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OPTIMIZING NEAR INFRARED REFLECTANCE SPECTROSCOPY TO PREDICT NUTRITIONAL QUALITY IN CHICKPEA HAULMS FOR LIVESTOCK FEED

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AUTHOR'S CONTRIBUTION

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ABSTRACT

The near infrared reflectance spectroscopy (NIRS) was used to develop calibration equations to predict chickpea haulm (*Cicer arietinum*) feed quality traits and mineral constituents. A total of 1348 cultivars of chickpea representing a nation-wide range of environments in Ethiopia and genotypic diversity (113 cultivars and 7 landraces) used in the framework of the Ethiopian National Chickpea Breeding and Genetics Program were scanned using a FOSS 5000 spectrophotometer. 130 samples representing the spectral characteristics of the chickpea haulms, selected using WinISI II software V.1.50, were chemically analyzed for the development of the calibration equations. A modified partial least-squares (MPLS) regression with cross validation was used to confirm the equations and identify possible spectral outliers (GH-value>3, where GH is the Mahalanobis distance). Values for coefficient of determination (R), standard error of prediction SEP(C) and ratio of performance deviation (RPD) were used for validation of the equations. Results showed ash (r =0.97; RPD=3.64), crude protein (r2= 0.99; RPD = 8.09), acid detergent fiber (r2 = 0.99; RPD = 6.43), neutral detergent fiber (r2=0.99; RPD = 6.65), lignin (r2 = 0.99; RPD =5), ME (r=0.99; RPD=24.3), IVOMD (r=0.99; RPD=26). These results show that the calibration equations can accurately predict nutritional quality traits of chickpea haulms. The use of the NIRS method can facilitate cost-effective and rapid decision making by researchers and farmers.

Keywords: Calibration equations; chickpea haulms; multi-location trial; NIRS; nutritional quality.

1. INTRODUCTION

Ethiopia is an agrarian country endowed with diverse ecosystems, edaphic and climatic conditions that are suitable for production of diverse crop, animal and microbial genetic resources [1,2,3]. Genetic diversity found in the Ethiopian landraces are being used worldwide for developing new crop varieties and addressing different production constraints [4].

Chickpea (*Cicer arietinum* L.) is one of the main pulse crops in the world. It ranks second in area and third in production among the pulses worldwide [5]. Chickpea is internationally cultivated in over 50 countries with about 13.2 Million hectare area and a production of about 11.6 Million tonnes (FAO, 2013). Chickpea is a good source of protein, carbohydrates, dietary fiber and minerals [6]. The average global chickpea yield is far below its presumed potential, and

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conventional breeding has not been able to increase the productivity as per its potential [7].

Chickpea haulm has been stated to have higher nutritive value than cereal straws [8,9,5] but lower than that of other legume straws [10,9]. Even though different scholars studied the nutritive value of chickpea haulms; there is no full information on the haulms quality of different chickpea varieties in Ethiopia. The present study was thus effort to bridge the gap. As the food-feed traits of chickpea crop in Ethiopia has not been thoroughly studied and based on high quality and yield of forage for livestock and primary food traits, finding prevailing genotypes which have these dual purpose traits would be a positive steps towards addressing food and feed gaps in the mixed crop-livestock systems to improve overall productivity and income of Ethiopian smallholder farmers.

Methods of feed evaluation has been adapted and sophisticated since the mid-1980s when Weende method was proposed. Since then various chemical, biological and physical methods have been recommended and applied for feed resource description. Near infrared spectroscopy (NIRS) is one of the new techniques being applied for the nutritional characteristics of animal feeds. The NIRS region is the wavelength range between 12000-4000 cm^{-1} in the electromagnetic spectrum. When a sample is evaluated, the radiant energy is absorbed selectively according to the specific vibration of the molecules presents, which produces an overtone in the spectrum [11].

The technique is, thus far, noted to be one of the vigorous applications to estimate chemical entity and parameters like *in-vitro* organic matter digestibility and metabolizable energy. Unlike most conventional analytical methods, NIRS technique is fast, low-cost, and nondestructive to the crop sample. NIRS requires very little sample preparation and no chemicals, is consistent and accurate [12], permits a larger range of samples to be tested, and can be used to analyze multiple properties at one time [13,14]. Although the reliability of NIRS has been investigated well for temperate feeds little work has been done for tropical feeds. Furthermore, the variation in ecological set up, the biological diversity in feed resources in the country requires quite robust and cost effective method for characterization. This research result intended to fill these gaps with objectives of developing and validate prediction equation for determining the nutritional quality of chickpea haulms as livestock feed using Near Infrared Reflectance Spectroscopy (NIRS).

2. MATERIALS AND METHODS

2.1 Description of the Study Areas

As indicated from the table, Chefe Donsa and Akaki sites were more highland than the other experimental sites.

2.2 Sample Description and Experimental Layout

One-thousand three hundred forty eight samples of chickpea haulms from preliminary and national variety trials were used in the experiment for NIRS analysis. However, total samples of 597 chickpea haulms and 48 genotypes of the crop from National variety trial were used for statistical analysis, because these varieties are already tested preliminarily on fields in terms of agronomic traits, diseases resistance, etc. by the breeders. Randomized complete block design (RCBD) with 4 replications was used in the experiment. The test genotypes consisted of both Desi and Kabuli chickpea types. All the agronomic practice of crop management were done from sowing the land

Table 1. Description of the experimental sites

Characteristics	Locations				
	Akaki	**Alem Tena**	**Chefe Donsa**	**Debre-zeit**	**Minjar**
Altitude	2200masl	1575masl	2450masl	1900masl	1810masl
Latitude	$08^0$53’ N	8° 18'N	08^0 57’ N	$08^0$44’N	$08^0$55’N
Longitude	$38^0$49′ E	38° 57'E	39^0 06’E	$38^0$58'E	39 ^{0}45’E
Annual max. Tem.	26.5^0c	29.8^0c	26^0c	28.3^0C	28^0C
Annual min. Tem.	7^0c	12.9^0c	7^0c	8.9^0C	10^0C
Mean annual RF	1025 mm	728 mm	843 mm	851 mm	867 mm
RF distribution	Bimodal, less erratic	erratic rainfall	Bimodal, less erratic	Bimodal	bimodal/uni-modal
Soil type	Vertisols	Light	Vertisols	Vertisols	Light

Sources: Befakadu [15]; Damitew et al. [16]; Abera and Kebede [17]; Kebede and Taddesse [18]; and Debre Zeit Agricultural Research Center

to harvesting and after full maturity (90% maturity) the harvesting had been conducted from 2 central rows of each plot (2.4 m^2) to calculate the yield in each location.

2.3 Sample Collection and Analysis

The haulm samples were collected after harvest from chickpea experimental sites (Akaki, Alem Tena, Chefe Donsa, Debre zeit and Minjar) and after threshing the samples in each location, the seed and the haulm was separated and the haulm for this was collected and put in paper bag and labeled it and after this, the haulm was transported to ILRI's Animal Nutrition laboratory, Addis Ababa, for analysis of chemical composition and mineral contents using NIRS and *in-vitro* gas production was done at ILRI Animal nutrition laboratory, India.

2.4 Scanning of Chickpea Haulm Samples Using NIRS

NIRS was performed on ground samples (1mm sieve size) using Foss NIRS 5000 with software package WinISI II in the 1108-2492 nm spectra ranges to scan chickpea haulm samples and the spectra of each sample was taken by scanning (Win Scan version 1.5, 2000, intrasoft international, L.L.C). Before scanning about two-spoonful of the samples was put in paper bag and pre-dried at 60^oC overnight in an oven to standardize moisture conditions. Partially dried chickpea sample was filled into NIRS cup and scanned.

2.5 Chemical Analysis Using Conventional Methods

A total of 130 representative chickpea haulm samples were selected using the software based on NIRS spectra data for laboratory analysis. The samples were analyzed for DM and total ash contents by the procedures of AOAC [19]. Nitrogen was determined by Kjeldahl method [19] and CP content was calculated as N x 6.25. Van Soest and Robertson [20] procedure was used to determine Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL). These all chemically determined data were used for calibration equations to perform regression with NIRS spectral data. I was involved, for all the laboratory works like NIRS, wet chemistry and mineral analysis but the *in-vitro* technique was done at ILRI Animal Nutrition Laboratory in India.

In-vitro gas production [21] test was carried out at ILRI Animal Nutrition Laboratory in India on 130 representative samples, which were used in the wet chemistry study, to estimate digestibility and metabolizable energy contents. The digestible organic matter and metabolizable energy were calculated using the equations as follows:

$$DOM = 15.38 + (0.483*GP) + (0.595*CP\ \%) + (0.181*ASH\ \%)$$

$$ME = 2.2 + (0.136*GP) + (0.0057*CP\ g/Kg)$$

$$GP = ((V_{24}-V_0-GP_0)*\text{altitude correction factor}*0.2/SW*DM*0.01)$$

Where

V_O= Blank
GP_O= Gas Produced without sample, i.e. gas produced for rumen fluid itself
GP = is 24 h net gas production (ml/200 mg),
CP = Crude protein,
V= Volume and
ME= metabolizable energy (MJ/Kg DM)
SW= Sample Weight

2.6 NIRS Equation Development

2.6.1 Calibration

Calibration is the procedure of creating a spectro-chemical prediction model [22]. In principle, the process relates chemical information contained in the spectral properties of a substance to chemical (or physical) information showed by reference laboratory methods [23]. The aim is to create a predictive equation by passing the laboratory reference method [13]. Partial least squares (PLS) regression was used to develop the calibration models. The sample population used in the calibration consisted of 130 chickpea haulm whereas 60 samples were used for validation. After the samples were scanned with NIRS and laboratory reference data were acquired and matched; and mathematical and statistical measures were performed. Calibration equations were developed using average spectral and wet chemistry data by stepwise multiple linear regressions based on this equation. Values for DM, CP, NDF, ADF, ADL, ash contents and IVOMD, OMD, ME of all the samples were predicted or calculated based on the developed prediction models. The best models obtained were selected for each constituent based on the highest calibration coefficients (r^2c), and the smallest standard error of calibration (SEC) [11].

2.6.2 Validation

Equation validation was conducted to assess the predictive ability of the selected calibration equation. Validation means prediction of either an independent set of samples, i.e. from a different population than the calibration set, with known reference values, or removing a certain number of samples from the

calibration set, and not using them in the calibration process. The standard error of prediction (SEP) is used to judge the predictive ability of a calibration equation. This method was described as the single best estimate of the predictive capability of NIRS equation [22]. The lowest standard error of prediction (SEP) assess the overall error between modeled and reference values [11]. The coefficient of determination in prediction (r^2p) and the ratio performance deviation (RPD) were also used as additional techniques to evaluate the predictive ability of the models. The RPD is a qualitative measure for the assessment of the validation results.

2.7 Statistical Analysis

Data obtained from predicted value of NIRS, chemical compositions regression results, mineral constituents and *in-vitro* gas production of fodder traits were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) and also correlated with primary food traits (agronomic characteristics) using statistical analysis system [24] software version 9.1.3. The statistical significance of the differences between means was tested using the Duncan's multiple range tests. A statistical model involved the effect of genotype, location and the interaction between location and genotype for chemical composition and agronomic traits of chickpea haulms. A statistical model used was:

Randomized Complete Block Design (RCBD)

Model:

$$Y_{ijkl} = \mu + L_i + G_j + LG_{ij} + B_k + E_{ijk},$$

Where:

Y_{ij}= the response variable
μ= Over all mean
L_i= effect of i^{th} location (i=5)
G_j= effect of j^{th} genotype (j=48)
LG_{ij}= interaction effect of i^{th} location and j^{th} genotype
B_k= effect of l^{th} block effect (l=4)
E_{ijk}= random error.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Chickpea Haulms and NIRS Analysis

The scanned chickpea haulm samples were different in their chemical compositions as shown in Table 2. There were significant differences among the samples for all entities which propose the presence of sufficient variation among the samples to develop NIRS equation.

The calibration and equation statistics for the constituents of chickpea haulms for DM, Ash, CP, NDF, ADF, Lignin, ME and TIVOMD in (Table 2) show high determination coefficient, high R-square value, low standard errors of calibration (SEC) and standard errors of prediction (SEP) and high ratio of prediction deviation (RPD) and hereafter, these traits could be predicted with good precision that means the composition predicted by NIRS agreed closely with that of chemical analysis for studied quality components. Higher SEC value was recorded for the traits (NDF and ADF) of feed sample may be due to the wider range of variation in the trait of respective sample. SECV is a basic statistics to measure correctness for a calibration equation [25]. In this study, the value of coefficient of determination (R^2) and coefficient of determination of cross validation (VR^{-1}) for Ash, CP, NDF and ADF, ADL were greater than the value which was described by Fikadu et al. [26] who got values of 0.83, 0.80, 0.98, 0.86, 0.98 and 0.46, 0.35, 0.93, 0.62 and 0.96 respectively.

The value of the coefficient of determination for most of the composition except % DM is greater than 0.92 [27], this displays the homogeneity of the samples collected. It was less accurate (r^2 value below 0.80) for DM. Procedures for clarification of *r*, it is generally accepted that models with an r^2 values of 0.66 to 0.81 can only be used for screening and perhaps some other approximate applications (quantitative predictions), models with r^2 value between 0.83 to 0.90, can be used for many applications, while models with values of 0.92-0.96 are suitable for most applications including quality assurance. A value of more than 0.98 is usable in any application [28,29]. So, in the current study, the result of r^2 for CP, NDF, ADF, ADL, ME and TIVOMD were greater than 0.98 and DM is in the range of 0.83 to 0.90.

The error calibration of CP obtained from in this study was lower than the values achieved by Lobos et al. [23], Decruyenaere et al. [30] and Fikadu et al. [31] who obtained RMSEC values of 0.46, 8.6 and 0.92, respectively. Besides this, NIRS calibration for CP indicated a RPD of 8.09, better than that of Alomar et al. [32], with a value of 3.7. Stuth et al. [13] professed that good prediction accuracy is typically obtained when measuring protein content in feeds and forages (with R^2 of 0.95 or higher), which is related to strong (-N-H-) absorptions in the NIR region. Hence, in the current study, r^2 value for protein content in chickpea haulm was 0.99 as indicated in Table 2. The correlation coefficients in

calibration (r^2c= 0.99) and validation (r^2c= 0.99) of CP content of chickpea haulm in this work were greater than previously experiential values by Fikadu et al. [26] who found 0.90 and 0.86 respectively.

The CP content of the haulm predicted by NIRS was 6.01, which was lower than the value reported by Fikadu et al. [26]. The r^2 (0.99) and low SEC (0.21) values found in this study were higher than the values reported by Fikadu et al. [31] who stated R^2 and SEC values of 0.83 and 0.92, respectively while defining chemical entities of natural pasture from Ethiopia using NIRS. This indicated that the calibration models in the current study were closely related to the wet chemistry (Kjeldahl method) values with a high degree of linearity. The coefficient of determination (r^2) used in this study was higher whereas SEC and SEP values lower than the corresponding values obtained in earlier [33,34,35,23].

3.2 NIRS Prediction of NDF, ADF and ADL

The mean values of NDF (53.79%), ADF (39.6%) and ADL (9.13%) predicted by NIRS were comparable to the wet chemistry values as shown in Table 2. Higher values of correlation coefficient for calibration (r^2c=0.99) and validation (r^2v >0.98), low SEC (0.22 - 0.85%) and SEP (0.36 -1.3%) and high RPD values (5.06% - 6.65%) were observed. The r^2c, SEC and RPD values found in this work (Table 2) were better than or comparable to the values reported by Stubbs et al. [36] for NDF (SEC=0.82, r^2= 0.94, RPD= 3.79); ADF (SEC= 0.74, r^2= 0.94, RPD=3.56) and ADL(SEC=0.43, r^2=0.72, RPD=1.72). Since the r^2 and RPD values of the three fiber component were greater than 0.98 and 5, respectively, the accuracy and prediction capability of the model can be considered excellent according to Saeys et al. [37].

Most of the obtained values in the current study were in range of previous findings that reported DM, Ash, OM, CP, NDF, ADF ADL and TIVOMD content of chickpea haulm in ranges of 87-93.3, 3.8-13.3, 86.7-95.3, 2.8-10, 46-78, 33-59.6, 8.5-15.8 and 42.7-62.7, respectively [38,39,40,9,41,42,43], but in the current study higher metabolizable energy of 7.86MJ/KG DM was recorded.

Dry matter of chickpea haulm in present study (90.41) was lower than earlier results which are reported by other researchers [44], (Fekadu et al., 2010), [45,46]. The Ash content in this study (8.99%) was lower than Aghajanzadeh et al. [44] who found 18%, 23.74% but within the range of Fekadu et al., (2010) who reported 8.67- 9%. The CP content of chickpea haulm in current study (5.95%) was lower than Aghajanzadeh [44] Bruno-Soares et al. [10] and Fikadu et al., (2010) who reported 6.05%, 6.1% and 6.19-6.37% but greater than Kafilzadeh and Maleki [43] (3.23%); and Seyoum et al. [46] (4.7%). The mean value of NDF (537.1 g/kg DM) content of chickpea haulm in this study was lower than the values of 615.5, 765, 669, 578, 563 and 580, g/kg DM reported by Kafilzadeh et al. [43]; Bruno-Soares et al. [10]; Lopez et al. [9]; Maheri-Sis et al. [45]; Fikadu et al. (2010) and Hadjipanayiotou et al. [47], respectively.

Table 2. Values obtained from the NIRS calibration and validation of chickpea haulms

Traits	Calibration set (n=130)		Validation set (n=60)		Laboratory Values			NIRS Predicted Values		
	RSQ	SEC (%)	1-VR	SEP (%)	RPD (%)	Mean (%)	SD	Mean (%)	SD	CV (%)
DM (%)	0.84	0.19	0.78	0.24	1.96	90.4	0.47	90.41	0.39	0.27
Ash (%)	0.97	0.35	0.96	0.56	3.61	9.01	2.02	9.01	1.93	6.22
CP (%)	0.99	0.21	0.99	0.425	8.09	6.04	3.44	6.01	3.39	7.04
NDF (%)	0.99	0.85	0.99	1.3	6.65	53.75	8.64	53.79	8.6	2.42
ADF (%)	0.99	0.64	0.99	1.09	6.45	39.66	7.03	39.6	6.97	2.75
ADL (%)	0.99	0.22	0.98	0.36	5.06	9.13	1.82	9.13	1.77	3.94
ME (MJ/ Kg DM)	0.99	0.06	0.99	0.036	24.4	7.86	0.88	7.88	0.87	0.46
TIVOMD (%)	0.99	0.45	0.99	0.218	26	53.79	5.69	53.85	5.67	0.4

*DM = Dry matter; CP = Crude protein; NDF = Neutral detergent fiber; ADF =Acid detergent fiber; ME= Metabolizable energy; TIVOMD= True in-vitro organic matter digestibility; n=number of samples, SEC= Standard Error of Calibration; RSQ= R-Square (coefficient of correlation in calibration); 1-VR= coefficient of determination of cross validation; SEP=Standard error of prediction; RDP= Ratio of Performance to Deviation (RPD=SD/SEP); SD=Standard Deviation; CV=Coefficient of Variation (CV=SEP/mean*100)*

Acid detergent fiber (395.9 g/kg DM) content of haulms were lower than those (596, 467 and 409.5 g/kg DM respectively) reported by Bruno-Soares et al. [10]; Kafilzadeh and Maleki [43]; Fikadu et al. (2010) but greater than Maheri-Sis et al. [45] who found 374 gm/kg DM while the acid detergent lignin content (9.09) greater than Fikadu et al. (2010) (8.28%) and lower than Bruno-Soares et al. [10] and Seyoum et al. [46] who reported 14.2% and 13.9% respectively. However, such differences in the chemical composition of chickpea haulms in various investigations can be due to the variation in the different chickpea varieties, leaf to stem ratio, growing conditions (geographic, seasonal variations, climatic conditions and soil characteristics), extent of foreign materials and impurities such as soil contamination, different measuring methods and laboratories procedures [39,45,9], (Fekadu et al. 2010), [5,43].

3.3 NIRS Prediction of ME and TIVOMD

The mean values predicted by NIRS for ME (7.88 MJ/kg DM) and TIVOMD (53.85%) were comparable to the wet chemistry values (Table 2). High values of coefficient of determination for calibration (RSQ=0.99 and 0.99) and validation (1-VR =0.97 and 0.97), low SEC (0.06 and 0.45), low SEP (0.036 and 0.218) and high RPD values (24.4 and 26) were observed for both ME and TIVOMD, respectively. The RSQ and 1-VR values for TIVOMD shown in the present study were higher than the RSQ and1-VR of 0.92 and 0.80, respectively, for TIVOMD in chickpea haulm previously reported by Fikadu et al. [26]. Even though, different scholars said that estimation of IVOMD or ME was difficult because of the variation with rumen fluid, etc. but in my study the estimation for both TIVOMD and ME was higher; this may be due to low standard error of calibration and prediction and this indicated that the higher value of ratio performance deviation. In general, the coefficients of determination (RSQ) and the ratio of prediction to deviation (RPD = SD/SEP) and standard errors of prediction corrected for bias, SEP(C) are measured for evaluating the accuracy of NIRS prediction [29,48]. High RSQ and RPD and low SEP(C) indicate good NIRS performance; a prediction with an r^2 >0.90 and RPD >3.0 is usually classified as successful. RPD: values below 1.5 are considered unusable, those between 1.5 and 2.0 can be used for rough predictions, those between 2.0 and 2.5 allow approximate quantitative predictions, while values above 2.5 and 3.0 are, respectively, considered being good and excellent predictive models. In the present study; the values of r^2 and RPD except for DM, all other chemical constituents (Ash, CP, NDF, ADF, ADL, ME and TIVOMD) are greater than 0.97 and 3.6 respectively.

4. CONCLUSION

The result indicated NIRS is a method of choice for prediction of chemical composition and mineral constituents of chickpea haulms. Hence, the technique is noted to be one of the more multifaceted robust applications to estimate chemical entity of chickpea haulms.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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