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RESEARCH ARTICLE

Estimating Roughage Quality with Near Infrared Reflectance (NIR) Spectroscopy and Chemometric Techniques

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ABSTRACT

Near-Infrared Spectroscopy has been commonly adopted in feed quality evaluations. The majority of studies have been performed on the detection of fibrous components with the help of NIR. Model development studies are not yet common for different roughage quality characteristics such as Relative Forage Value (RFV), Relative Forage Quality (RFQ), and Net Energy Lactation (NEL), which are used in the evaluation of roughage quality. The purpose of this study is the detection of RFV, RFQ, and NEL values with NIR spectroscopy in different roughage samples, and to investigate the effect of wavelength selection on the model's success. In this study, spectral data belonging to silage, dry alfalfa, dry oat, and wheat straw samples and laboratory analysis results were used to develop estimation models according to the partial least square regression (PLSR) method. A variable importance projection (VIP) method was used as the wavelength selection method. Estimation models, which were developed according to study results, were obtained from the VIP-PLS model combination (RMSE=12.7, Bias=0.000, R2=0.804, RPD=2.28) for RFV. VIP method has increased the estimation of success for all variables. Based on the study results, it was recognized that it is possible to use NIR in the calculations used in roughages quality evaluation parameters.

Keywords: Near-İnfrared Reflectance (NIR), Roughage quality, Wavelength Selection, Chemometrics

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Kaba Yem Kalitesinin Near Infrared Reflectance (NIR) Spektroskopisi ve Kemometrik Teknikler ile Tahmin Edilmesi

ÖZ

Yakın Kızılötesi Spektroskopisi yem kalite değerlendirmelerinde yaygın olarak kullanılmaya başlanmıştır. Yapılan çalışmaların büyük kısmı lifli bileşenlerin NIR ile tespitine yönelik olarak yürütülmüştür. Kaba yem kalitesinin değerlendirilmesinde kullanılan Nisbi yem değeri (Relative Forage Value, RFV), Nisbi yem Kalitesi (Relative Forage Quality, RFQ) ve Net Enerji Laktasyon (Net Energy Lactation, NEL) gibi farklı kalite özelliklerine yönelik ise model geliştirme çalışmaları henüz yaygınlaşmamıştır. Bu çalışmada farklı kaba yem örneklerinde NFV, RFQ ve NEL değerlerinin NIR spektroskopisi ile tespiti ve dalgaboyu seçiminin model başarısı üzerine etkisinin araştırılması amaçlanmıştır. Araştırmada silaj, yonca kuru otu, yulaf kuru otu ve buğday samanı örneklerinde alınan spektral veriler ve laboratuvar analiz sonuçları kullanılarak kısmı en küçük kareler regresyon (PLSR) yöntemine göre tahmin modelleri geliştirilmiştir. Dalga boyu seçim yöntemi olarak variable importance projection (VIP) metodundan yararlanılmıştır. Araştırma bulgularına göre geliştirilen tahmin modellerinin RFV için VIP-PLS model kombinasyonundan (RMSE=12.7, Bias=0,000, R2=0,804, RPD=2,28) elde edilmiştir. VIP yöntemi bütün değişkenler için tahmin başarısını yükseltmiştir. Araştırma bulgularına dayanarak, kaba yem kalite değerlendirme parametrelerinde kullanılan hesaplamaların NIR ile tespitinin mümkün olduğu anlaşılmıştır.

Anahtar Kelimeler: Yakın Kızılötesi Yansıma, Kaba yem kalitesi, Dalga boyu seçimi, kemometri

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INTRODUCTION

Near-Infrared Reflectance (NIR) spectroscopy is one of the analytical devices that has widespread use in the content analysis of different agricultural products, and it has been commonly used in feed analyses (Sinnaeve et al. 1994). In feed quality analyses, characteristics such as moisture, protein content, and digestibility has been the main center of focus. As an alternative to reference analyses that are used to evaluate feed quality, NIR spectroscopy allows us to analyze multiple variables simultaneously without using chemicals and in a shorter time (Undersander 2006).

Measuring feed quality with NIR spectroscopy has originatedat at the end of the 1970s and the first years of the 1980s. In this period, various studies have been performed, which showed it was possible to use NIR spectroscopy devices for the analysis of protein and fibrous materials in feed samples (Norris et al. 1976, Shenk et al. 1981, Marten et al. 1983, Jones et al. 1976). These studies are still ongoing in our day, and it is investigated whether it is possible to use NIR to measure feed quality in different plant types with newly developed devices. In this scope, estimation models have been developed to ensure the measurement of parameters of feed quality such as ADF, NDF, CP and crude fat in soybean (Asekova et al. 2016), Italian ryegrass (Yang et al. 2017) , corn (Gümüştaş and Bayram 2018) and wheat straw (Nielsen et al. 2019) with the help of NIR. These parameters are determined with wet chemical methods recognized by international standard organizations, history of which depends on the detergent system of analysis recommended by Von Soest (1991) and Wendee's system of analysis (Ergün et al. 2004). Instead of wet chemical methods used in the determination of feed quality parameters, NIR determination methods have been adopted, and these methods have been accepted by ISO (ISO 12099). The number of NIR spectroscopy studies about novel statistical methods has been increasing on chemometric approaches covering efficient wavelength selection methods and pretreatment on spectral data.

Chemometric methods are used mainly in three stages in the development of calibration models with NIR devices. These stages consist of the pretreatment for the removal of noise and unwanted changes in spectral data, the selection of wavelengths related to the target variable, and the formation and validation of the estimation model (Kahrıman and Egesel 2018). While each of these stages has a separate importance on the model's success, the use of efficient wavelength selection methods to enable the determination of variables that should be used in the model can noteworthily increase the model's success. Different wavelength selection methods have been used on scientific literature in studies about spectral analyses. Some of those are Variable Importance Projections (VIP), Selectivity Ratio (SR), Competitive Adaptive Resampling Method (SPA), and Random Frog (RFOG) methods (Wold et al. 1993, Galvão et al. 2008, Li et al. 2014). There is a high number of studies reporting that the success of the established model increases by applying wavelength selection methods on data obtained from NIR devices (Cécillon et al. 2009, Kahrıman et al. 2017).

In the literature review, it was observed that mostly general parameters such as fiber analyses (ADF, NDF) or total protein content are used as target variables in the studies that use NIR on the determination of feed quality. From this point forth, it was considered that it would be beneficial to investigate whether it is possible to estimate RFV, RFQ, and NEL parameters directly with NIR and to study how wavelength selection will affect the success of the established model.

The purpose of this study is to determine whether it is possible to determine RFV, RFQ, and NEL values with NIR in different roughage samples, and to study the effect of wavelength selection on the estimation success of the models established for these parameters.

MATERIALS and METHODS

Samples

The samples of roughage used (n=52) in the study (Corn silage, Alfalfa, Oat grass hay, Wheat straw) were obtained fresh from farms in 0.5 kg weight. They were transported to the laboratory after being placed in airtight bags, and stored at -20 °C until analyses were performed. Dried feed samples were milled (Retsch ZM 200 ultra centrifugal mill, on 1 mm screen) before reference and spectral analyses.

Reference (REF, Chemical Analyses) Analyses

Reference (REF) (chemical) analyses of roughage samples from the study were performed in Balıkesir University Faculty of Veterinary Animal Nutrition and Nutritional Diseases Laboratory by using dry matter (method 934.01), ash (method 942.05), crude protein (method 990.03), crude oil (method 920.39) AOAC (1997) methods in compliance with the reported method, and reference analysis values (nutrient values) were determined. NDF and ADF analyses of feed samples were performed according to the methods reported by Van Soest et al. (1991) by using automated Gerhard FT12 Fiber Analyzer (Gerhardt Analytical Systems Documents, 2012). RFV, RFQ, and NEL levels were calculated according to equations reported in NRC (2001) by using nutrient values obtained from the chemical analyses of roughages samples.

Development of NIR Models and Evaluation

Spectral measurements were performed on a desktop NIR device (Spectrastar 2400D, Unity Scientific, USA) with the samples after completing laboratory analyses. Rotational measurement cup mode was used in measurements, and spectral data were recorded at each 1 nm between 1200-2400 nm with approximately 50 g of ground sample. For model development, sample files were compiled in jdx format by using the InfoStar program.

Spectral data were imported to an Excel file with Unscrambler 10 x (Camo, Oslo, Norway). Afterwards, these files were transferred into the program named R (R Development Team, 2018), and modeling studies were performed by using the mdatools package (Kucheryavskiy, 2020). First of all, spectra were converted to line charts in order to observe the change in spectral data (Figure 1). Partial least square regression (PLSR) method was used as the modelling method, and two separate models were established with spectral data for each variable. In the first model, all spectral data was used to develop the estimation models. In the second model, efficient wavelengths were selected according to the VIP method (Wold et al. 1993), and estimation models were developed by using these wavelengths. Estimation success and reliability of established models were evaluated according to the following parameters.

$$RMSEP = \sqrt{\frac{\sum (Ypred - Yref)^2}{n}}$$

$$SEE = \sqrt{\frac{n}{n-1}(RMSEP^2 - Bias^2)}$$

$$RPD = \frac{STD_{ref}}{SE_{pred}}$$

Where; RMSEP: the square of the mean value of estimated squares; Ypred: the calculated value; Yref: the value obtained by standard analyses; n: the number of observations; Bias: the mean difference between estimated and standardized analyses; SEE: the standard error of the predicted values in the calibration set; STDref: the standard deviation of reference analyses, and SEpred, the standard error of prediction values. Moreover, the coefficient of determination was calculated for the models. These calculations were performed separately for the calibration and validation set, and the internal validation method was used as the validation method.

Determination of Relative Feed Value (RFV), Relative Forage Quality (RFQ) and Net Energy Lactation (NEL) Values

The estimated values of dependent variables in prediction models were calculated by the equations in Table 1. Proposed equations were obtained from previous studies (Samiei et al. 2015, Romero et al. 2014; Jaranyama and Garcia 2004, Van Dyke and Anderson 2000).

Table 1. Calculation formulas for RFV, RFQ, and NEL values and the related equation components

Equation for Target Varibale	Equaiton Components
RFV = DDM x DMI x 0.775	$DMI = \frac{120}{NDF\%}$ $DDM = 88.9 - (0.779xADF\%)$
$\boldsymbol{RFQ} = \frac{(DMIxTDN)}{1.23}$	$DMI = \left(\frac{120}{NDF\%}\right) + (NDFD - 45)x\left(\frac{0.374}{1350}\right)x100$ $TDN = (NFC \times 0.98) + (CP \times 0.93) + (FA \times 0.97 \times 2.25) + (NDFn\ x(NDFD/100)) - 7$ $NFC = 100 - (NDFn + CP + EE + ash)$ NDF = else estimated as NDFn= NDF x 0.93 FA= fatty acid (% of DM)= ether extract – 1 NDFD = 45 is an average value for fiber digestibility of alfalfa and alfalfa/grass mixtures
NE$_L$= $[0.866 - (0.0077xADF)] * 2.2$	

DDM = Digestible Dry Matter, DMI = Dry matter intake (% of Body Weight), DM=Dry Matter, TDN = Total Digestible Nutrients (% of DM), NDF= Neutral Detergent Fiber (% of DM), ADF=Acid Detergent Fiber (% of DM), NDFD = 48-hour in vitro NDF digestibility (% of NDF), CP = Crude Protein (% of DM), NDFn = Nitrogen Free, NFC = Non Fibrous Carbohydrate (% of DM), NE$_L$= Net Energy Lactation.

RESULTS

Descriptive statistics of the laboratory analysis results are presented in Table 2 for the samples used in the study. A noteworthy variation was also observed in the raw spectral data obtained from the samples (Figure 1). Depending on those, it can be stated that both the variation in spectral data and the variation in target variables were suitable for estimation model development in the established models.

The evaluation parameters are presented in Table 3 for the estimation models established in the study. While 99.9% of the variation in spectral data can be explained by models for all feed quality parameters, 34.20% to 80.43% of the variation independent variables could be explained in established models (Table 3). Root-mean-square error (RMSE) was found to be at an acceptable level for the measured feed quality evaluations. While slope values of the estimations varied between 0.60 and 0.80, estimations without any deviation could be provided in calibration models (Bias=0.00), and in contrast, various levels of deviations were observed in internal validation sets although it changed according to models. RPD values belonging to calibration and validation sets were determined to be between 1.25 and 2.22 (Table 3). The studies on spectral model development reported that models with RPD values above 2 could be used for scans. It was observed that the wavelength selection method (VIP) used in the study increased the estimation success in calibration sets for NEL and RFV parameters, and reduced it for RFQ parameter (Table 3). These data are being confirmed with the relation between reference and NIR estimations of the models developed by using all spectral data (Figure 2 a, b, c) and the results of the models that used wavelengths selected with VIP (Figure 2 d, e, f). There are various studies on literature that reported a positive correlation between RFV and RFQ (Undersander and Moore 2004). Accordingly, a positive connection may be expected in the NIR estimations of RFV and RFQ values. It was observed in the study that the use of the VIP method did not meet this expectation for the external validation set, and it was understood that wavelength selection might lead to deviations in NIR estimations for the RFQ parameter.

On the other hand, the fact that there were different main components in RFQ and RFV calculation formulas may have an important effect on NIR estimations. While DMI and TDN are main estimators in RFQ calculations, ADF and NDF are effective in RFV calculation. It is considered that the wavelength selection method poses a positive effect on the selection of areas related to ADF and NDF, however, it leads to a number of deviations in the detection of wavelengths related to DMI and TDN.

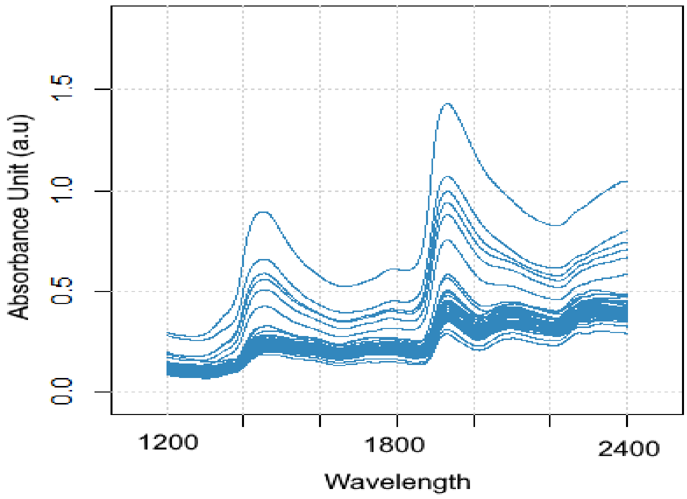

Figure 1. The plot of spectral data obtained from roughage samples.

Table 2. Descriptive statistics for RFV, RFQ, and NE_L calculations in the sample set used

	n	Mean	Minimum	Maximum	Standard Deviation
RFV	53	91.4	47.9	158.0	29.0
RFQ	53	86.1	48.3	160.9	27.0
NE_L	53	1.23	0.90	1.56	0.19

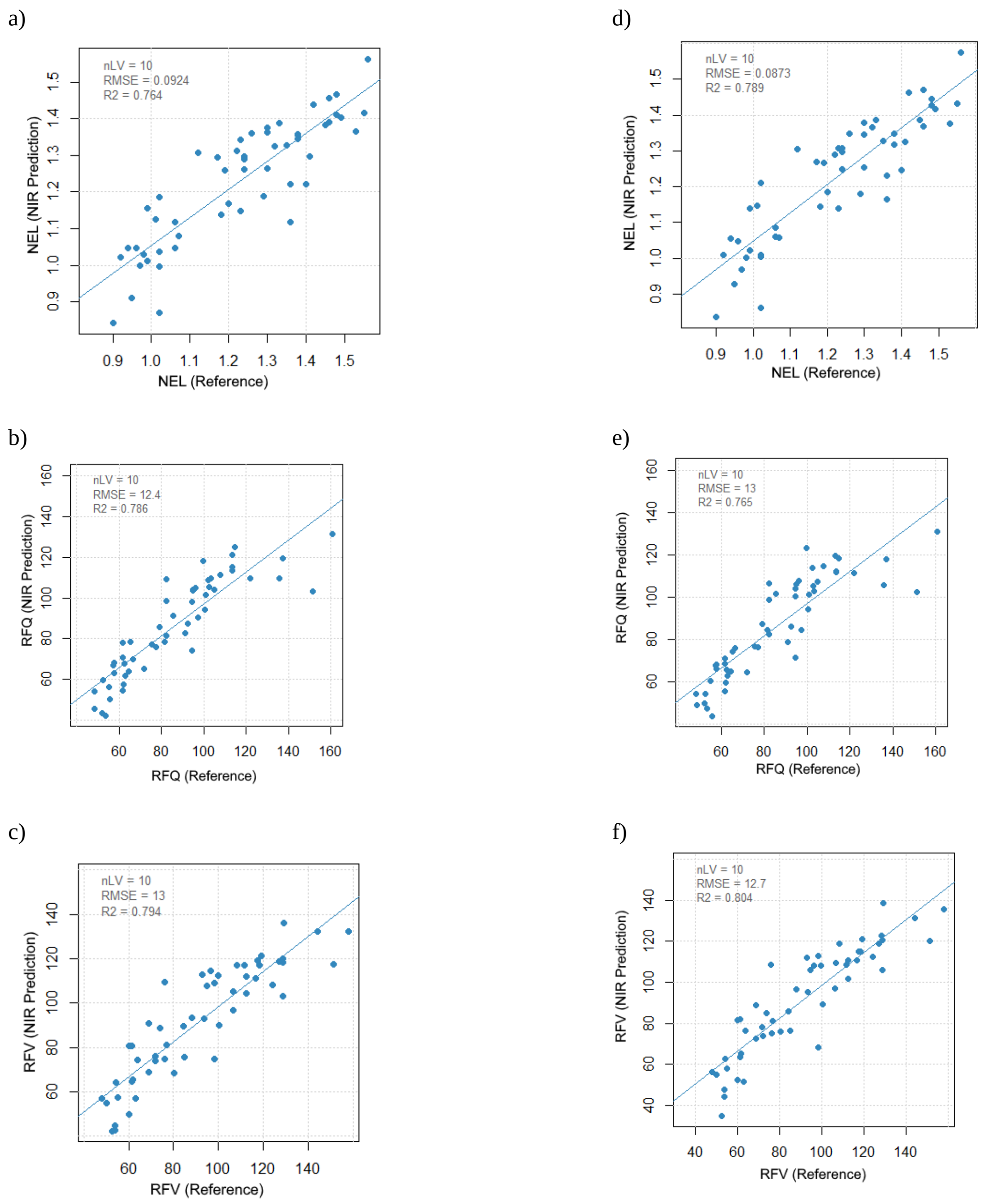

Figure 2. The relation between reference and NIR estimations in regression models established with the entire spectral region (a, b, c) and selected wavelengths (d, e, f).

Table 3. Evaluation parameters for PLS models established with all spectral data and wavelengths selected with the VIP method

		X cumexpvar	Y cumexpvar	RMSE	Slope	Bias	RPD
RFV	PLS-Cal	99.99	79.36	13.043	0.79	0.0000	2.22
	PLS-Val	99.97	45.40	21.080	0.66	1.0494	1.38
	VIP-Cal	99.99	80.43	12.700	0.80	0.0000	2.28
	VIP-Val	99.98	36.17	22.859	0.70	0.6319	1.27
RFQ	PLS-Cal	99.9	78.61	12.371	0.79	0.0000	2.18
	PLS-Val	99.97	35.10	20.883	0.61	0.7463	1.29
	VIP-Cal	99.99	76.48	12.973	0.76	0.0000	2.08
	VIP-Val	99.99	34.86	21.056	0.63	0.5881	1.28
NE_L	PLS-Cal	99.99	76.35	0.092	0.76	0.0000	2.08
	PLS-Val	99.98	34.20	0.154	0.62	0.0062	1.25
	VIP-Cal	99.99	78.90	0.087	0.79	0.0000	2.20
	VIP-Val	99.98	39.88	0.147	0.67	0.0014	1.31

DISCUSSION

It was determined that there was a sufficient variation for RFV, RFQ, and NEL values in the sample set that was used as a dependent variable in the modelling study (Table 2). The variation determined in target variables overlaps with the limit values reported in previous studies for the calculation of these characteristics with roughage samples (Undersander and Moore 2004, Güney et al. 2016).

The results of the study are similar to the results obtained from other studies on feed quality evaluations in roughage samples. Nevertheless, there is a prominent change in calibration success according to the sample type used in scientific literature. It was observed in the study of Doğusoylu and Bayram (2019) that estimation success was low in the calibration model they established for determining NDF content in corn kernel, and it was determined that R-value was 0.3353, R2 was 0.1124, and the standard deviation was 3.4691 in the calibration set. In another study, NDF, ADF, crude ash, crude fat, and crude protein values were compared in locust bean with chemical and spectroscopic (NIRS) methods, and it was stated that different results had been obtained ($P< 0.05$, $P<0.01$) (Pehlevan and Özdoğan 2015). Asekova et al. (2016) found R2 values above 80% and RPD values above 2 for the calibration models established for crude protein, crude fat, and NDF content in soybean, and they have calculated R2 value as 78.9% and RPD value as 1.79 for ADF content. While RFV, RFQ, and NEL parameters were not addressed in these studies, it is observed that there are prominent changes due to sample type in the analysis results belonging to the characteristics used in the calculation of these parameters. In our study, roughages of different plant types have been used as the material. Among these materials, silage samples have relatively different characteristics compared to other roughage samples and were separated from other samples in spectral data (Figure 1). This may have caused deviations in the NIR estimations of RFV, RFQ, and NEL parameters. In the study performed by Rushing et al. (2016), apart from important feed characteristics, the estimation success for RFV value ($n = 55$, $R2 = 0.916$, $RSCD = 3.45$, $RSCIQ = 3.04$) was found to be higher than the model established in our study for the determination of RFV value with NIR. This may have caused by the fact that samples of only one plant type (Elymus glabriflorus) were used in that study. These results suggest that calibration models should be established separately for samples belonging to different roughage types. The number of samples in this study did not allow this to be performed.

CONCLUSION

In this study, it was investigated whether it was possible to determine the indirectly calculated RFV, RFQ and NEL parameters with NIR spectroscopy in roughage feed quality measurements, and the effect of wavelength selection on the model's success was examined. According to study results, it was recognized that RFV, RFQ, and NEL parameters could be determined with NIR at an acceptable accuracy. It was observed that the wavelength selection that was used (VIP) affected estimation success positively, and it will be useful for estimation models to only use the wavelengths related to the target variable in the scanned spectral area as the indicator variable. Since the number of samples used in this study is limited, the samples that negatively affected the model success could not be eliminated. It is estimated that increasing the number of samples in the calibration set and using different chemometric methods in future studies may allow determining RFV, RFQ, and NEL values more accurately in roughage samples.

Conflict of Interest: The authors declare that they have no conflict of interest.

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