



## RESEARCH ARTICLE

### Nutritional Evaluation of Commercial Broiler Feeds by Using Near Infrared Reflectance Spectroscopy

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#### ABSTRACT

Broiler feeds from different commercial feed mills were collected and NIR spectrum of feed samples were obtained in duplicate (scanning number 32, resolution 8) with an FT-NIRS (Bruker, MPA, Germany) systems monochromator (700-2400 nm) using a Quartz cup sampling device. Multivariate analysis were performed for the development of calibration equations of nutrient content by an Optical User Software (OPUS) and Opus-Lab to relate the spectral data and corresponding concentration values of broiler and layer feeds. Data were centered using Partial Least Squares (PLS) algorithm and spectral outliers were identified from each calibration. The calibration models were validated by RMSECV (Root Mean Square Error Cross Validation), RMSEE (Root Mean Square Error of Estimation) and correlation coefficient ( $r^2$ ) between the measured values of nutrient component determined by analytical laboratory versus predicted values by the NIRS. The standard error of estimation (RMSEE) for the determination of moisture, CP, CF, EE, Ca and P in broiler feeds was 0.230, 0.351, 0.361, 0.350, 0.056 and 0.021% respectively with correlation coefficient ( $r^2$ ) of 86.09, 95.77, 86.28, 96.28, 80.50 and 95.80. After cross validation, the standard error (RMSECV) for the prediction of moisture, CP, CF, EE, Ca and P in broiler feeds was 0.242, 0.371, 0.406, 0.390, 0.066 and 0.031% respectively having correlation coefficients ( $r^2$ ) of 84.32, 92.20, 81.84, 95.46, 75.00 and 94.71. From the results of the present study it may be concluded that NIRS could potentially be used to predict the moisture, CP, CF, EE, Ca and P contents in commercial broiler feeds.

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#### INTRODUCTION

Broiler farming is one of the fastest growing and most promising industries in Bangladesh. Its steady growth (15-20%) results in attaining country's economic growth which also contributes to improve the nutritional status by supplying meat. The government is getting interested in broiler farming and is encouraging both urban and rural people to work here and enhance capacity. Intensive production system, however, depends solely on compound feeds the cost of which represents 65-70% of the total cost of broiler production. However, proper attention should be given to evaluate the nutritional quality of feed ingredients in order to supply the adequate amount of balanced diet to poultry for maximizing the productivity cost effectively.

Near Infrared Reflectance Spectroscopy (NIRS) offers the potential for obtaining a rapid, nondestructive and accurate estimate of the chemical composition of feedstuffs. The technique has extensive application for the analysis of constituents of agricultural crops, feeds and foods (Osborne *et al.*, 1993; Williams and Norris, 2001; Roberts *et al.*, 2004). NIR spectroscopy, while much simpler and more rapid than traditional analytical methods typically requires grinding a sample to a fine particle size to give a smooth and homogeneous surface for reflection and increased precision. This analytical tools requires no chemical reagents, therefore, avoids the problems of organic and other chemical waste disposal. Once calibrations are in place, it takes just minutes to have the result of one or more constituents which by conventional chemistry may take hours or days. Currently, NIRS of

whole grains at the grain elevator is used widely in the USA, Canada, Australia and Europe for evaluation of protein and moisture content of grains (Sandra *et al.*, 2005). However, little is known about the potential of NIR spectroscopy for the nutritional evaluation of locally available ingredients in Bangladesh as well as other parts of Southeast Asia and quick prediction of nutritional quality of feed ingredients in the feeds is necessary for achieving sustainable poultry production. Therefore, the aim of this study was: a) to develop calibration procedures using NIRS and validation of calibrations for the evaluation of locally available broiler feed samples accurately and b) to determine the nutritive value of large number of commercial broiler feeds within shortest possible time.

## MATERIALS AND METHODS

### Sample collection and preparation

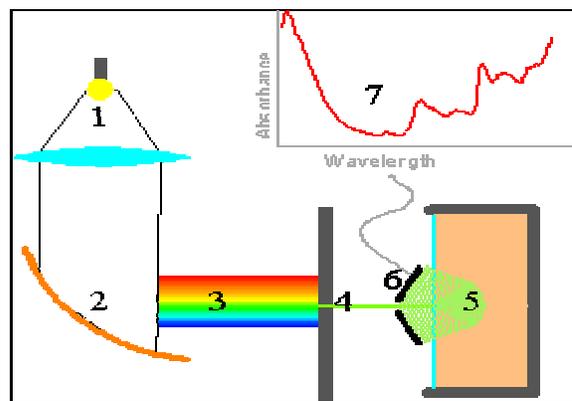
Broiler feed samples were collected from the different commercial feed mills and feed markets available in Bangladesh from 2006 to 2009. Samples were ground through 2.0 mm screen for the homogeneous particle size using Cemotec Grinding Mill (Foss Tecator, Sweden) before reflectance measurements and dried at 60°C for wet chemistry analysis.

### Spectroscopic and laboratory analysis of the sample

Identification of appropriate samples was the first step in utilizing a combination of NIRS and laboratory analysis. Ground broiler feed samples were scanned in duplicate (scanning number 32, resolution 8) with an FT-NIRS (Bruker, MPA, Germany) systems monochromator (700-2400 nm). Samples were packed into a Quartz cup sample holder which holds the sample into a clear glass window and to maintain good contact between the granular sample and window. Monochromatic light was focused on to a concave mirror which separated the light into its composing wavelengths (Figure 1). Required wavelength was selected by which the near infrared light was fallen on to the feedstuff. The amount of this light which was reflected by the feedstuff was measured to obtain the absorption corresponding to selected wavelength. The graph depicting the wavelength tested versus the absorption is called the spectrum, and an example spectrum is presented in the top right corner (Figure 1). Spectral reference curves were measured each of the broiler feed sample and the data were stored in selected folder. Sample moisture, CP, CF, EE, Ca and P were determined according to the procedure of AOAC (2000).

### Development of calibration model

For the development of calibration model in the present experiment, multivariate analysis was performed by a commercial analysis program Optical User Software (OPUS) and Opus Lab provided by Bruker, MPA, Germany to relate the spectral data and corresponding concentration values for each nutrient component (Moisture, CP, CF, EE, Ca and P) of broiler feed samples. The model was developed using Partial Least Squares (PLS) algorithm and the spectral data were processed by a suitable mathematical method e.g. first derivative, second derivative, vector normalization, subtraction of straight line etc. PLS uses eigenvectors and eigenvalues to



**Fig. 1:** Schematical layout of NIRS machine. Light is focused (1) on to a concave mirror (2), which separates the light into its composing wavelengths (3). One wave-length is selected (4), and falls onto the feedstuff (5). The amount of this light which is reflected by the feedstuff is measured (6) to obtain the absorption corresponding to one wavelength. By changing the orientation of the mirror (2), different wavelengths of light are selected to obtain absorption measures for all wavelengths of interest. The graph depicting the wavelength tested versus the absorption is called the spectrum, and an example spectrum for coconut meal is presented in the top right corner (7).

perform a decomposition of the spectral and constituent concentration data simultaneously. The decomposition process is a systematic means to determine the most important variations in the data. PLS uses constituent concentration information during spectral decomposition, which weights spectra containing higher constituent concentrations more heavily. The term “factor” or “rank” is used to describe a linear combination of spectral data. PLS reconstructs a spectrum that represents the predicted constituent values. This predicted spectrum is subtracted from the actual spectrum to determine residuals. Therefore, the residual (*Res*) is the difference between the true and fitted value. Thus the sum of squared errors (*SSE*) is the quadratic summation of these values:

$$SSE = \sum [Res_i]^2$$

The standard error of calibration or root mean square error of estimation (*RMSEE*) is calculated from this sum, with *M* being the number of standards and *R* the PLS factors or rank:

$$RMSEE = \sqrt{\frac{1}{M-R-1} SSE}$$

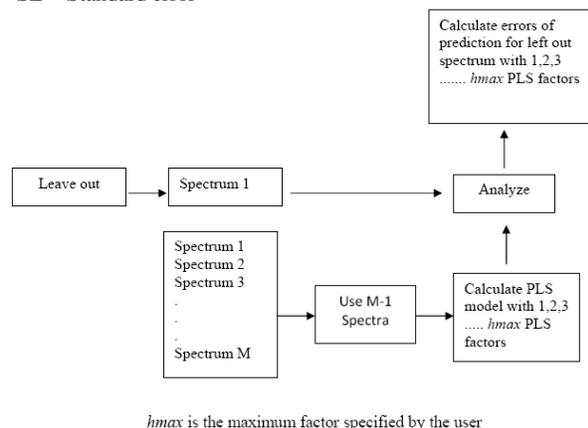
Appropriate frequency range of the spectrum was selected to get good correlation between the changes in spectral and the concentration data.

### Validation of the calibration model

The suitability of the chosen data processing method and the frequency range for method development was evaluated during validation. In the case of cross validation, individual samples were taken from the calibration set. Using the remaining samples, a calibration model was established and used to analyze the previously extracted samples. This procedure of removing samples,

**Table 1:** General statistics for the chemical composition of broiler feed samples

Nutrients	Sample No	Min.-Max.	Mean	SE <sup>1</sup>
			%	
Moisture	543	10.90-13.11	12.23	0.023
CP	352	13.12-22.96	17.96	0.090
CF	215	1.78-7.20	4.05	0.065
EE	374	3.09-11.35	7.02	0.094
Ca	230	0.63-1.27	0.94	0.007
P	241	0.38-0.83	0.66	0.006

<sup>1</sup>SE = Standard error**Fig. 2:** Steps in cross validation

analyzing them, and returning them to the calibration data set was continued successively until all the samples had been analyzed once (Figure 2).

A comparison of the resulting analysis values with the original raw data allowed the calculation of the predictive error of the complete data system, the root mean square error cross validation (*RMSECV*):

$$RMSECV = \sqrt{\frac{1}{M} \sum_{i=1}^M (Differ_i)^2}$$

Besides, coefficient of determination ( $r^2$ ) from the linear regression of measured values of nutrient component determined by analytical laboratory versus predicted values by the NIR calibration was calculated to give the accuracy of the model. During the validation, potential outliers could be detected easily and only after all outliers had been removed from the calibration data set, and finally after the optimum parameters had been found, the calibration model was established.

## RESULTS AND DISCUSSION

The overall range of moisture, CP, CF, EE, Ca and P contents in broiler feeds were 10.90 to 13.11, 13.12 to 22.96, 1.78 to 7.20, 3.09 to 11.35, 0.63 to 1.27 and 0.38 to 0.83 with standard error (SE) of 0.023, 0.090, 0.065, 0.094, 0.007 and 0.006 respectively (Table 1). The number of samples in developing calibration equations for the prediction of CF (n=215), Ca (n=230) and P (n=241) were relatively lower than the number of samples used in developing equations for predicting moisture (n=543), CP (n=352) and EE (n=374). The SE of CP, CF and EE contents in broiler feeds were slightly higher than that of moisture, Ca and P contents which probably due to the higher variations of CP, CF and EE contents in broiler feeds.

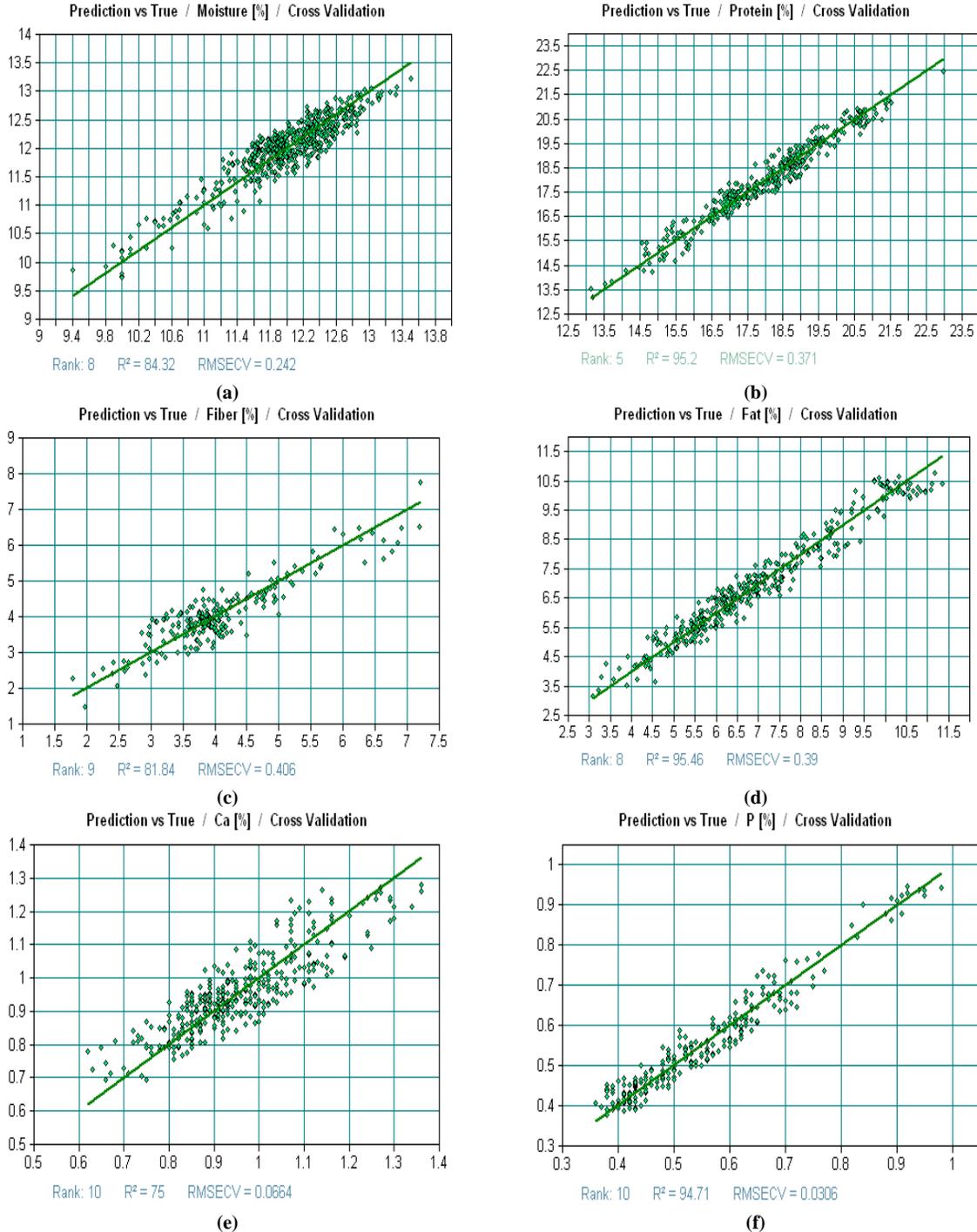
The standard error of estimation (RMSEE) in developing calibration equations by using NIRS for the determination of moisture, CP, CF, EE, Ca and P of broiler feeds were 0.230, 0.351, 0.361, 0.350, 0.056 and 0.021% respectively with correlation coefficient ( $r^2$ ) of 86.09, 95.77, 86.28, 96.28, 80.50 and 95.80 (Table 2). After cross validation, the standard error (RMSECV) for the prediction of moisture, CP, CF, EE, Ca and P in broiler feeds were 0.242, 0.371, 0.406, 0.390, 0.066 and 0.030% respectively. Besides, the correlation coefficients ( $r^2$ ) between measured values of nutrient component determined by analytical laboratory versus predicted values determined by the NIR calibrations for the determination of moisture, CP, CF, EE, Ca and P in broiler feeds were 84.32, 95.20, 81.84, 95.46, 75.00 and 94.71 respectively (Table 2 and Figure 3). The standard error in calibration (RMSEE) and after cross validation (RMSECV) for the prediction of CP, CF and EE contents in broiler feeds were slightly higher than that of calibrations for the prediction of moisture, Ca and P in broiler feeds which could be due to higher variations observed in laboratory determinations of CP, CF and EE contents in boiler feeds (Table 1). Holeček *et al.* (1982) reported that the high errors of analysis could be due to differences in botanical composition, maturity of grains, method of collection and preparation of samples as well as host environmental interactions. However, the correlation coefficient ( $r^2$ ) between measured CP, CF and EE contents of broiler feeds in the laboratory and predicted nutrient contents by NIR calibration equations were 95.77, 86.28 and 96.28 were satisfactory. Similarly, after cross validation, the correlation between laboratory values and NIR predicted values of CP, CF and EE in broiler feeds were satisfactory also (Figure 3b, 3c and 3d). Melchinger *et al.* (1986) found the SE of prediction and correlation coefficient ( $r^2$ ) for the prediction of CP in maize grains to be 0.29% and 96.00 respectively. Therefore, the model developed for CP, and EE determination in broiler feed samples in the present experiment appears to be sufficiently accurate and latter for quality control applications. Besides, the correlation coefficient ( $r^2$ ) for the prediction of CF contents in broiler feeds could be improved by the addition of large number of samples in calibration set.

In predicting moisture, Ca and P contents in broiler feeds, the RMSEE were 0.023, 0.056 and 0.021% respectively and after cross validation the RMSECV were 0.242, 0.066 and 0.031% respectively. The SE for the prediction of moisture, Ca and P by using NIRS were almost similar nature to the SE of laboratory determination of moisture, Ca and P in broiler feeds. According to the procedure of Conzen (2003), multivariate calibration was developed and in the present experiment the commercial software analysis program OPUS (Optical User Software) had the opportunity to use all possible mathematical models i. e. first derivative, second derivative, vector normalization etc. to develop calibration equations with least errors. In addition, the correlation coefficient ( $r^2$ ) between measured moisture and Ca contents of broiler feeds in the laboratory and predicted moisture and Ca contents by NIRS after cross validation were 84.32 and 94.71 respectively indicate the accuracy of the model (Figure 3a and 3e). However, the

**Table 2:** NIR statistics for the prediction of moisture, CP, CF, EE, Ca and P contents in broiler feeds

Nutrients	Sample No	Cross validation statistics			Calibration statistics		
		Rank	RMSECV <sup>1</sup>	r <sup>2</sup>	Rank	RMSEE <sup>2</sup>	r <sup>2</sup>
Moisture	543	08	0.242	84.32	08	0.230	86.09
CP	352	05	0.371	95.20	05	0.351	95.77
CF	215	09	0.406	81.84	09	0.361	86.28
EE	374	08	0.390	95.46	08	0.350	96.28
Ca	230	10	0.066	75.00	10	0.056	80.50
P	241	10	0.031	94.71	10	0.021	95.80

<sup>1</sup>RMSECV = Root Mean Square Error Cross Validation; <sup>2</sup>RMSEE = Root Mean Square Error of Estimation; r<sup>2</sup> = correlation coefficient



**Fig. 3:** Prediction (NIR) vs true (laboratory), RMSECV, PLS factors (Rank) and correlation coefficient (r) for the prediction of (a) moisture, (b) CP, (c) CF, (d) EE, (e) Ca and (f) P determination in broiler feed samples.

correlation coefficient ( $r^2$ ) of P between laboratory and NIRS determination was 75.00 (Figure 3f) which was relatively lower after cross validation. In PLS regression, if too many factors (rank) are chosen (10 for Ca) the model tries to account even the smaller changes in data set which creates spectral noise ("overfitting") and leads to decrease correlation coefficient after validation (Conzen, 2003). However, this could be increased by incorporation large number of homogeneous samples in calibration set.

### Conclusions

In the present experiment, the samples were collected from different feed mills and locations having variations in nutrient contents and thus the improvement of calibrations has not been as great as that observed with homogeneous calibrations. The correlation coefficient ( $r^2$ ) for the prediction of CF and Ca contents in broiler feeds could be improved by the addition of large number of homogeneous samples in calibration sets. However, from the above discussion it can be said that it is obligatory to predict animal feeds for quick manufacturing process. NIRS should be preferred as the most convenient tool in this regard, because this non-destructive analytical method needs no chemical reagents and hence it is environmentally sound enough as it does not have any organic or chemical waste.

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