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CALIBRATIONS USING NEAR-INFRARED SPECTROSCOPY TO DETERMINE THE NUTRITIONAL CONTENT AND CHEMICAL COMPOSITION OF HAY AND SILAGE OF PEARL MILLET

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Abstract

Forages display essentially identical fodder; but ensiling procedures may alter the spectra of forages. The aim of this study was to predict the forage properties of pearl millet (*Pennisetum glaucum* [L.] R. Br.) and monitor the performance of pearl millet silage calibration models. Crude protein, crude ash, NDF, ADF, and ADL were all examined in the samples using NIR spectroscopy. High accuracy was demonstrated by the relative prediction determinant for validation of the NDF and ADF contents of pearl millet forages and silages. The equations predicted with a comparatively high degree of accuracy for protein, ash, and ADL. Even though the protein, ash, and ADL equations were utilized for screening, the relative prognostic prediction rates for calibration demonstrate that the NDF and ADF equations were suitable for the quantitative prediction of pearl millet silage fodder quality. The calibrations for pearl millet silage protein, ash, NDF, ADF and ADL resulted in R^2_C between 0.853 and 0.997; SECV of 0.544, 0.474, 0.673, 0.728, and 0.385, respectively. We confirm the potential of NIRS to predict pearl millet and silage chemical composition. Future steps include determining the effect of pearl millet forage and silage on growth performance, digestibility potential, and feed potential in cattle.

Key words: calibration, near-infrared spectroscopy, pearl millet, reference values, silages

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List of abbreviations: ADF – acid detergent fibre, ADL – acid detergent lignin, D – detrend, GH – distance from a sample point to centre, Mahalanobis distance, MPLS – modified partial least squares, NDF – neutral detergent fibre, NIRs – Near-Infrared Spectroscopy, r^2 – coefficient of determination, RPD – ratio of performance to deviation (SD/SECV), R_C^2 – coefficient of determination for calibration, R_V^2 – coefficient of determination for validation.

Introduction. Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is an annual crop of the *Poaceae* family that grows best in warm weather. It has the advantage of being more heat- and drought-tolerant than other prevalent forage grasses in the area, such as fodder maize, and it also grows and yields well in poor, sandy, and saline soils in arid, hot, and dry conditions [1]. It may be fed at any growth stage, has a relatively high protein content (10–12%), and is highly capable of tillering and ratooning. According to reports, pearl millet has a great resilience to drought, making it a valuable crop for providing high-quality animal feed in arid and semi-arid regions as well as other parts of the world with comparable agro-ecologies [2]. Pearl millet has received more attention lately as a multi-cut fodder crop, particularly in Brazil, the Middle East, and Central Asia [3], for both fresh feeding and silage production [1]. The most popular method for preserving fodder in semiarid areas is ensiling, which helps to preserve plant uniformity as well as the food's moisture and nutritional content [4].When hard stems in silage ferment and become soft, the palatability of the fodder crop increases, making it easier for animals to digest [5].

NIR spectroscopy is quick and nondestructive compared to most traditional analytical techniques. Since there was no use of chemicals or waste generation that needs to be disposed of, this was an environmentally friendly approach and it was possible to measure more than one parameter at the same time [6], but the disadvantages include the need for a large, recent and sample-specific calibration dataset in addition to accurate reference analyses for calibrations [7]. Specific calibrations based on a small range of samples typically performed better than broad-based calibrations as long as the samples to be analyzed were represented in the calibration set [8].

The effects of salt and drought stress on production and forage quality [9,10], the composition of pearl millet fodder [11,12], and NDF digestibility have all been the subject of some research [13]. The NDF, ADF and ADL content of forage crops have been investigated using NIR spectroscopy for switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardi*), Indiangrass (*Sorghastrum nutans*) [14], and hay meadows, *Nardus stricta* grasslands [15]. NIR spectroscopy has not, however, been used in any study to assess the quality of pearl millet silage and feed fodder in order to improve feed industry programmes and cultivars. To assist farmers and breeders, chemical compositions in pearl millet need to be determined quickly and accurately. The aim of this study was to evaluate the accuracy and

application of using NIR spectroscopy to quickly analyze the ash, protein, NDF, ADF, and ADL for pearl millet and its silage.

Materials and methods. ***Samples and reference methods.*** Crops were produced at the Eastern Mediterranean Agricultural Research Institute in Adana, (36°54′25″ N, 35°34′43″ E) and GAP Agricultural Research Institute Talat Demirören Research Station in Akçakale/Şanlıurfa (36°54′10″ N and 38°55′23″ E and 378 m altitude) between 2022–2023. A total of 310 samples (distribution of samples: 52% in 2022 and 48% in 2023) were used from Adana and Şanlıurfa locations. The harvest was made on the date corresponding to the milk-dough formation period of the grains in bunches of each genotype. Approximately 500–1000 g of fresh herb samples (stem, leaves, and bunches) taken from each plant in each plot during harvest were dried under greenhouse conditions and then dried in the drying cabinet at 60 °C until the weight stabilized, and then the dry herb proportions were determined by weighing.

A total of 288 samples were used for silage. Fresh plant samples weighing between 500 and 1000 g gathered from each experiment plot were dried, processed in a mill that was made specifically for the purpose, and then put through a 2 mm sieve before being examined for quality. Using a 3–5 cm stem-branch leaf grinding machine, 1.5–2.0 kg of fresh plant samples were removed from each plot during harvest. The samples were then packed into 1 kg vacuum bags that had been carefully prepared, and the air was removed from the bags using a suction device to remove 95% of the air. The vacuumed silage material was labelled and kept for fermentation at room conditions for 60 days. No inoculants were applied. At the end of the fermentation period, a 250 g wet sample was taken from the opened silages and dried in the drying cabinet at 60 °C until its weight stabilized. Samples of matured silage were dried for 48 h at 60 °C. After being dehydrated, the samples were put back together and pulverized in a mill that had a 2 mm screen. All of the samples were processed through a 1-mm screen in a mill before being subjected to standard wet chemistry techniques for analysis. To preserve quality and avoid bug infestation, samples were kept in deep freezers in closed plastic containers. The protein content (calculated as $N \times 6.25$) was ascertained using the conventional Kjeldahl method [16] and expressed on a dry basis. To determine the dry matter content, a 3-g subsample was oven-dried at 105 °C until it attained a consistent weight [16]. Within two hours of the samples being weighed to ascertain their dry matter concentration, the spectra were acquired. The methods presented in Van Soest et al. [17] were used to calculate NDF, ADF, and ADL. At 550 °C, organic materials were burned to calculate the amount of ash. The proportion of ash that was still present was stated [16].

Acquisition of spectra. The Foss NIRS-XDS NIR spectroscopy was the equipment used in this investigation, and it had a scanning range of 400–2500 nm. There were a total of 20 NIR data points per sample, with the NIR data being interpolated at 2-nm intervals (ABD, Port Matilda, PA, Infrasoft International software).

Calibration development and data analysis. The WinISI III program (version 1.61) was used to create the calibration models. A suitable equation was found by experimenting with different combinations of the segment length (over which the difference is calculated) and the degree of smoothing (to reduce noise). To improve the smoothness, the modest smoothing over 2 nm was carried out once. The suggested MPLS method was used for the calibration in order to create comparable calibrations for the fodder components made of pearl millet. The calibration spectra were arranged using two distinct outliers: GH and T [18, 19]. There were three outlier passes. After removing high outliers, a new calibration was carried out [20]. To predict these values more precisely and maximize calibration, eight mathematical processes from the first derivative (1-4-4-1, 1-6-8-1, 1-8-8-1, 1-10-10-1) and the second derivative (2-4-4-1, 2-6-8-1, 2-8-8-1, 2-10-10-1) were utilized. For these equations, standard normal variable techniques were applied. Using ISI scan software (Infrasoft International Port Matilda, PA, USA), the spectra were gathered and organized. To test the equations, the software selected a random sample for each component that represented one-fifth of the calibration population samples. The laboratory-gathered validation set values' standard deviation and mean were compared to the calibration set's values [19].

The best equation was determined by combining the bias and slope of the calibration and validation sets in the best possible way, together with the coefficient of determination (r^2) and SE. When feasible, the equations further assessed by determining which wavelengths match known absorbance peaks were associated with the components under investigation. It has been expected to have low SEC and SECV values and high r^2 values for the reliability model to give accurate prediction [21].

Thirty samples of silage and 45 samples of pearl millet were chosen at random for external validation in order to independently verify the NIR calibration equations. As a result, every model was verified on a separate test set encompassing the entire spectrum of proximal (ash, protein) and fibre (NDF, ADF, ADL) components.

To ascertain whether there is a significant bias and a notable rise in unexplained error, there are two control limits. The results provide the values and deviation limits for the local and global spectral distances. The predictive power of the models was assessed using the range to error ratio, SEP, SEPC, bias (mean difference between NIR expected and reference concentration), RPDv to assess prediction accuracy, and the coefficient of determination in validation [21].

Results. Table 1 provides the mean, standard deviation, and min–max values for the chemical composition variables for the calibration sets of silage and fodder. Compared to the forage set, the silage calibration set included a wider range. In the calibration set, the coefficients of variation for protein, ash, NDF, ADF, and ADL forage were 0.18, 0.14, 0.03, 0.06, and 0.18; in the silage set, the coefficients of variation were 0.27, 0.25, 0.11, 0.12, and 0.46, respectively.

Table 1

Descriptive statistics for protein, ash, NDF, ADF and ADL within pearl millet hay and silage samples calibration set (results are expressed in % dry matter)

	Pearl millet hay calibration set					Pearl millet silage calibration set				
Groups	*N*	Max	Min	Mean	SD	*N*	Max	Min	Mean	SD
Protein	306	3.01	10.22	6.62	1.20	281	2.85	10.07	6.46	1.80
Ash	308	4.40	11.44	7.92	1.17	273	3.63	10.85	7.24	1.80
NDF	288	58.84	72.40	65.62	2.26	263	35.93	71.45	53.69	5.92
ADF	289	32.55	45.65	39.10	2.18	264	20.74	43.85	32.29	3.85
ADL	303	2.44	8.34	5.31	0.98	268	1.44	5.88	3.66	1.70

N – Number of samples; Max – maximum; Min – minimum; SD – standard deviation

Tables 2 and 3 display the calibration statistics for the protein, ash, NDF, ADF, and ADL readings for the pearl millet and silage samples. R^2_C and RPD values were greater than 0.83 and 2.15 for all pearl millet variables, however, they

Table 2

Statistics of calibration and cross-validation of predictive models used for determination of fibre contents in pearl millet forages by NIRS analysis

	N	[a]Math treatment	[b]Scatter correction	SEC	R^2_C	SECV	RPD
Protein	300	1681	SNV	0.491	0.833	0.558	2.154
Ash	300	2441	SNV	0.451	0.852	0.489	2.398
NDF	275	2681	D	0.372	0.984	0.409	5.528
ADF	272	1441	SNV	0.256	0.986	0.288	7.572
ADL	284	1441	SNV	0.287	0.857	0.389	2.529

[a]Math treatment: derivative order, subtraction gap, first smoothing, second smoothing
[b] SNV – standard normal variate, D – detrend

Table 3

Statistics of calibration and cross-validation of predictive models used for determination of fibre contents in pearl millet silages by NIRS analysis

	N	[a]Math treatment	[b]Scatter correction	SEC	R^2_C	SECV	RPD
Protein	271	2681	D	0.460	0.853	0.544	3.312
Ash	269	2441	SNV	0.419	0.879	0.474	3.805
NDF	252	2881	SNV	0.636	0.995	0.673	8.797
ADF	243	2681	D	0.598	0.997	0.728	5.289
ADL	248	1441	SNV	0.359	0.879	0.385	4.425

[a]Math treatment: derivative order, subtraction gap, first smoothing, second smoothing
[b] SNV – standard normal variate, D – detrend

were 0.85 and 3.31 for silage samples, respectively. The first derivative (1-4-4-1 and 1-6-8-1) was the best pre-treatment for ADF, ADL, and protein; the second derivative (2-4-4-1 and 2-6-8-1) was the best for ash and NDF in forage (Table 2). The first derivative (1-4-4-1) of ADL was the best treatment for silage samples, whereas the second derivative (2-4-4-1 and 2-6-8-1) was the best for protein, ash, NDF, and ADF (Table 3).

High predictive power for NDF and ADF determination was demonstrated by the calibration and validation findings of the forage and silage samples ($R^2_C \geq 0.98$ and $R^2_{VAL} > 0.97$; Tables 1–3). The range of the RPD values for silage was 2.06 to 4.49, whereas the range for pearl millet was 2.19 to 6.00. Predictive values are highest for ADF content and lowest for ADL content. The pearl millet validation set showed a bias > 0 in the ash and ADL contents, while the silage validation set showed a bias> 0 in the protein, ash, and ADF contents.

With the exception of ADL contents, the calibration models applied to the pearl millet silage samples produced accurate predictions (Table 3), with R^2 coefficients that were comparable to those found for the validation set.

Figure 1 and 2 show the link between the predicted values using NIRS models and the reference chemical composition (protein, ash, NDF, ADF, and ADL) of the forages and silages used as external validation. The protein, ash, NDF, ADF,

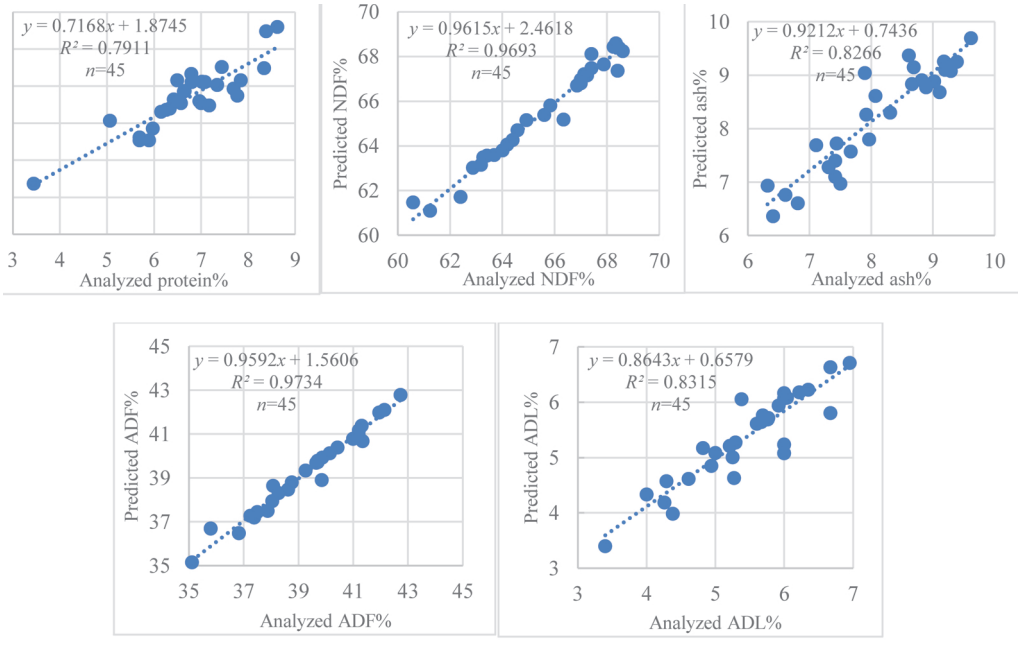

Fig. 1. Evaluation of NIR spectroscopy calibration equations in terms of comparisons of NIR spectroscopy – predicted values of protein, ash, NDF, ADF and ADL with corresponding measured values obtained by wet chemical analyses (analyzed) of pearl millet samples obtained independently of the calibration (predicted) samples

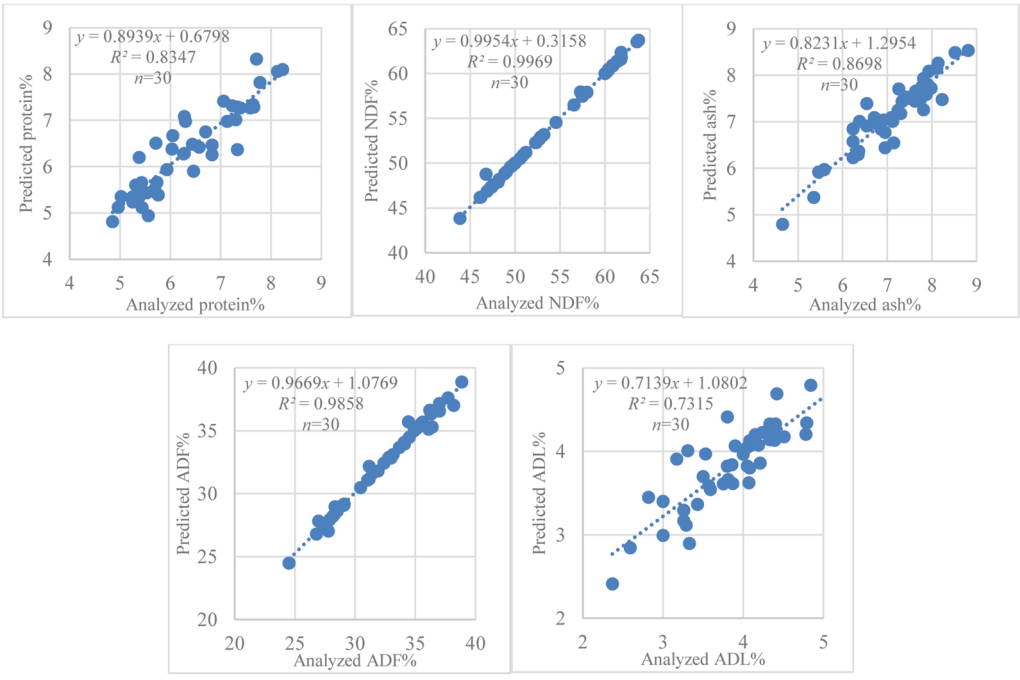

Fig. 2. Evaluation of NIR spectroscopy calibration equations in terms of comparisons of NIR spectroscopy -predicted values of protein, ash, NDF, ADF and ADL with corresponding measured values obtained by wet chemical analyses (analyzed) of pearl millet silage samples obtained independently of the calibration (predicted) samples

and ADL (Fig. 1, 2) equations' validation R^2 values for pearl millet varied from 0.791 to 0.973. For ADF (0.973), NDF (0.969), ADL (0.83), and ash (0.826), high R^2 values were noted; protein (0.791) showed a moderate value. The range of silage validation R^2 values is from 0.73 to 0.996. NDF (0.996), ADF (0.985), ash (0.869) and protein (0.834), all had high R^2 values, although ADL had a moderate value (0.731).

Discussions. The study's pearl millet samples are thought to accurately reflect the nutritional value and chemical makeup of plants that can be found growing in the Mediterranean region. The calibration database included variables like location, botanical composition, maturity stage, and management circumstances that affect nutritive value [22]. Although significant, the variability linked to the pearl millet forage validation set was still less than that seen in the silage calibration set.

The scale of variability in protein, ash, NDF, ADF and ADL of pearl millet [23,24] and silage [25] used in this study are similar to those reported by other authors. The nutritional value of pearl millet generally determines the nutritive value of silage, although additional sources of heterogeneity in the chemical composition and nutritive value of silage samples result from the processing needed to

extract fodder. The smaller sample size and the features of the independent samples databases may account for the reduced variability of the prediction databases revealed here.

Anaerobic acid fermentation serves as the foundation for the preservation technique of silage forage. Proteolysis, degradation of hemicellulose, and the disappearance of soluble carbohydrates are the changes in fodder composition that occur during this process [26]. Moreover, variations in the visible segment between the spectra of silage and fodder may be linked to variations in the amount of chlorophyll (around 680 nm). R_V^2 and RPD values were greater than 0.97 and 2.09 for all variables as well as forage and silage, respectively, indicating that they were appropriate for quality control [21]. The prediction equations presented in this study for silage quality and pearl millet forage are either stronger or comparable to those previously published [10, 11].

Conclusion. Our results suggest that NIR could be a useful technique for determining several important silage and pearl millet quality parameters, such as protein, ADF, and NDF. This study is the first step in creating more chemometric models and applying the NIR equation to the analysis of the chemical composition of pearl millet silage and hay. However, it needs to be improved or extended by future researches. The NIR calibration equations created in this work are reliable and will be helpful in estimating the NDF, ADF, and protein levels in pearl millet, even though more research is required to increase the accuracy of the ash and ADL calibrations in this crop.

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