Prediction of Crude Protein, Extractable Fat, Calcium and Phosphorus Contents of Broiler Chicken Carcasses Using Near-infrared Reflectance Spectroscopy

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ABSTRACT : Near-infrared reflectance spectroscopic (NIRS) calibrations were developed for accurate and fast prediction of whole broiler chicken carcass composition. The Feed and Forage Foss systems Model 5000 Reflectance Transport Model 5000 with near-infrared reflectance spectroscopy (NIRS)-WinISI II windows software was used for this purpose. One equation was developed for the prediction of each carcass component. One hundred and fifty freeze dried broiler whole carcass samples were ground in a Cyclotech 1,093 sample mill and analyzed for dry matter, protein, fat, calcium and phosphate. Samples were divided into two sets: a calibration set from which equations were derived and a prediction set used to validate these equations. The chemical analysis values (mean±SD) were calculated based on dry matter basis as follows: dry matter: 33.41±2.78 (range: 26.41-43.47), protein: 54.04±6.63 (range: 36.20-76.09), fat 35.44±8.34 (range: 7.50-55.03), calcium 2.55±0.65 (range: 0.99-4.41), phosphorus 1.38±0.26 (range: 0.60-2.28). One hundred and three samples were used to calibrate the equations and prediction values. The software used was modified to obtain partial least square regression statistics, as it is the most suitable for natural products analysis. The coefficients of determination (R^2) and the standard errors of prediction were 0.82 and 1.83 for the dry matter, 0.96 and 1.98 for protein, 0.99 and 1.07 for fat, 0.90 and 0.30 for calcium and 0.91 and 0.11 for phosphorus, respectively. The present study indicated that NIRS can be calibrated to predict the whole broiler carcass chemical composition, including minerals in a rapid, accurate, and cost effective manner. It neither requires skilled operators nor generates hazardous waste. These findings may have practical importance to improve instrumental procedures for quick evaluation of broiler carcass composition. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 7 : 1036-1040)***

Key Words : Near-infrared Reflectance Spectroscopy, Carcass Composition, Broilers

INTRODUCTION

Evaluation of chemical parameters such as fat, protein, calcium and phosphorus of poultry meat are essential to determine its nutritive values. The poultry industry has become more aware of the problem created by carcass components such as excessive fat, that increases pressures to meet consumer demands. To respond to these pressures, it is essential to collect as much information as possible on raw materials using analytical laboratory techniques. Traditionally, the AOAC methods have been the most used chemical reference for this purpose. However, it is laborious, time consuming, generates toxic hazardous waste and is not well suited for routine large-scale industry use.

Near-infrared reflectance spectroscopy (NIRS) has been widely used as a rapid and accurate method for measuring some constituents of materials without requiring extensive sample preparation (Norris et al., 1976). Components measured include protein, moisture, fibre, starch, individual sugars, amino acids, and fats (Renden et al., 1986; Valdes and Summers, 1986; Foley et al., 1998; Smith et al., 2001; Fontaine et al., 2002). The principle of NIRS is based on the use of selective absorption of electromagnetic radiation from 800 to 2,500 nm in accordance with the characteristic vibration frequencies of functional groups (De Boever et al., 1994). It has the capability to measure CH, NH, OH bonds, which form the functional groups in moisture, protein, amino acids, fibre, sugar and fat in biological substances. Although, organic complexes may be detected (Shenk et al., 1992), no absorption bands for minerals exist in the near-infrared region. Every biological substance has a unique NIRS composite spectrum, depending on their characteristic functional groups; the NIRS spectrum of a sample is the composition of all the physical and chemical information of the sample (Murray and Williams, 1987; De Boever et al., 1994). Although, NIRS has been widely used to measure components in different products, little information is available on the evaluation of whole broiler carcass composition (Renden et al., 1986; Valdes and Summers, 1986; Cozzolino et al., 1996; Chen and Marks, 1998; Windham et al., 2003). An early attempt was made by Renden et al. (1986) and Valdes and Summers (1986) to predict fat, protein and moisture in poultry meat. Renden et al. (1986) found a high accuracy of prediction of fat and moisture in whole mature dwarf hen carcasses with NIRS, while Valdes and Summers (1986) found a low accuracy of NIRS in predicting protein and fat content of broiler breast muscle. Cozzolino et al. (1996) and Windham et al. (2003) have obtained higher accuracy of prediction with NIRS estimation of broiler breast fat. The current study aimed to calibrate NIRS equations to evaluate its accuracy to predict

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a wide range of chemical composition of whole broiler chicken carcasses.

MATERIALS AND METHODS

Birds

Four hundred and forty, 1-day old male and female chickens, of a commercial Cobb 500 strain were used to evaluate the effect of four levels of organic acid added to water on carcasses and meat quality characteristics. Five birds were assigned randomly to each of 88 suspended wire cages (62×62×37 cm), such that all pens had nearly a similar average initial weight. The 88 pens were randomly assigned to four different treatment groups (0.0, 0.2, 0.4, 0.6 and 0.8% of ascorbic acid added to drinking water), of which each treatment had 22 replicates. Feed and water were available *ad libitum*. The cages were kept in an environmentally controlled shed. Light was provided for 23 h each day.

Sample collection

One hundred and fifty whole carcass samples were randomly collected from 6-week-old whole de-feathered broiler chickens. The frozen carcasses were cut into smaller sections and ground sequentially passing twice through a Hobart meat grinder. A 250 g sub-sample has been randomly collected from different parts of the whole minced carcass. Broiler carcass samples were placed in individual airtight containers at -20°C and then freeze dried at -60°C under 1.2 mm vacuum pressure till they attained a steady dry weight. Moisture was determined by the difference in weight before and after freeze-drying. The samples were ground in a Cyclotech 1093 sample mill with a 0.5-mm screen and were placed in sealed plastic bags until analysed. The grinder was steam cleaned between carcasses.

Chemical analysis

The freeze-dried samples were used for the chemical analysis. Calcium and phosphorus were estimated by ashing of dry samples at 450°C for 12 h in a muffle furnace. Ether extract was estimated using petroleum ether in a Soxhlet apparatus. Nitrogen was determined by Kjeldahl method. Calcium in the ash was measured with an atomic absorption spectrophotometer (PHILIPS model PU9100, single beam) according the procedures of AOAC (1990). Phosphorus was measured by spectrophotometeric Molybdovanadphosphate method (AOAC, 2000). Analysis for all items was done in duplicates on dry matter basis.

Near-infrared reflectance spectroscopy analysis

Samples were divided into calibration and prediction sets, which consisted of 150 and 103, respectively. Prediction samples were randomly selected from the calibration samples. All the calibration samples were analysed by conventional chemical methods in the laboratory and then scanned in the NIRS for comparison.

Feed and Forage Foss NIRS systems Model 5000 Reflectance Transport Model Analyzer was used for scanning samples and collecting spectra. The software used was WinISI II version 1.50. Interpretation of NIRS spectra was done by calibrating with reference values obtained from laboratory analysis of the sample and correlating them to NIRS measurements of these samples. The reference laboratory data and the sample spectra were used to develop predictive equations. Samples were uniformly mixed and loaded in the NIRS sample cups. About 5-6 g of ground sample were placed in sample cup to about 8 mm depth then covered to be processed on the holder gently. Consistency in sample preparation and packing is important for accuracy. The NIR spectrum for each sample was recorded as log 1/R (reflectance) for each wavelength in the NIRS range.

Calibration

The mathematical relationship between chemical reference values and the NIRS spectral data was analysed using regression equation. Calibrations were made using reference values on a dry matter basis, so the NIRS predicted results are also expressed on a dry matter basis. Spectra of 150 broiler carcass samples were collected in the NIRS region (800-2,800 nm). Calibration equations were computed for dry matter, protein, fat, calcium, and phosphorus using modified partial least squares regression using the WinISI II software. The program calculates the mathematical relationship between the spectral data from the scanned sample and its reference values obtained by standard chemical procedures. The program calculates the cross validation errors for dry matter, protein, fat, calcium, and phosphorus, and then calculates the modified partial least square means for each of the component. Upon completion of the calibrations for components, the NIRS equation was developed to predict the constituents in the product.

Statistically, the equation was evaluated using the monitor results program, which performs statistical comparison between chemical reference values and NIRS predicted values for the data set. It also provided listing and graphical comparison of the two values. Model performance was reported as the coefficient of determination (R^2), standard error of prediction (SEP), and linear regression of components reference method against predicted values (slope) (Hurschka, 1987).

RESULTS AND DISCUSSION

The descriptive statistics for the chemical composition of broiler carcasses are summarized in Table 1. There was a

Table 1. Values for chemical analysis (on dry matter basis) in whole broiler carcass samples used for near-infrared reflectance equation and validation

Variable	Mean	SD	Range	SEC	SECV	R^2	1-VR
Dry matter (%)	33.94	2.78	26.41-43.47	0.76	0.87	0.93	0.91
Crude protein (%)	54.04	6.63	36.20-76.09	0.96	1.14	0.98	0.97
Fat (%)	35.44	8.34	7.50-55.03	0.62	0.73	0.99	0.99
Calcium (%)	2.55	0.65	0.99-4.41	0.21	0.23	0.90	0.88
Phosphorus (%)	1.38	0.26	0.60-2.28	0.11	0.12	0.80	0.76

SD: Standard deviation, SEC: Standard error of calibration, SECV: Standard error of cross validation.
R^2: Coefficient of determination, 1-VR: 1- variance ratio.

Table 2. Near-infrared reflectance calibration and validation statistics for whole broiler carcass samples (on dry matter basis)

Variable	Mean	SD	Range	SEP	Bias	R^2	Slope
Dry matter (%)	34.06	3.33	25.25-41.87	1.83	0.12	0.82	0.81
Crude protein (%)	53.92	8.24	35.50-78.42	1.98	0.55	0.96	0.87
Fat (%)	35.94	9.50	5.82-54.52	1.07	0.18	0.99	0.98
Calcium (%)	2.66	0.65	0.77-4.21	0.30	0.03	0.90	0.95
Phosphorus (%)	1.40	0.27	0.54-1.98	0.11	0.00	0.91	0.91

SD: Standard deviation, SEP: Standard error of prediction, Bias: Average difference between reference and NIRS values, R^2: Coefficient of determination, Slope: Slope of reference vs. NIRS.

considerable variation in sample composition as shown by the wide range of values. The widest range and variation was in fat. It is essential for any calibration procedure to ensure that a range of spectral variation in the whole population is represented in the samples selected for calibration development (Foley et al., 1998). Ranges of fat and protein percentage in the present study were wider than those reported by (Valdes and Summers, 1986; Min and Lee, 2004) and this may be due to treatment effect. The results in the present study (data are not presented) showed that different levels of organic acid had a significant effect on fat content of broiler chicken carcasses. However, the use of a wide variation in carcass composition is a valuable factor to evaluate the accuracy of the NIR procedure.

Calibration and validation statistics are shown in Table 1 and 2, respectively. The range of protein and fat in the prediction set are somewhat broader than the corresponding ranges in the calibration set. Also, the minimum and maximum values in the calibration set are at least as extreme as the corresponding levels in the prediction set. Calibration from this data set should be valid for samples within the ranges of approximately 28-44% dry matter, 36-76% protein, 8-55% fat, 1-4% calcium, 1-2% phosphorus.

There is little available information on the NIRS calibration to predict calcium and phosphorus in poultry meat. Abeni and Bergoglio (2001) concluded that ash content in breast meat of broiler chicken was unpredictable by NIRS technique. However, there have been some other studies conducted utilizing NIRS to analyse the calcium and phosphorus content of poultry meat and excreta. Smith et al. (2001) attempted to calibrate NIRS to predict the calcium and phosphorus content of chick excreta. They obtained a R^2 of 0.86 and a SEP of 0.14 for calcium and a R^2 of 0.93 and a SEP of 0.13 for phosphorus. They concluded that NIRS is a good predictor for calcium and total phosphorus content of chick excreta. There are no absorption bands for minerals in the near-infrared region, but organic complexes and chelates may be detected (De Boever et al., 1994). Mineral analysis studies have indicated that calcium and phosphorus may exist in forms detectable by NIRS at least in some grasses and legumes (Clark et al., 1987; Saiga et al., 1989). The calibration values obtained for phosphorus in forages were acceptable by Clark et al. (1987) but were not successful in a similar study by Vasquez Aldana et al. (1995). De Boever et al. (1994) reported that phosphorus of feedstuffs could be predicted by NIRS with a R^2 of 0.94 to 0.96 and a SEP of 0.08. In grass samples, Dealdana et al. (1995) found that calcium could be predicted with a R^2 0.73 to 0.92 and a SEP 0.15 to 0.22. The estimation of mineral elements by NIRS is generally dependent on the occurrence of those elements in organic or hydrated molecules (Clark et al., 1987b; Vasquez de Aldana et al., 1995), or mineral levels may simply be correlated to some organic material that the NIRS can easily measure, or may be due to differences in bone content, which would affect particle size and NIRS light scattering. The results of the present study confirmed that NIRS is a good predictor for calcium and phosphorus with a R^2 of 0.90 and 0.91, respectively.

There have been some studies utilizating NIRS to analyse the fat and protein contents in carcass, breast or leg muscles in poultry (Renden et al., 1986; Valdes and Summers, 1986; Cozzolino et al., 1996; Chen and Marks, 1998; Abeni et al., 2001; Windham et al., 2003). Renden et al. (1986) concluded that NIRS was a rapid and effective method for determining carcass fat (R^2 = 0.96) and moisture (R^2 = 0.95) percentages in whole mature dwarf hens. Valdes and Summers (1986) reported an R^2 of 0.98 and 0.91 for the prediction of crude protein and fat in broiler carcass muscle using NIRS. They concluded that NIRS was equally accurate to predict broiler carcass composition as other laboratory procedures. Similarly, high correlation of determination and relatively low SEP were obtained in the

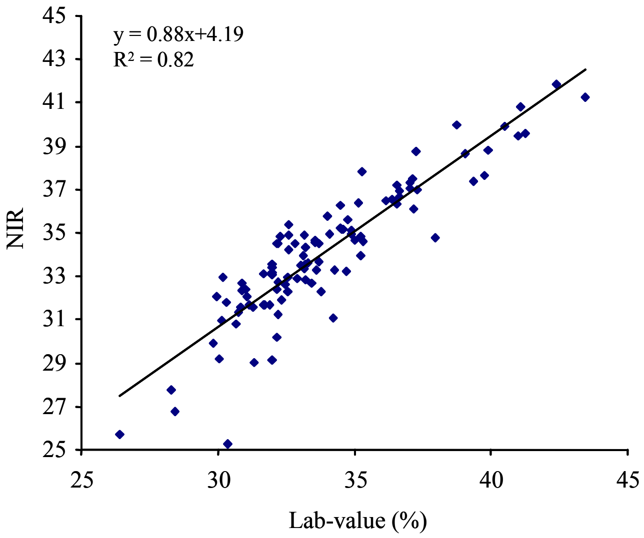

Figure 1. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined dry matter values (on dry matter basis) of 150 whole broiler carcasses.

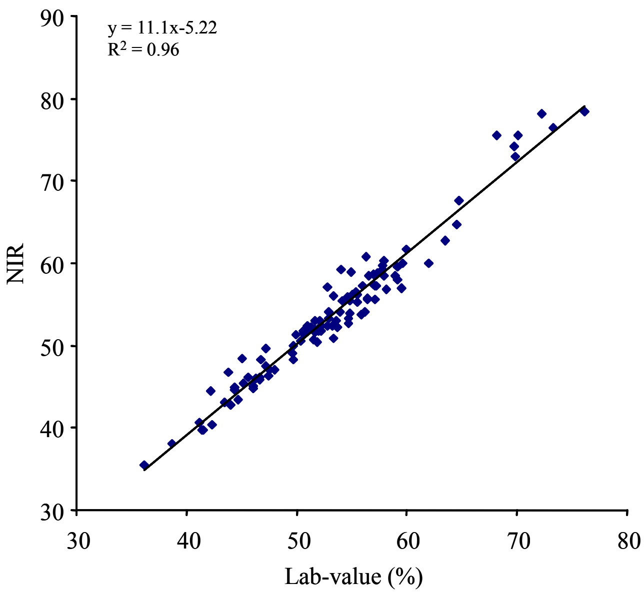

Figure 2. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined protein values (on dry matter basis) of 150 whole broiler carcasses.

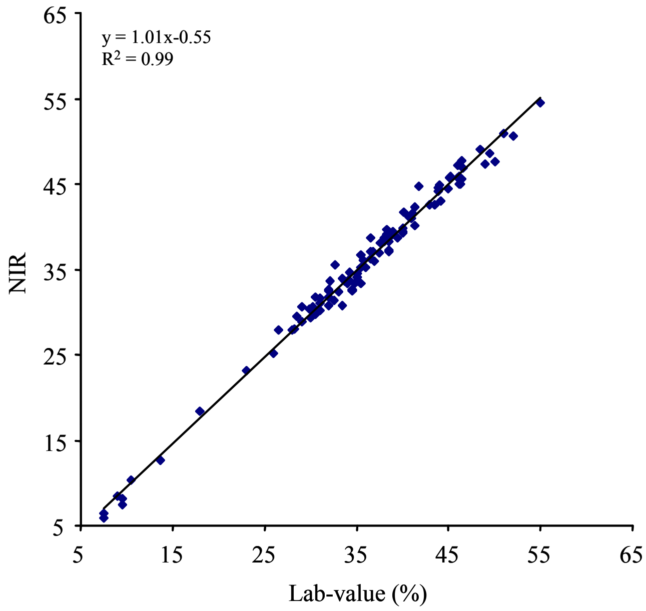

Figure 3. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined fat values (on dry matter basis) of 150 whole broiler carcasses.

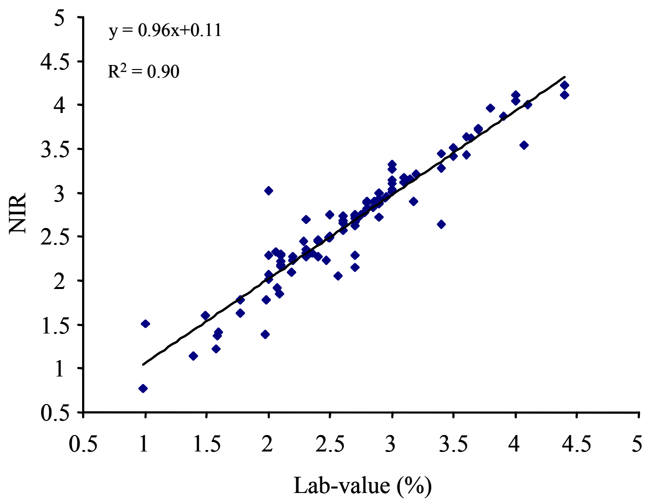

Figure 4. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined calcium values (on dry matter basis) of 150 whole broiler carcasses.

present study for dry matter (R^2 = 0.82, SEP = 1.83) (Figure 1), crude protein (R^2 = 0.96, SEP = 1.98) (Figure 2), fat (R^2 = 0.99, SEP = 1.07) (Figure 3), calcium (R^2 = 0.90, SEP = 0.30) (Figure 4) and phosphorus (R^2 = 91, SEP = 0.11) (Figure 5). In the present study, the result obtained by NIRS analysis was for whole broiler carcass fat content, which was higher than those reported by Valdes and Summers (1986) but similar to those reported by Renden et al. (1986). Windham et al. (2003) reported an SEP of 0.32 and R^2 of 0.98 using NIRS to predict fat content of boned poultry breast muscle, trimmings and finished product samples. Similar values were found in the present study.

Comparing to referenced laboratory methods, the NIRS uses less time and cost of chemicals for determination of dry matter, protein, fat, calcium and total phosphorus. Approximately 24-36 h are needed to determine the dry matter, 4 h for protein, 8 h for ether extract, about 6 h for ash and another 2 h to determine Ca and P. Traditional analyses methods also require various reagents and specialized equipments as well as well-trained technicians. Ten broiler carcass samples can be scanned by NIRS system to determine similar analysis within one h, therefore, more samples can be run in less time for several constituents utilizing NIRS to obtain an acceptable estimate of the mean.

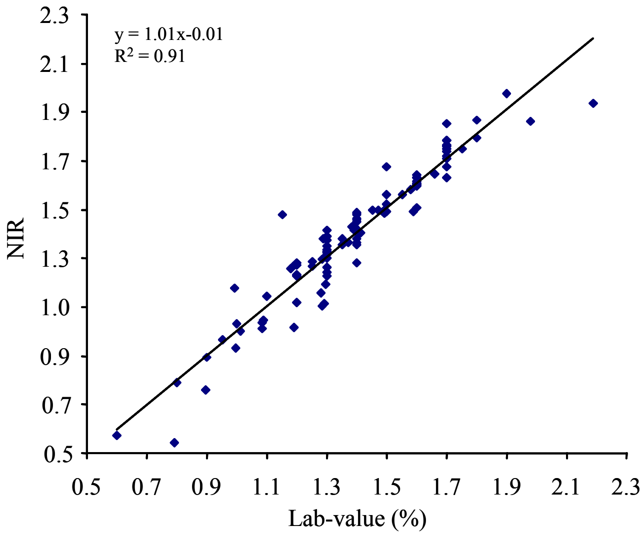

Figure 5. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined phosphors values (on dry matter basis) of 150 whole broiler carcasses.

Conclusion

The results of this study demonstrate that NIRS is an effective alternative to standard chemical analysis of whole carcass dry matter, protein, fat, calcium and phosphors contents in broiler chickens, With the inclusion of more samples in the calibration set that covering a broader range of constituents and with some refinement in the sampling technique, it seems likely possible to develop a robust calibration. There are many benefits using NIRS for this purpose. It uses no consumables as well as it is environment friendly. In addition the use of NIRS involve no extensive sample preparation (only drying and grinding to uniform size), it is a cost effective and reproducible analytical procedure. Therefore, NIRS may be used to replace wet chemistry in quantifying many compositional parameters of poultry carcass samples to a high accurate level, once the initial calibration and equation model for the sample has been established.

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