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Provisional assessment of forage quality in common vetch using near-infrared reflectance spectroscopy*

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Abstract

Techniques involving wet chemistry are time- and money-consuming, and the waste they generate is harmful to the environment. The near infrared technology not only makes it feasible to quickly examine various parameters, but also to determine all of these parameters at once. For the purpose of identifying the forage quality components of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), dry matter (DM), and ash, a calibration of Near Infrared Reflectance (NIR) spectroscopy was obtained. In this study, it was aimed to develop a common vetch specific near infrared (NIR) spectroscopy calibration by using 197 samples consisting of different common vetch lines and varieties. For the calibration of DM, CP, ADF, and NDF, second derivative pretreatment was utilized; for ash, first derivative pretreatment was used. The coefficients of determination (R^2) in the calibration set were 0.977 for DM, 0.856 for ash, 0.823 for CP, 0.754 for ADF and 0.630 for NDF. The validations performed over the DM, ash, CP, NDF and ADF parameters of mean values and the coefficient of determination in the external validation (r^2) were 92.44, 0.974; 1.953, 0.822; 17.04, 0.784; 42.24, 0.659; 30.56, 0.644, respectively. The equations for DM (RPDv = 6.247) showed high accuracy, while the equations for ash (RPDv = 2.367), CP (RPDv = 2.146), NDF (RPDv = 2.037) and ADF (RPDv = 2.010) showed relatively high accuracy. The dry matter equations were suitable for quantitative prediction of common vetch quality, according to the relative predictive determinant ratios (RPDv) for calibration, whilst the CP, ADF, NDF, and ash equations were helpful for screening. It was determined that NIR spectroscopy may be used to successfully predict CP, ADF, DM, and ash in samples of common vetch.

Keywords: acid detergent fibre, ash, fodder plant, neutral detergent fibre, *Vicia sativa*

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INTRODUCTION

Common vetch (*Vicia sativa* L.) is an annual plant which is grown in pasture and used as hay or grain for the livestock diets. As its grains contain high crude protein, it is used as a concentrate feed for ruminants. It naturally grows to the Mediterranean environment and can be found in Türkiye within the natural vegetation (Seydosoglu 2014). Common vetch increases soil fertility with its ability to produce its own nitrates. Common vetch is a suitable plant in crop rotation. It can be planted at different sowing times and can also be used as green manure (Abbasi et al. 2014).

Common vetch is similar to alfalfa in terms of feed value, has a short vegetative period, and can thrive in practically any type of soil and environment. This grass does not swell when given to animals as green grass (Seydosoglu 2014). Vetch grains are broken and given to animals as a concentrate feed. Also, straw left over from plants harvested for grain yield is a good animal feed. (Kaya et al. 2013). Low temperatures that occur in the winter months cause a decrease in the grass and seed yield of the common vetch. According to Tenopala et al. (2012), common vetch could survive a water shortage for up to 24 days before fully recovering its biotic activity when regular watering had begun. Determining common vetch varieties that are appropriate for the local climate was crucial (Seydosoglu 2014). Researchers reported that there were statistically significant differences in genotype x environment interactions in terms of hay and seed yield of common vetch species (Yucel et al. 2009, Seydosoglu 2014, Georgieva et al. 2015). In Türkiye, common vetch is the most important plant of the legume-based pastures (4.132.686 da). In areas where the plants are well adapted, planted legume-based pastures are also widely employed in the dairy industry (Huang et al. 2017) and are regarded as superior pastures.

In the electromagnetic spectrum, the NIR region is defined as the wavelength range between 700 and 2500 nm. When a sample is analyzed, radiant energy is selectively absorbed based on the specific vibration of the molecule, resulting in an overtone in the spectrum. Prominent absorptions in the NIR spectra of the forages include two bands water at 1940 nm and 1450 nm; aliphatic carbon bands (lipids) at 2310, 1725, 1400 and 1210 nm; and oxygen bands (carbohydrates) around 2100 and 1600 nm (García-Sánchez et al. 2017). Unlike most conventional analytical methods, NIR spectroscopy is fast and nondestructive; it does not use chemicals, produces no chemical waste that must be disposed of, and is multiparametric, meaning that multiple parameters can be determined simultaneously in the same measurement (Acosta et al. 2020). Some studies have been conducted using NIR spectroscopy for nutrient compositions of forage crops such as legume-grass mixtures (Locher et al. 2005), switchgrass (Vogel et al. 2011), alfalfa-grass mixtures (Karayilanli et al. 2016) and winter pea (*Pisum sativum* L., Saha et al. 2018). NIR spectroscopy has not yet been used in any studies to evaluate the

common vetch fodder quality for forage cultivar improvement and feed industry initiatives.

Chemical compositions of common vetch must be determined quickly and accurately to assist farmers and breeders. In order to quickly examine the CP, ADF, NDF, DM, and ash for common vetch, this study set out to ascertain the applicability and accuracy of the NIR spectroscopy technique.

MATERIALS AND METHODS

The crops were grown at the Eastern Mediterranean Agricultural Research Institute (36°5101800 N, 35°2004900 E) in Adana, Türkiye, during the 2019-2020 season (average temp. 17.8°C and relative humidity 70.6%). A total of 197 samples were collected from common vetch cultivation areas in the Cukurova region of Türkiye (52% in 2019 and 48% in 2020). The harvest took place between the end of March and the beginning of April, when the sub pods began to form grain. Fresh grass samples weighing about 500 g were taken from the green grass harvest plots and dried at 65°C until they reached constant weight.

Laboratory chemical analyses

Before any samples were subjected to conventional wet chemistry analysis, they were all ground in a mill to pass through a 1-mm screen. Samples were kept in closed plastic containers in a deep freezer to preserve quality and prevent insect infestation. The CP content (calculated as $N \times 6.25$) of common vetch samples was determined using the standard Kjeldahl procedure (AOAC 2000-990.03) and expressed on a dry matter basis. To determine dry matter, a 3-g subsample was dried in an oven at 105°C until it attained constant weight (AOAC 2000-930.15). Two hours after the samples were weighed to determine their DM content, the samples' spectra were collected. ADF and NDF were determined using the method developed by Van Soest et al. (1991). For the purpose of determining the amount of ash, organic components were burned at 550°C; the remainder was expressed as a percentage (AOAC 2000-930.05).

A Foss NIRS-XDS was the instrument used in this investigation for NIR spectroscopy. A small, round metal cup (3.75 cm diameter) was used for scanning of a sample within the full spectrum (400-2498 nm), taking about 5 g of each sample. The reflectance spectra ($\log_{10} 1/R$) from 400 to 2498 nm collecting 1050 data points per sample were recorded at 2 nm intervals. The software used was Infrasoft International Port Matilda, PA, ABD.

Calibration

WinISI III software was used to create the calibration models (version 1.61). Different combinations of a segment length over which the difference is calculated and a smoothing amount which dampens noise were employed in the quest for an acceptable equation. To increase the smoothness, little smoothing across 2 nm was carried out once more. The calibration was carried out using suggested modified partial least squares (MPLS) to create appropriate calibrations for typical vetch forage components (Barnes et al. 1989, Daszykowski et al. 2002). All multivariate regression equations were developed for the spectral ranges 400-2498 nm and 1100-2498 nm. Standard normal variable (SNV) + detrending (DT) (Barnes et al. 1989) algorithms were used for scatter correction. Additionally, three derivative mathematical treatments were tested in the development of NIRS calibrations: “1-4-4-1”; “2-4-4-1” and “2-6-8-1” (Table 1). ISI scan software was used to gather

Table 1
Calibration and prediction statistics in equation development for analysis of quality components of common vetch with NIR spectroscopy

Mathematical	Treatment [†]				Range (%DM) [‡]	Calibration set			
	ND	NC	NP1	NP2		N	SD	R [¶]	SEC [§]
CP	2	4	4	1	(12.56-23.68)	197	2.164	0.823	0.909
ADF	2	6	8	1	(18.00-41.17)	197	6.301	0.754	2.589
NDF	2	4	4	1	(30.00-61.53)	197	9.293	0.630	2.828
DM	2	4	4	1	(75.44-95.84)	197	2.556	0.977	0.312
Ash	1	4	4	1	(7.09-15.56)	197	1.834	0.856	0.711

[†] Mathematical treatment: ND – the difference number, NC – segment length in nm over which the difference is calculated, NP1 – the first smoothing in nm, NP2 – the second smoothing in nm;

[¶] Squared simple correlation of NIRS predicted values vs. known quality values from conventional laboratory assays;

[§] Standard error of calibration – V(mean square error) from the least squares regression of known laboratory values on NIRS values;

[‡] Range in chemical data (calibration and validation sets combined).

and manage the spectra (Infrasoft International Port Matilda, PA, USA). The software tested the equations by randomly selecting one-fifth of the data from the calibration population for each component. The standard deviation (SD) and mean of the laboratory validation set data were compared to the SD and mean of the calibration set data (Patil et al. 2010).

External validation

By computing the ideal ratio of the calibration and validation sets' standard errors (SE), coefficients of determination (r^2), biases, and slopes, as well as those of the validation sets, the best equation was chosen. When it was feasible, equations were further confirmed by making sure that the wavelengths corresponded to known absorbance maxima related to the components being studied. It was anticipated that the reliability model would have low standard error calibration (SEC), standard error cross validity (SECV), and high r^2 values in order to provide accurate prediction (Williams et al. 2017).

The SD of the reference data was compared for the 39 samples used for the validation sample set (Williams et al. 2017). As a result, each model was validated using an independent test set that included a wide range of proximate (CP, DM, ash) and fiber (NDF/ADF) compositions.

There are two control limits that can be used to determine whether there is a significant bias and an increase in unexplained error. The output includes deviation limits as well as values for global and neighborhood spectral distances. To assess the predictive ability of the models, the coefficient of determination in validation, standard error of performance (SEP), standard error of prediction corrected for bias (SEPC), bias (mean difference between NIR predicted and reference concentration), relative predictive determinant [SD of the external validation set data/SEP(C)] (RPD_v), and range to error ratio were used (Williams and Norris 2001).

RESULTS AND DISCUSSIONS

Reflection appearances and normalized spectra as a result of the preliminary applications applied to common vetch spectra are shown in Figure 1.

The scale of variability in CP, ADF, NDF and DM of the samples used in this study are similar to those reported by other authors (Larbi et al. 2011, Uzun et al. 2011). Table 1 displays the calibration and validation (prediction) statistics. The first derivative mathematical analysis of the log (1/R) signal produced the best equations for ash. Second derivative mathematical techniques were necessary to produce appropriate equations for CP, ADF, NDF, and DM. The SEC and SEP values, sometimes referred to as the standard deviation of the difference between laboratory and NIR spectroscopy data, were in good agreement with results from prior research on common vetch. Absorbance of spectra peaked between 1450-1934 nm wavelength (Figure 1a). This wavelength gave information about O-H and C-H molecule bonds. The first and second derivative of common vetch spectra are shown in Figure 1b,c. Particle size effects have been reduced using derivative adjustments of the spectra. A single derivative might not have provided the most accurate predictions for all constituents (Shenk et al. 2008, Andueza et al. 2016).

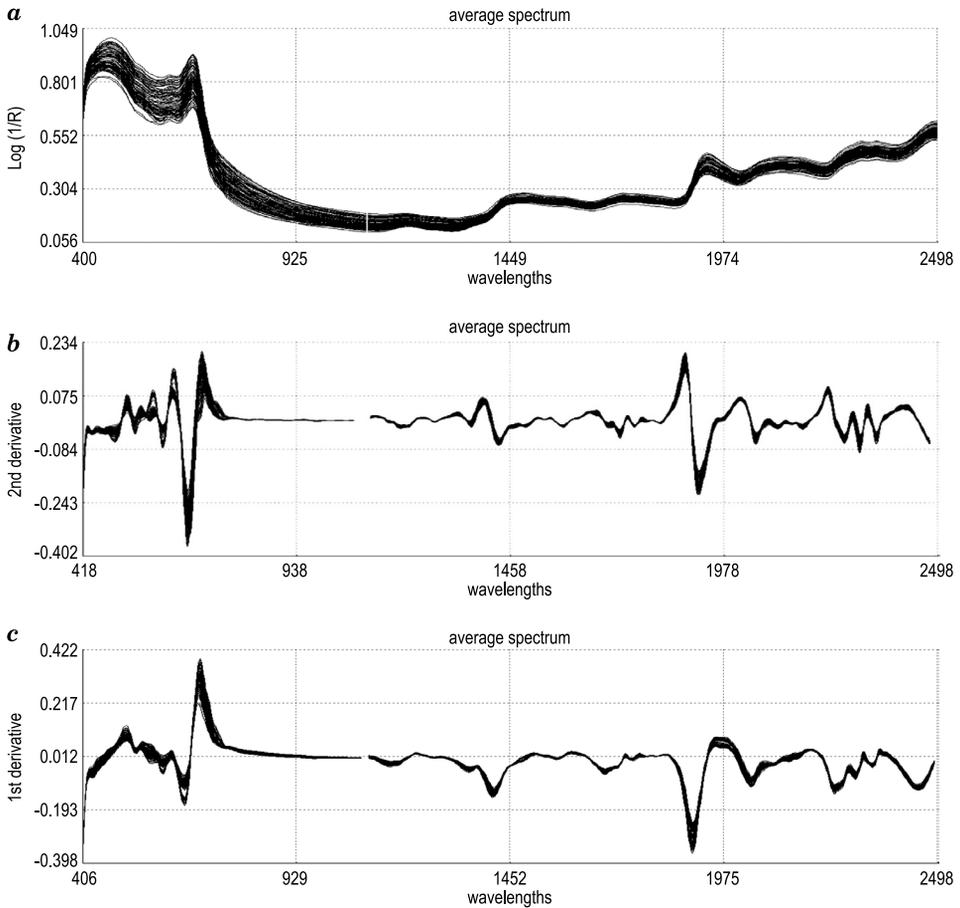


Fig. 1. Raw data (a), 1st order derivate (b), 2nd order derivate (c)

Maxima were found at 514, 622, 692, 1360, 1510, 1790, 1960, 2188, 2284 and 2356 nm and minima were found at 602, 658, 1414, 1682, 1898, 2042, 2244, 2296, and 2450 nm. The absorption bands at 1360 nm in the NIR segment of the spectrum have been attributed to the C-H combination of methylene groups; 1510 nm related to N-H stretch first overtone of protein, 1790 nm which has been assigned O-H combination of water; 1960 nm was due to O-H stretch/O-H bend combination band of starch (Shenk et al. 2008). The range in calibration sets for CP, ADF, NDF, DM and ash were, respectively, 12.56%-23.68%, 18.00 %-41.17%, 30.00%-61.53%, 75.44%-95.84% and 7.09%-15.56% (Table 1).

The validation sample sets' respective mean values for CP, ADF, NDF, DM, and ash were 17.04%, 30.56%, 42.24%, 92.44%, and 10.19%. The calibration set's respective standard errors of calibration for CP, ADF, NDF, DM, and ash were 0.91, 2.58, 2.82, 0.31, and 0.71. When calibrations were tested

on the prediction sets, the GH-distance between independent samples and the center of the common vetch calibration population was less than 3. (Patil et al. 2010). This approach might therefore be relevant to samples of common vetch.

Since it was difficult to find calibrations unique to the common vetch species under study in the literature that was available, references were made to mixtures of legume species and grass and legume mixtures. Standard errors of 0.83-1.45 for CP; 2.15-2.45 for NDF; 1.06-1.66 for ADF, 0.74-1.88 for DM and 0.73-1.50 for ash were reported by Despal et al. (2020), Serrano et al. (2021) and Parrini et al. (2022).

External validation of calibration models

Table 2 displays the findings of the external validation of the regression-based calibration models for the CP, ADF, NDF, DM, and ash common

Table 2

Monitoring statistics of the external validation set to predict forage quality by using NIR spectroscopy[†]

Constituents	N	Mean	SD [¶]	SEP	r^2	SEP (C) [§]	Bias	Slope	RPD _v ^p
CP	39	17.04	2.144	0.99	0.784	0.99	0.081	0.959	2.146
ADF	39	30.56	6.396	3.16	0.644	3.18	0.332	0.917	2.010
NDF	39	42.24	9.440	4.57	0.659	4.63	0.000	1.000	2.037
DM	39	92.44	2.049	0.32	0.974	0.33	0.028	0.990	6.247
Ash	39	10.19	1.953	0.82	0.822	0.83	0.085	1.017	2.367

[†] Reported values were calculated from the number of samples (N) in the external validation set, bias – the average difference between the reference and NIR spectroscopy values, slope – the steepness of a straight line curve;

[¶] Standard deviation of quality values;

[§] Standard error of prediction corrected for bias;

^p SD of the external validation set data/SEP(C).

vetch forage quality parameters. The NIR spectroscopy predictions accurately portrayed the variation in the samples. The NIR calibration equation models correctly predicted the CP, ADF, NDF, DM, and ash constituents of the validation set, according to the bias, standard error of prediction corrected for bias SEP(C), and r^2 statistics (Table 2). The constituents' validation r^2 scores ranged from 0.644 to 0.974. High r^2 values for DM (0.974) and ash (0.822) were observed, whereas moderate values for CP (0.784), NDF (0.659), and ADF were also noted (0.644). The related metric SECV of the cross-validation statistics was in good agreement with the standard error of prediction and r^2 , as well. Figure 2*a,b,c,d,e* shows the correlations between the wet chemistry results for CP, ADF, NDF, DM, and ash and the NIR spectroscopy predictions.

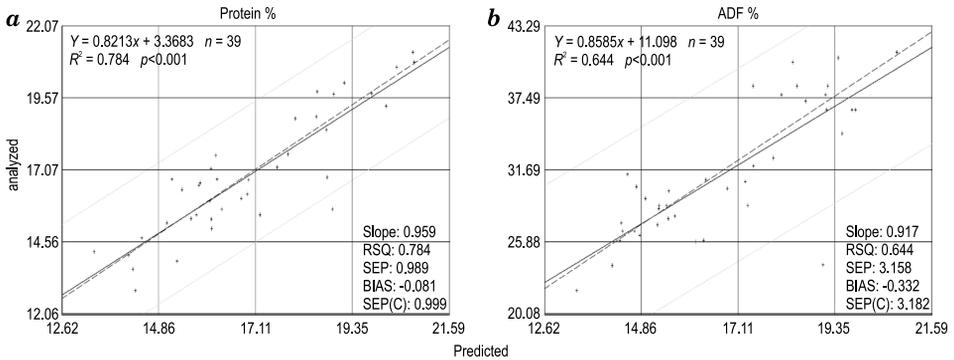


Fig. 2a,b. Evaluation of NIR spectroscopy calibration equations in terms of comparison of NIR spectroscopy predicted values of CP and ADF with corresponding measured values obtained by wet chemical analyses of common vetch samples obtained independently of the calibration samples. Analysis results showed vertical line. The prediction results showed horizontal line.

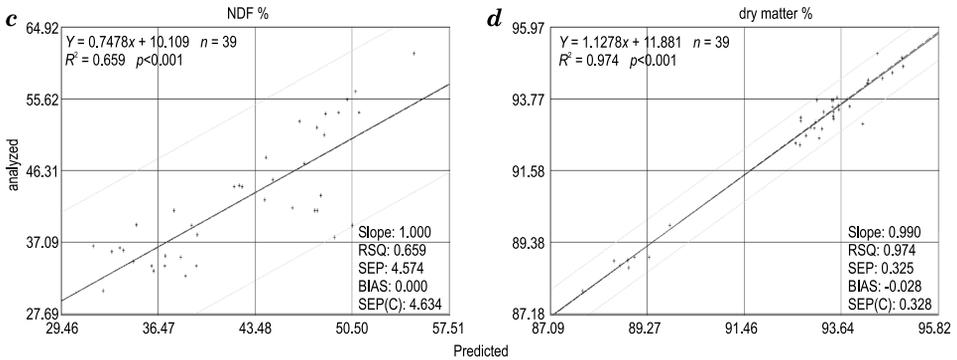


Fig. 2c,d. Evaluation of NIR spectroscopy calibration equations in terms of comparison of NIR and DM with corresponding measured values obtained by wet chemical analyses of common vetch obtained independently of the calibration samples. Analysis results showed vertical line. The prediction results showed horizontal line.

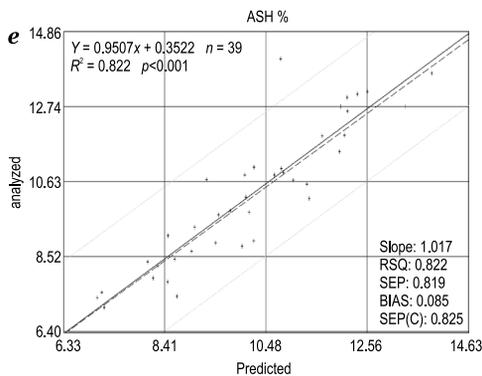


Fig. 2e. Evaluation of NIR spectroscopy calibration equations in terms of comparison of NIR spectroscopy predicted values of ash with corresponding measured values obtained by wet chemical analyses of common vetch samples obtained independently of the calibration samples. Analysis results showed vertical line. The prediction results showed horizontal line.

The common vetch sample analyses employed calibration formulae. For the variables CP, ADF, DM, and ash, a high degree of prediction accuracy was found, although the NDF parameter showed an adequate level of accuracy. Calibration equations with low SEC and high r^2 values were accepted but they lacked the precision needed to examine unidentified samples. This could be explained by the possibility that an excessive number of wavelengths included in the equation has a relationship to the physical and chemical properties of the calibration samples, such as particle size and moisture content, rather than component concentration. At this stage, just like in calibration and cross-validation, ideal equations are chosen for external validation based on low SEP and high coefficients of determination (r^2 – Shenk et al. 2008). All other equations had low SEPs, with the exception of the ADF and NDF equations, which had quite significant error values.

When more common vetch samples from the subsequent harvests in the same area were validated, Figure 2a,b,c,d,e shows the connections between NIR spectroscopy and wet chemistry results for CP, ADF, NDF, and ash.

The variations in r^2 values for each element of the common vetch calibration show that the range in chemical data had an impact on the coefficients of determination. This is consistent with the findings of Despal et al. (2020) and Parrini et al (2022). In this study, NIR equations were created for CP, ADF, NDF, DM, and ash to assess fodder quality and be used for breeding common vetch. The coefficients of determination ($r^2 > 0.8$) for DM and ash were generally higher than those for the ADF, NDF, and CP constituents ($r^2 < 0.8$), which was consistent with previous research (Starks, Brown 2010). Rabotnikof et al. (1995) reported lower prediction accuracy for NDF and CP using NIR spectroscopy. The findings for 75 warm-season grass samples from La Pampa, Argentina, revealed that NIRS was substantially more effective at predicting CP than NDF. In a study using samples from *Brassica napus* L., RPDc and RPDv values were 1.95 and 1.92 and 1.86 and 2.22, respectively, in the NDF and ADF MPLS (1,4,4,1) prediction model (Dimov et al. 2011). These findings were comparable to those obtained in our study. According to Williams and Norris (2001), equations with an RPD greater than 2.4 are preferable for good calibration, whereas equations with an RPD less than 1.5 are unusable. Galvez-Sola et al. (2010), classified calibration performance into four categories as follows: I) $r^2 > 0.95$ and an RPD > 4 for excellent calibrations, II) $r^2 = 0.90-0.95$ and an RPD of 3-4 for successful calibrations, III) $r^2 = 0.80-0.90$ and an RPD of 2.25-3.00 for moderately successful calibrations, IV) $r^2 = 0.80-0.90$ and an RPD of 2.25-3.00 for moderately successful calibrations, V) $r^2 = 0.80-0.90$ and for moderately useful calibrations, $r^2 = 0.7-0.8$ and an RPD of 1.75-2.25 are acceptable. Calibration equations for the prediction of common vetch chemical composition were successful for the DM and ash models, but only moderately useful for the ADF, NDF, and CP models (Table 2). Indicators of statistical significance r^2 and RPDv for DM were 0.78 and 2.15, respectively, and 0.71 and 2.04 for ash. The laboratory and NIR analyses were also mutually consistent. The slopes and bias intercepts

were nearly 0 and 1, respectively (Table 2). For CP, DM, NDF, ADF, and ash in this investigation, the prediction ability of NIR equation models was demonstrated by decreased SEC and SECV with comparatively high r^2 values. Our findings suggested that NIR would be a practical method for measuring several important common vetch quality indices, including NDF and CP. This study is an initial step toward creating additional chemometric models and applying the NIR equation to the chemical components of common vetch. Although the crude protein and NDF levels can be predicted using the NIR calibration equations created in this study, more research is required to enhance the efficacy of the calibrations for DM, ash, and ADF in common vetch.

CONCLUSION

This study is the initial step toward creating additional chemometric models and applying the NIR equation to the chemical components of common vetch. Although the dry matter, crude protein, and ash contents can be accurately predicted using the NIR calibration equations created in this study, more research is required to enhance the performance of the calibrations for ADF and NDF in common vetch. Scanners can make use of the ADF and NDF results. These findings imply that NIRS may be utilized effectively as a standard screening approach for the components of these three traits in large-scale breeding projects for common vetch. The inclusion of common vetch cultivars of different geographical origins in future study may increase the robustness of the equations and lead to the sequence of a global calibration for these properties in common vetch.

Conflict of interest

The authors declare no conflict of interest

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