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Research Article

Development of Local Calibrations for the Nutritional Evaluation of Corn Silage Available in Bangladesh by Using Near Infrared Reflectance Spectroscopy

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Abstract

This study was conducted to calibrate and validate the 700 - 9500 nm monochromator of near-infrared spectrophotometer (Bruker-MPA, Germany) equipment for the fast analysis of proximate components (CP, CF, EE, and Ash) in commercial corn silage at the Feed Quality Control Laboratory, DLS, Savar, Dhaka. A total of 52 samples were examined in the animal nutrition section of QC lab to determine the nutrients that were available. In order to connect the spectral data and associated wet chemistry values, local calibration equations were created in the NIRS utilizing OPUS (Optical User Software) in the second phase of this study. On the infrared light scanner, a quartz sample cup was utilized to hold the sample and MPAII spherical macro sample_64_rotating_Res16-DLS.XPM was employed. Fresh samples were ground through a 2 mm screen after being dried for the analysis. The typical values in the lab for % DM, % Ash, % CP, % EE, and % CF are 95.32, 5.82, 10.19, 2.72 and 26.64, respectively. For the formulation of the NIRS equation, the value for each component was assigned to the calibration group. Following calibration in NIR, the measurements of % DM, % Ash, % CP, % CF, and % EE showed a root mean square estimation errors (RMSEE) of 0.29, 0.35, 0.63, 0.08 and 0.45, respectively, and correlation coefficients (r2) of 95.51, 94.58, 91.79, 97.19 and 98.71, respectively which indicate the close relationship to the mean laboratory values. RMSECV (Root Mean Square Error Cross-Validation) values after cross-validation were 0.397, 0.488, 0.988, 0.107 and 0.713, respectively with r2 values of 89.59, 86.88, 75.19, 93.7 and 95.92, respectively. The NIRS projected values' accuracy %age ranged from 98.94 to 100.16%, which indicates that they are quite near to the mean laboratory values. It is concluded that NIR may be a useful tool for estimating the proximate nutritional value of corn silage in Bangladesh.

Keywords: *Near-Infrared Spectrophotometer (NIR); Cross-Validation; Calibration; Corn Silage; Proximate Analysis; FT-NIR*

Introduction

Silage is a method of preserving forage in an anaerobic environment that results in the breakdown of proteins and amino acids, and the generation of a number of nitrogen compounds, such as amines and ammonia, through a chemical reaction by plant or microbiological enzymes present in this environment [1]. The demand for maize silage is strong among all types of fodder silage, which has led to an increase in maize output in Bangladesh. For instance, the production of maize increased by 45.97% from the 2015-16 fiscal year's estimated 24.45 lac metric tons to the 2019-20 fiscal year's estimated 35.69 lac metric tons [2]. It indicates that with the expansion of corn growth, green corn feed is now accessible. The entrepreneur was able to create business-related silage production and marketing because of the readily available maize fodder (major). In addition to encouraging the production of fodder silage, it is crucial to preserve and watch over its quality at the farm and market levels. The quick screening tool known as near-infrared reflectance spectroscopy (NIRS) is well known worldwide. It is frequently used in the feed industries and can quickly evaluate feed samples. The phrase "proximate composition" refers to the six components and is frequently used in the feed/food industry. As a %age of the feed, moisture, crude protein, ether extract, crude fiber, and crude ash are all expressed. The broad application of near-infrared spectroscopy for the prediction of proximate components in forages has substantially boosted the simplicity of obtaining proximate analysis of forage samples. However, Bangladesh does not regularly use NIR for fodder proximate analysis in ruminant nutrition. The database utilized for this inquiry on corn silage is not accessible in Bangladesh. The many international organizations responsible for validating various test methods have already developed the analysis of proximate components of animal feed and feedstuff. Nevertheless, the purpose of this work was to calibrate the FT-NIR against wet chemistry data for assessing five nutritional qualities of the widely available corn silage in Bangladesh (DM %, CP %, CF %, EE %, and Ash %). The FT-NIR-MPAII-validation Advanced was completed by calibrating and validating the analytical values (Table 1) using the OPAS Lab software. In NIRS, the calibration model is created utilizing a variety of statistical traits. The correlation coefficient (r2), RMSECV (Root Mean Square Error Cross-Validation), RMSEE (Root Mean Square Estimation Error), etc. are some of the indicators of linear validation.

Materials and Methods

Experimental place and date

Eight districts overall, including larger Dhaka, Rajshahi, and Rangpur in Bangladesh, were chosen for the study. These districts are: Manikganj, Munsiganj, Sherpur, Dhaka, Faridpur, Bogura, and Lalmonirhat. The laboratory research was conducted at the Feed Quality Control Section, QC Laboratory, Savar, DLS, Dhaka, Bangladesh from July 2021 to February 2022 with the approval of the Project-Establishment of Quality Control Laboratory for Livestock Production Inputs and its Food Products, Department of Livestock Services, Bangladesh.

Sample collection and preparation

52 samples of corn silage total, with an average weight of 4 - 5 kg, were taken during sample collection from each pit while adhering to the sampling technique (ISO 6497-2002). To prevent rotting and maintain temperature to prevent exposure to high temperatures, samples of corn silage were sealed in plastic bags or vacuum-packed and transported to the lab as soon as feasible. Each fresh corn silage sample had one portion ground using a 1 mm sieve and immediately dried in an air-forced oven at 65°C for 24 hours.

Laboratory analysis

Analytical standard procedures were utilized to value the close-proximate components of corn silage, as shown in table 1.

Evaluation of DM and moisture (%)

The QC lab analysis that is probably performed the most frequently is the determination of dry matter, or more specifically, moisture. This is a crucial analysis because the concentration of other nutrients is typically represented on a dry matter basis (as a %age of the dry matter). The residue remaining after drying for three to four hours at 103°C in a vented oven served as the dry matter of the collected sample, which was determined gravimetrically. Three copies of each sample were analyzed, and mean values were computed.

Evaluation of crude ash (%)

The residual was measured gravimetrically after the sample (5g sample) was burned at 550°C for three hours in a preheated muffle furnace and all the organic material was oxidized.

Evaluation of crude fiber (%)

Crude fiber analysis serves as the foundation of the Weende system. Originally, the analysis was supposed to separate plant carbohydrates into sections that were easier to digest and harder to digest (crumble fiber) (nitrogen-free extract; NFE). The fiber crucible was filled with 1g of material and 0.5 - 1g of celite. The substance was then broken down using a 1.25% potassium hydroxide and 1.25% sulfuric acid solution. The weight of the dried ash sample was determined.

Evaluation of ether extract/crude fat by soxhlet apparatus

When referring to a wide range of substances that are insoluble in water but soluble to varying degrees in "fat solvents" or "organic solvents," such as ether (diethyl ether), chloroform, alcohol (methanol, ethanol, etc.), acetone, benzene, and "petroleum ether," the terms "lipid" and "fat" are frequently used interchangeably.

A 5g test piece of the sample was inserted in the soxhlet sample thimble cup. 100 mL of diethyl ether was then poured into the sample cup. Ether Extract was collected below the sample cup as a result of the distillation and filtration, which took place at a boiling temperature of 60 - 68°C. After drying, the residue was weighed.

Evaluation of crude protein (CP) by DUMAS method

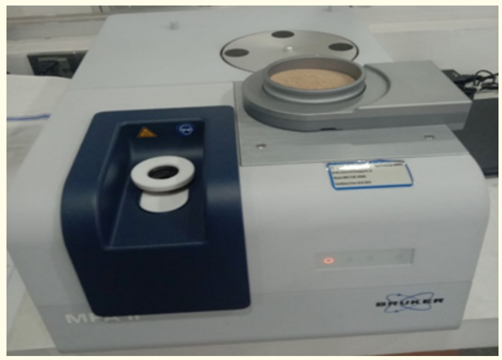
Utilizing the Dumas method (total combustion method), crude protein analysis for animal feed was verified. This method is one of the most frequently used analyses in the nutrition laboratory. The nitrogen is converted to nitrogen oxides (NOx) gas at 1030 degrees Celsius during the quantitative combustion digestion step of the Dumas procedure for measuring nitrogen. NOx is changed into N_2 in a thermal conductivity cell, and the N_2 is then measured. Pour around 0.2 - 0.5g of EDTA to the nearest 0.1 mg (W) in a tin cup to determine the amount of crude protein. Set the tin cup in Dumas' contraption after carefully sealing it so that it is airtight. The temperature used to burn the silage was 10300°C for combustion and 650°C for reduction. By dividing the observed nitrogen amount by the necessary factor (6.25), the crude protein content was calculated and displayed as a %age. The Dumas apparatus, which can make a comprehensive determination, automatically determines % Nitrogen (% N). To determine N content, the areas of the peaks found in the samples and the calibration standard (EDTA) are compared. Finally, the nitrogen % and factor 6.25 were multiplied to find the CP %.

Constituents (%)	Method	Major instruments
Dry Matter (DM)	AOAC 930.15.2000	Forced air Oven
Crude Ash	AOAC 942.05.2000	Muffle furnace
Crude protein (CP)	AOAC 990.03	DUMAS
Crude Fiber (CF)	AOAC 978.10	Velp Scientific -FIWE Advance Automatic Fiber Extractor
Ether Extract (EE)	AOAC 920.39	SER 158, solvent extractor, Velp scientific.

Table 1: *Analytical standard method.*

Calibration and validation procedure

NIRS- Advanced (Bruker, MPAII, Germany) systems monochromator (700 - 9500 nm) range was utilized for the creation of an ideal database of corn silage, and the results were compared to those determined by wet chemical testing on 52 samples of dry ground corn silage. On the infrared light scanner, a quartz sample cup was utilized to hold the sample, and MPAII spherical macro sample 64 rotating Res16-DLS.XPM was employed. The 52 corn silage samples were gathered from three major divisions (greater Dhaka, Rajshahi, and Rangpur) and examined in the chemistry lab at QC Lab, DLS, Savar, Dhaka for the determination of DM %, CF %, CP %, EE %, and Ash %. In order to establish the NIRS calibration equation, these analytical results were further calibrated and validated in the NIRS MPAII-Advanced equipment using the spectrum of each analyte. All calculated information is presented below in tabular form (Table 2). The accuracy, correlation coefficient, RMSECV, and RMSEE had been measured to confirm the NIR test results described above (Table 1).



NIR machine

NIR working Flow chart (Sample measurement)

Figure

Results and Discussion

In the samples of corn silage, the % of crude protein (CP), DM, EE, CF, and Ash were examined. There were 52 samples including calibration and prediction sets. The RMSECV (Root Mean Square Error Cross-Validation), RMSEE (Root Mean Square Error of Estimation), and r2 (Correlation coefficient) methods were used to evaluate the predictions' accuracy. Root mean square error cross-validation (RMSECV) and the correlation coefficient (R2) between the measured values obtained from the analytical laboratory and the predicted values of NIRS were used to verify the correctness of the calibration models. The calibration was adequate for screening for the proximate analysis since the RPD to identify the % of DM, Ash, CP, EE, and CF in corn silage were more than 3 [3] with ranking 7-10 (Table 2). The evaluation's correlation coefficients for the % of DM, Ash, CP, EE, and CF in corn silage were 95.51, 94.58, 91.79, 97.19, and 98.71, respectively (Figure 1-5). Since there was an unsatisfactory correlation (R2 = 91.79) between the actual and predicted value for the determination of the CP levels in corn silage, this could be because there were only 52 samples in the calibration set (Table 1). The inclusion of numerous homogenous samples in the calibration sets, however, could enhance this association. Dry matter and crude protein were the parameters predicted by NIR, with r2, RMSECV, and RMSEE ranging between 95.51 and 91.79, 0.397 and 0.988, and 0.29 and 0.63, respectively, with biases of 0.0109 and -.019. However, according to Eduardo V. Valdes [4] opinion on crude protein (CP), dry weight basis, the standard error in cross-validation (RMSEE) was 0.54%. Unlike soybean forage, where the RPD of CP was 3.34 [5], this experiment's RPD was 3.49.

SL	Analytes	No. of samples	Laboratory values %	NIRS values (%) (predicted)			Accuracy% (98-101)	Cross-Validation Statistics						Calibration Statistics		
			Mean	Mean	Data range %	Frequency region		Rank	Calibration accepted spectra	r2	RMSECV	Bias	RPD	r2	RMSEE	RPD
1	DM% (2nd)	51	95.334	95.323	91.97-97.64	4248-6104, 7496-9400	99.99	8	48	89.59	0.397	0.0109	3.1	95.51	0.29	4.72
2	Ash%	51	5.819	5.819	2.835-9.891	4424-4600, 7504-6800	100.00	9	46	86.88	0.488	-0.0232	2.76	94.58	0.35	4.29
3	CP%	52	10.189	10.191	6.745-15.37	4600-6104, 9400-7496	100.02	10	40	75.19	0.988	-0.019	2.01	91.79	0.63	3.49
4	EE%	51	2.750	2.721	1.576-3.631	5448-5776, 8448-7496	98.94	7	38	93.7	0.107	0.00011	3.98	97.19	0.08	5.96
5	CF%	51	26.595	26.637	19.81-32.31	4424-4600, 5448-6104, 7496-9400	100.16	9	47	95.92	0.713	0.00632	4.95	98.71	0.45	8.82

Table 2: *General and calibration (NIR) statistics for the chemical composition of corn silage:*

RMSECV = Root Mean Square Error Cross-Validation, RMSEE = Root Mean Square Error of Estimation, r2 = Correlation Coefficient, RPD= Relative %age Difference. DM= Dry Matter, CF= Crude Fiber, CP= Crude Protein, EE= Ether Extract.

The primary determinant of feed energy content is fat. Fat content is more challenging to forecast with any degree of accuracy than moisture and CP, particularly in plant protein components. Nevertheless, in the current experiment, the correlation between the actual and predicted value for determining the fat content in corn silage (R2 = 97.19, SECV = 0.107, and RPD= 5.96) is relatively better than the fat content predicted by NIRS in [5] soybean grass protein materials (R2 = 94.2, SECV = 0.467, and RPD= 3.45). From a spectral perspective, fat content may be accurately assessed because the NIR area has some signature wavelengths connected to fat.

The Correlation coefficient of Ash% of corn silage is 94.58. The correlation between minerals and organic components, either through interactions with organic molecules or by producing salts, is said to make it possible for NIRS to be used to assess mineral constituents [6]. This indirect association might also be responsible for the experiment's successful predictions of the ash content of corn silage.

For the determination of CF and EE in broiler feeds [7], the standard error of estimation (RMSEE) was 0.361 and 0.350, respectively, with correlation coefficients (r2) of 86.28 and 96.28. In contrast, the RMSEE for corn silage is 0.45 and 0.08, which are better at 98.71 and 97.19 with a correlation coefficient. With correlation coefficients (r2) of 95.92 and 93.7, the standard error (RMSECV) for the prediction of CF and EE in corn silage was 0.713% and 0.107%, respectively, after cross-validation.

The chemical composition of feed protein materials is influenced by a number of elements, including species, particle sizes, and processing methods, and as a result, the price can vary greatly. Here, table 2 displays the NIR calibration and cross-validation data for the estimation of nutrients in samples of corn silage. Multivariate calibration was created in accordance with process 2, and OPUS, a commercial software analysis application, had access to all available. Ranking 7-10 (Table 2) has acceptable r2 values for calibration and cross-validation [8]. However, bias in each component was lower than 1, demonstrating the model's correctness. First, a matrix representation of the spectral and concentration data was encoded. To prevent the tiny spectral changes in the data set that can lead to over fitting, few parameters were chosen (noise). In order to obtain the best prediction for each component, the appropriate rank, factors using RMSECV were tuned by cross-validation [9].

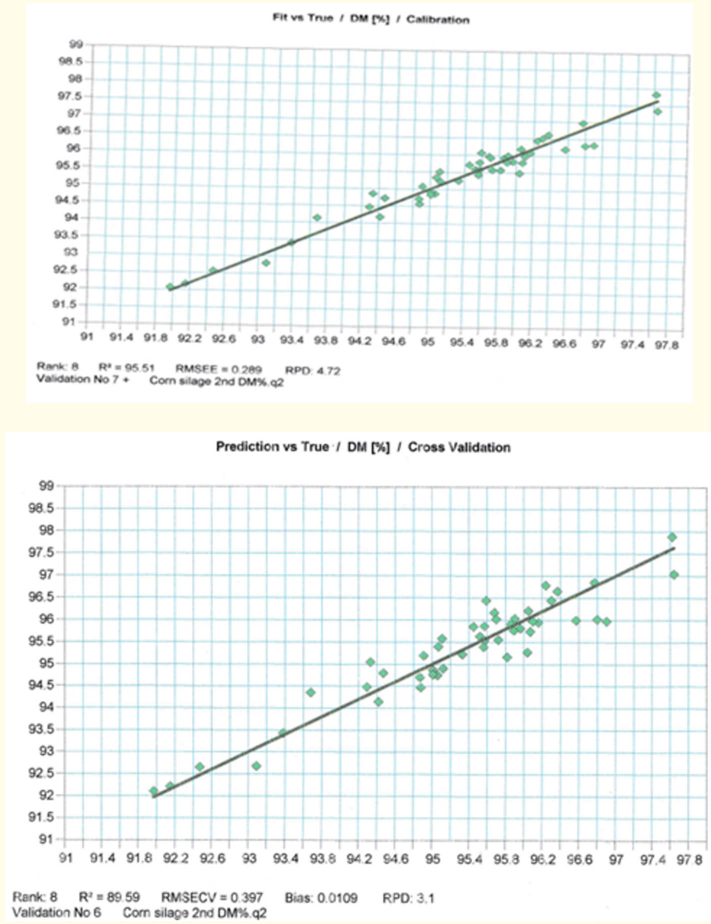
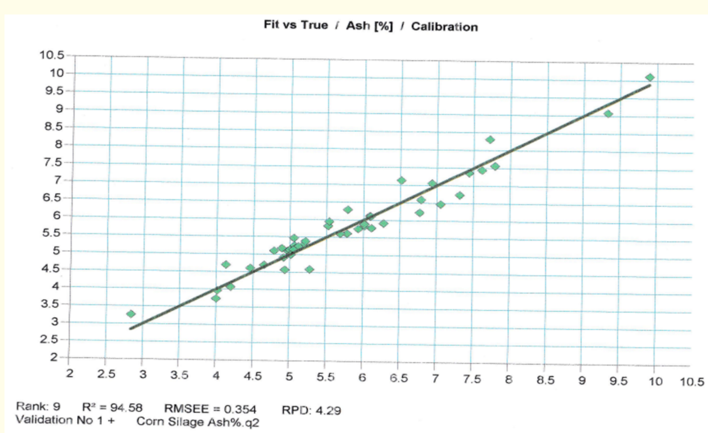


Figure 1: *Regression equations of prediction (NIR) vs true (laboratory) values for DM % Calibration and cross validation.*



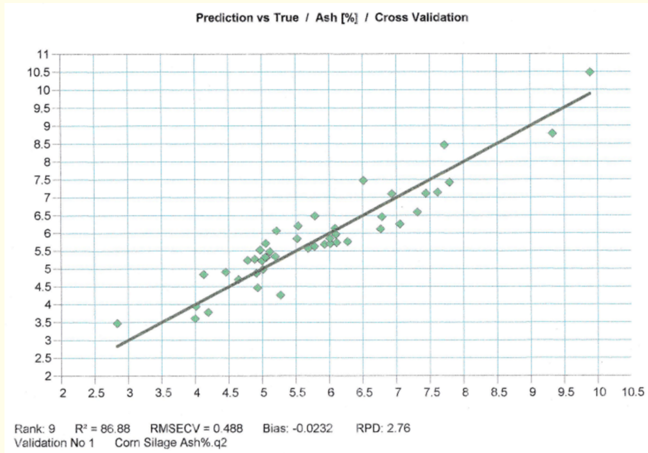


Figure 2: *Regression equations of prediction (NIR) vs true (laboratory) values for Ash % Calibration and cross validation.*

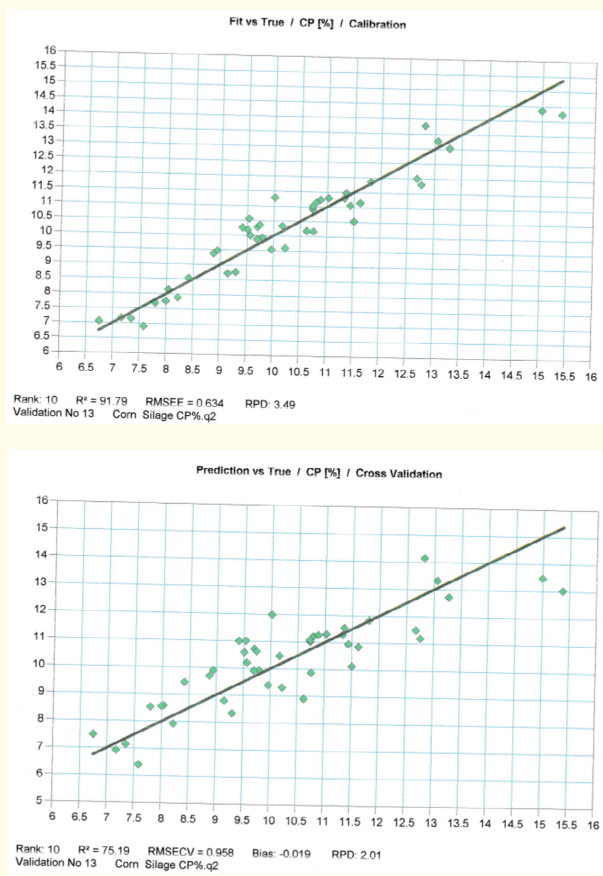


Figure 3: *Regression equations of prediction (NIR) vs true (laboratory) values for CP % Calibration and cross validation.*

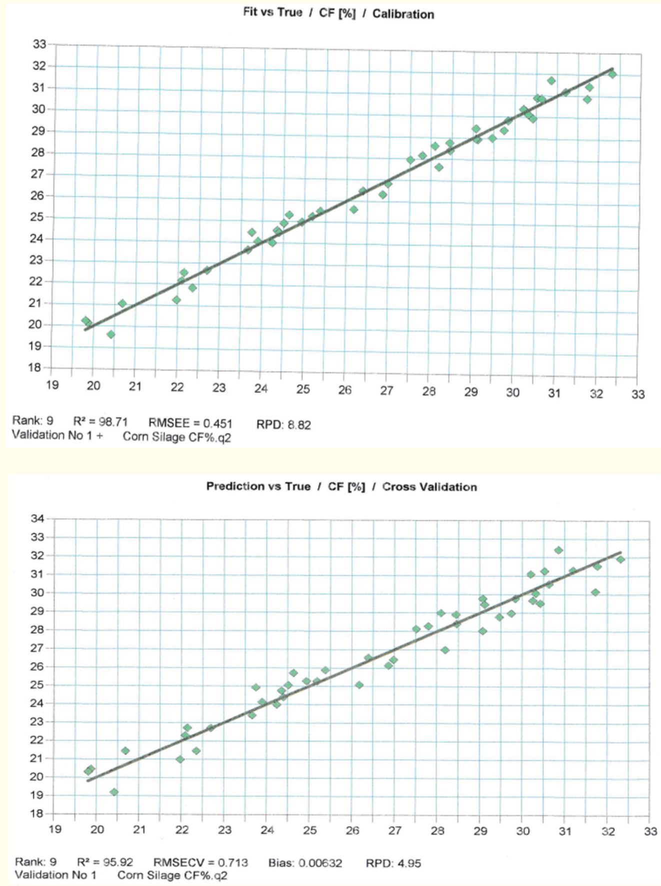
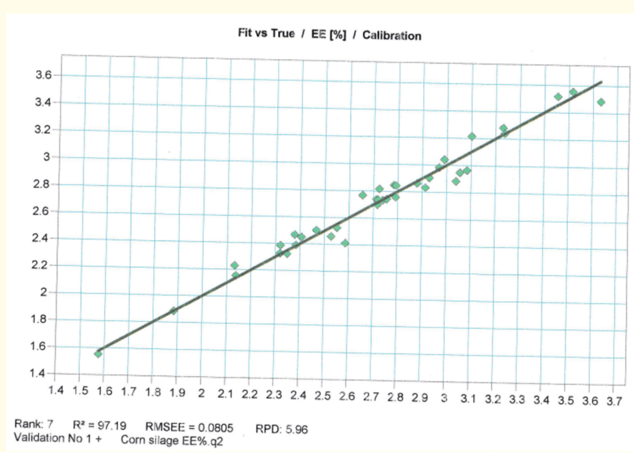


Figure 4: *Regression equations of prediction (NIR) vs true (laboratory) values for CF % Calibration and cross validation.*



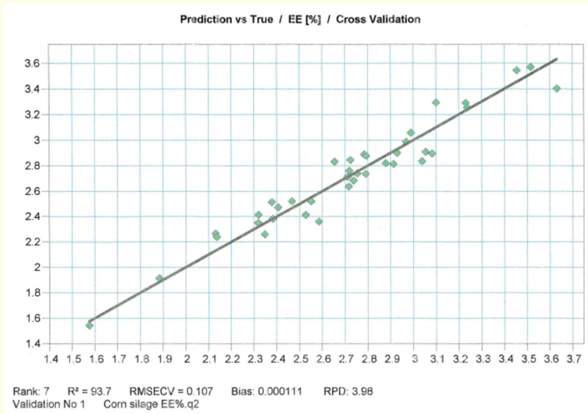


Figure 5: *Regression equations of prediction (NIR) vs true (laboratory) values for EE % Calibration and cross validation.*

Conclusion

It is concluded that the wet chemistry values of the closest components of dry corn silage and the mean anticipated values by FT-NIR are very closely correlated. Therefore, NIRS could be effectively applied to forecast the nutritional value of corn silage.

Limitation of the Study

It is recommended that further research with ample number of samples may upgrade the database of Corn silage in NIR.

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