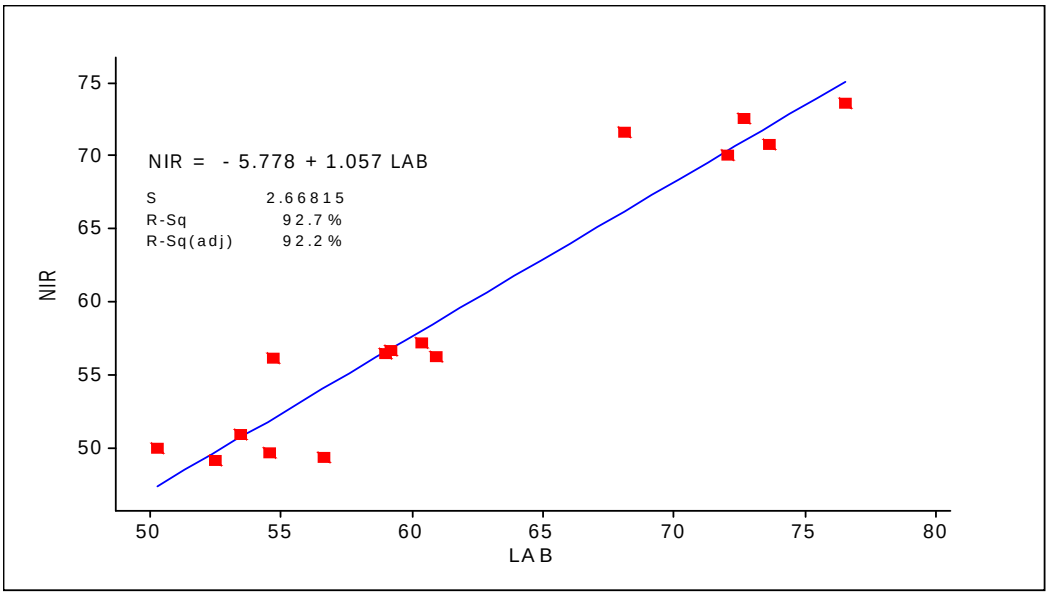

Fig. 4. Regression equation between laboratory and NIR methods for measuring DMD in *Agropyron cristatom.*

The calibration equation for ADF was able to estimate a series of 45 samples (other than the calibration series) with the correlation coefficient of 0.98 and the standard error of estimation of 1.23 (Table 5 and Diagram 3). The fitted calibration equation was more accurate for raw protein compared to the other two characteristics so that it was able to estimate a series of 45 samples with the correlation coefficient of 0.99 and the standard error of estimation of 1.1 (Table 5 and Diagram 1).

Table 5: Average forage quality indicators according to phenological stages in different species.

RFV	ME (MJ)	DMI (%)	DMD (%)	NDF (%)	ADF (%)	CP (%)	Species	Growth stage
265.01	10.73	4.56	74.91	26.35	24.61	26.62	*Agropyron cristatom*	1
133.27	8.19	2.87	59.92	41.90	34.10	10.55	*Agropyron cristatom*	2
120.45	7.77	2.70	57.48	44.51	35.53	7.57	*Agropyron cristatom*	3
252.46	10.62	4.39	74.21	27.49	24.51	25.78	*Dactylis glomerata*	1
131.80	8.23	2.83	60.16	42.51	33.87	10.68	*Dactylis glomerata*	2
106.83	7.62	2.43	56.56	49.43	37.42	9.08	*Dactylis glomerata*	3
266.73	10.52	4.66	73.66	25.81	23.81	23.09	*Festuca ovina*	1
139.56	8.30	2.97	60.61	40.41	33.94	11.90	*Festuca ovina*	2
127.29	8.10	2.76	59.39	43.54	34.06	9.21	*Festuca ovina*	3

Table 6: Summary of laboratory results of qualitative traits in species of grasses.

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 7: Calibration results and NIR assessment to measure the quality traits in species of grasses.

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	1.88	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

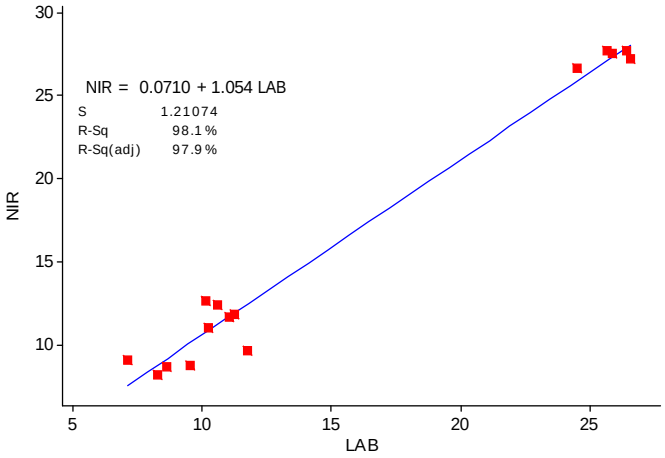

Fig. 5. Regression equation between laboratory and NIR methods for measuring crude protein in *Dactylis glomerata.*

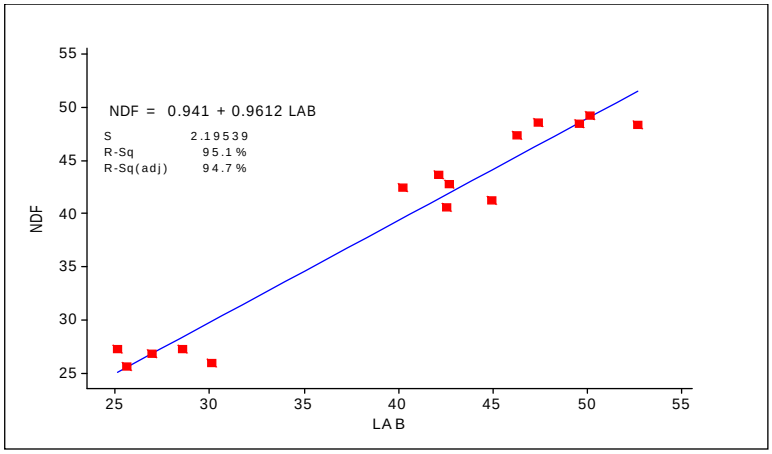

Fig. 6. Regression equation between laboratory and NIR methods for measuring NDF in *Dactylis glomerata.*

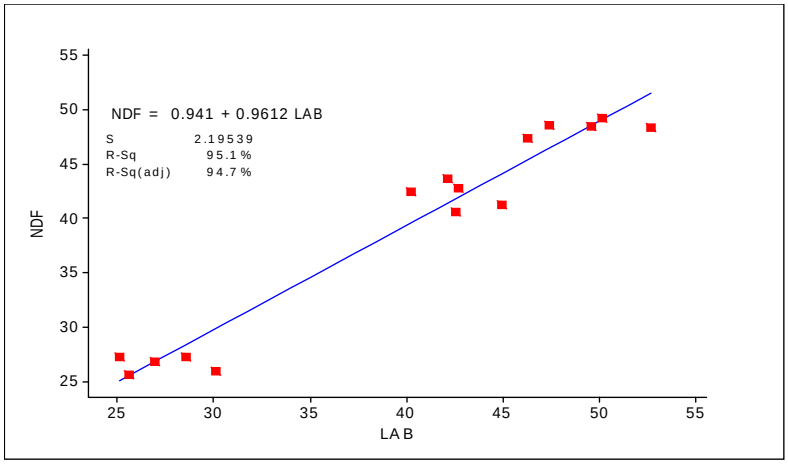

Fig. 6. Regression equation between laboratory and NIR methods for measuring NDF in *Dactylis glomerata.*

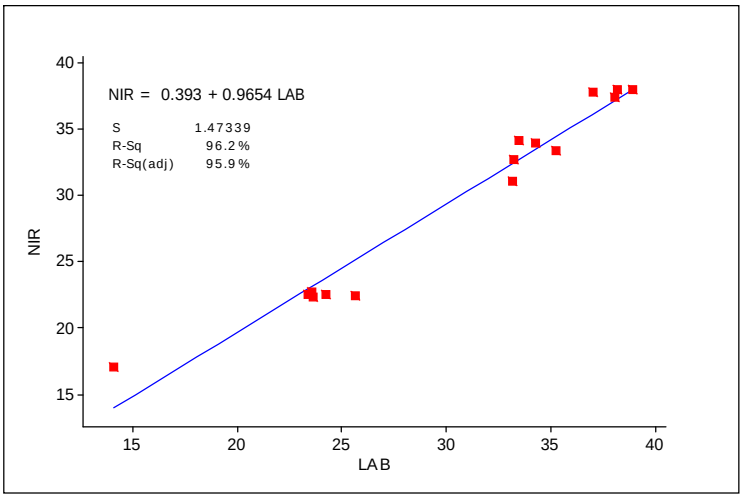

Fig. 7. Regression equation between laboratory and NIR methods for measuring ADF in *Dactylis glomerata.*

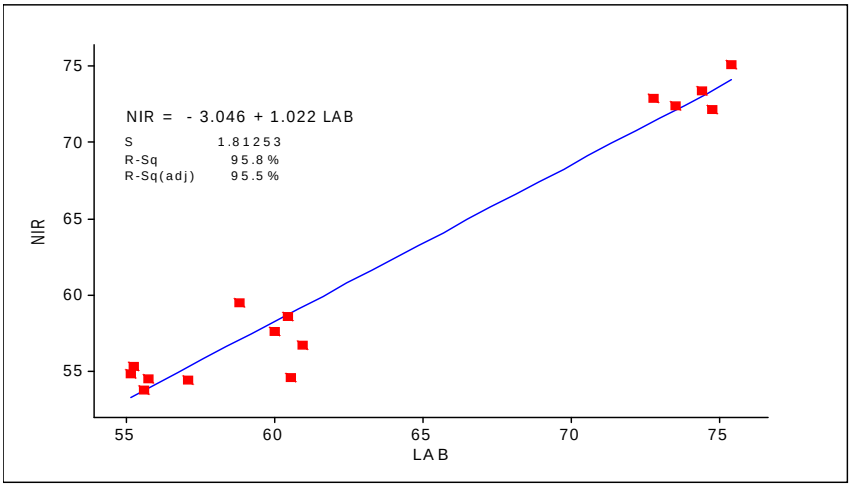

Fig. 8. Regression equation between laboratory and NIR methods for measuring DMD in *Dactylis glomerata.*

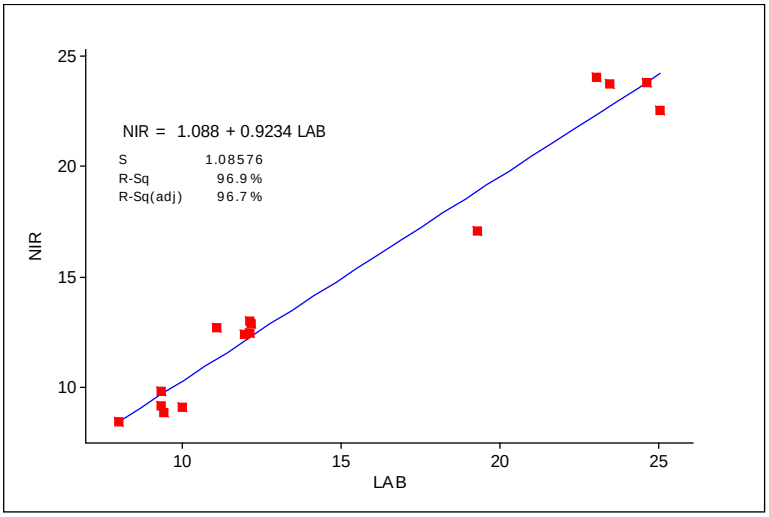

Fig. 9. Regression equation between laboratory and NIR methods for measuring crude protein in *Festuca ovina.*

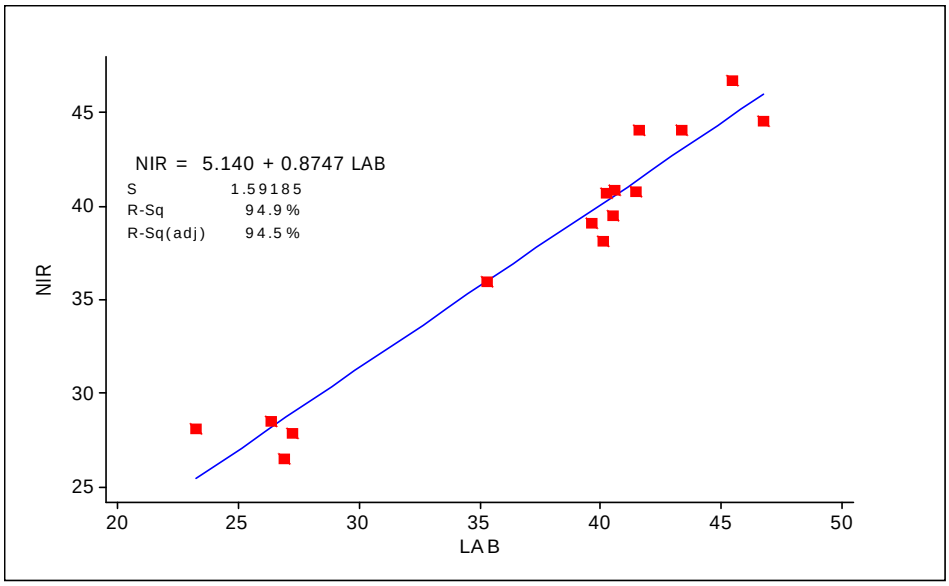

Fig. 10. Regression equation between laboratory and NIR methods for measuring NDF in *Festuca ovina.*

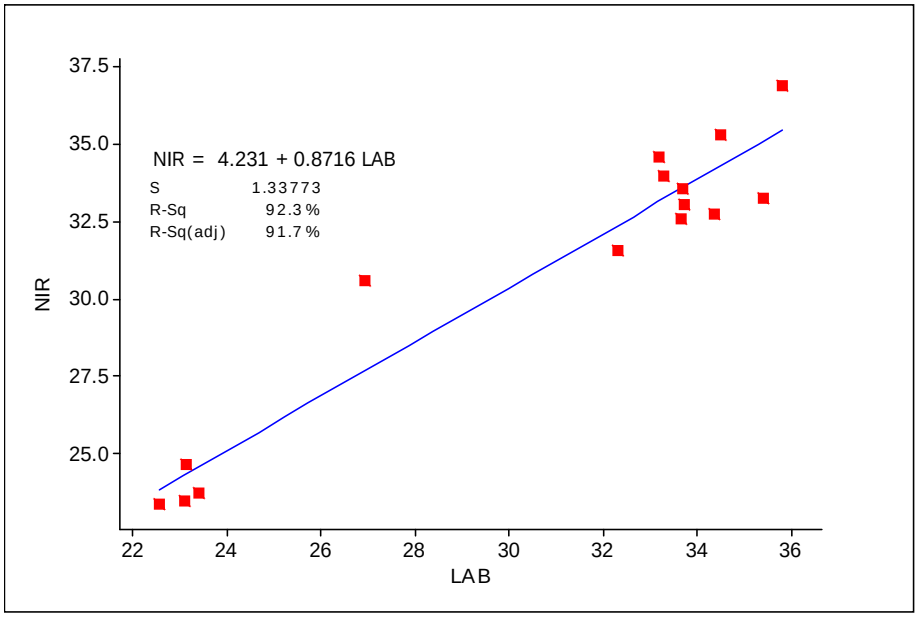

Fig. 11. Regression equation between laboratory and NIR methods for measuring ADF in *Festuca ovina.*

Results of estimations made on other samples by the calibration equation for the NDF characteristic showed that the obtained statistical parameters including the standard error of estimation were similar to results reported in various references that are mentioned in Table 1.

Colelho *et al*. (1987), Norris *et al*. (1976), and Lin and Martin (1986) presented results for ADF that are similar to those found in our study and presented in Table 2.

The standard error of calibration and the standard error of estimation for Crude protein found in our research are similar to those reported in various references that are presented in Table 3. The standard error of measurements related to the two measurements made using the chemical and the NIR methods are compared in Table 4 and 5, respectively. Considering these comparisons, and the regression equation between results obtained from these two methods, we can conclude that the NIR method can be used as an accurate, rapid, and suitable method for measuring qualitative characteristics and chemical elements in plant breeding programs, animal sciences, and rangeland science.

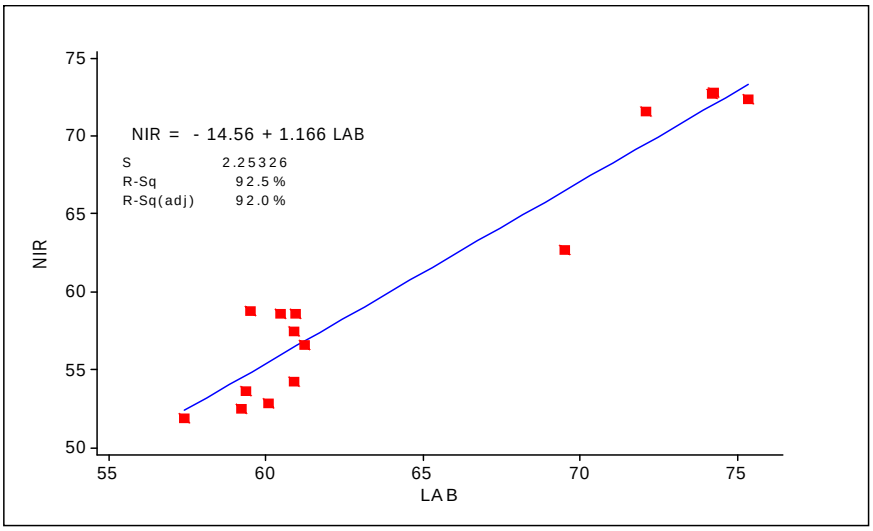

Fig. 12. Regression equation between laboratory and NIR methods for measuring DMD in *Festuca ovina.*

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