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Prediction of Composition of Broiler Chicken Excreta Using Near-infrared Reflectance Spectroscopy

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التنبؤ بالمكونات الكيميائية لفضلات الدواجن بأستخدام جهاز معامل الانعكاس بالاشعة تحت الحمراء

عصام توفيق كاظم

الخلاصة: تم تطوير معايير جهاز معامل الانعكاس بالأشعة تحت الحمراء باستخدام المطياف للتنبؤ بمحتوى المادة الجافة والنيتروجين والدهون والألياف الذائبة في القواعد والحوامض والطاقة الكلية والكالسيوم والفوسفات في عينات من فضلات الدجاج اللاحم. ثم تحليل التكوين الكيمائي لبراز الدجاج اللاحم بطرق التحليل الكيميائية التقليدية بالمختبر ومقارنتها بنتائج جهاز المطياف تم تجفيف اثنان وسبعون عينة من برا فضلات الدجاج اللاحم بدرجة حرارة 60 درجة مئوية تم تحليلها لتحديد مستوى المادة الجافة والنيتروجين والدهون والألياف الذائبة في القواعد والحوامض والطاقة الكلية والكالسيوم والفوسفات وكانت قراءات المتوسط العام ومعامل الإنحراف القياسي كالآتي :للمادة الجافة 7.65±31.46 (المدى: 19.14-44.51) والناتروجين 5.85±2.88)تامدى 4.85-7.00)والدهون 1.37±0.25 (المدى: 0.88-1.99)(والالياف الذائبة في المحاليل الحامضية :16.7±1.99 (المدى: 12.11-19.97) و الالياف الذائبة في المحاليل القفاعدية 26.26±1.63 (المدى: 22.03±30.21) والطاقة الكلية: 15.27±0.99 (المدى: 14.52-16.11) والكالسيوم: 2.57±0.22 (المدى: 2.16-3.01) و الفوسفات: 1.79±0.15 (المدى: 1.41-2.11). ثم فحصت العينات باستخدام المطياف موديل 5000 وحدد الخط الطيفي لكل عينة. طورت معادلات المعايرة وقيم التنبؤ لعينات براز الدجاج اللاحم كما تم تطوير أنظمة البرنامج لحساب التراجع التربيعي الاقل التي تعتبر اكثر ملائمة للمنتجات الطبيعة. وكانت معامل التقدير(R^2) والخطأ المعياري المتوقع لعينات من فضلات دجاج الاحم للمادة الجافة 0.97 و 1.27وللناتروجين 0.95 و 0.72 وللدهون 0.92 و 0.07 و للاليف الذائبة في الحوامض 0.87 و 0.78 و للالياف الذائبة في القواعد 0.88 و 0.72 و للطاقة الكلية 0.89 و 0.24 و للكاسيوم 0.96 و 0.06 و للفسفور 0.93 و 0.09 باتتابع. أشارت نتائج هذا البحث إلى امكانية استخدام معيار المطياف للتنبؤ بمكونات فضلات الدجاج اللاحم.

ABSTRACT: Near-infrared reflectance spectroscopic (NIRS) calibrations were developed for the prediction of the content of dry matter (DM); nitrogen (N), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), gross energy (GE), calcium (Ca) and phosphate (P) in broiler excreta samples. The chemical composition of broiler excreta was determined by the conventional chemical analysis methods in the laboratory and compared with NIRS. Excreta samples (n = 72) were oven dried (60 °C) and analyzed for DM, N, EE, NDF, ADF, GE, Ca and P. The determined values (mean ± SD) were as follows: DM: 31.46 ± 7.65 (range:19.14 - 44.51), N: 5.85 ± 2.88 (range: 4.85 -7.00), EE: 1.37 ± 0.25 (range: 0.88-1.99), ADF: 16.71 ± 1.99 (range: 12.11-19.97), NDF: 26.26 ± 1.63 (range: 22.03-30.21), GE: 15.27 ± 0.33 (range: 14.52-16.11), Ca: 2.57 ± 0.22 (range: 2.16-3.01), P: 1.79 ± 0.15 (range: 1.41-2.11). The samples were then scanned in a NIRS model 5000 analyzer and the spectra obtained for each sample. Calibration equations and prediction values were developed for broiler excreta samples. The software used modified partial least square regression statistic, as it is most suitable for natural products. For broiler excreta samples, the coefficient of determination (R^2) and the standard error of prediction (SEP) was DM = 0.97, 1.27, N = 0.95, 0.72, EE = 0.92, 0.07, ADF = 0.87, 0.78, NDF = 0.88, 0.72, GE = 0.89; 0.24, Ca = 0.96, 0.06, P = 0.93, 0.09, respectively. The results indicate that it is possible to calibrate NIRS to predict major constituents in broiler excreta samples.

Keywords: Spectroscopy, broiler, excreta, calibration, Oman.

Introduction

Poultry manure, which is a pollutant in soil and water, is produced in large quantities by the poultry industry. An accurate and fast analytical procedure to detect poultry manure components is becoming more critical in areas with intensive poultry production (Reeves, 2001). In some countries, restrictive manure management guidelines have been adopted, which

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need a quick, easy and reliable method for determining excreta composition to assist those abiding by and enforcing regulations. To manage these pressures, it is necessary to collect as much information as possible on raw materials, which depend on analytical laboratory techniques. Traditionally, chemical reference methods are laborious, time consuming and generate hazardous wastes.

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 successfully measured include nitrogen, moisture, fibre, starch, individual sugar, amino acids, and fat (Foley *et al*., 1998; Fontaine *et al*., 2002; Smith *et al*., 2001; Valdes and Summers, 1986), The principle of NIRS is the selective absorption of electromagnetic radiation from 800 to 2500 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 samples. 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 (De Boever *et al*., 1994; Murray and Williams, 1987).

The objectives of the current study were to calibrate NIRS spectrometer equations and to evaluate their accuracy to predict chemical composition, moisture, nitrogen, ether extract, neutral detergent fibre, acid detergent fibre, gross energy, calcium and phosphorus in broiler excreta.

Materials and Methods

Seventy-two broiler excreta samples (500 g) were randomly collected from a broiler feed evaluation project. The excreta samples were dried in a forced air oven at 60 °C until no decrease in weight occurred. All samples were then left on the laboratory bench for over 48 h to equilibrate with atmospheric moisture. The samples were ground in a Cyclotech 1093 sample mill with a 0.5-mm screen and were stored in plastic bags until analysis.

Laboratory analysis

Excreta samples were analysed for dry matter (DM) by drying in an oven at 60 °C until no decrease in weight occurred, and for organic matter (OM) and ash by ashing dry samples at 450 °C for 12 h in a muffle furnace (AOAC, 1990). Triplicate samples of approximately 2 g each were freeze-dried for 4 days for ether extraction (EE) by petroleum ether in a Soxhlet apparatus. Nitrogen (N) was determined by the Kjeldahl method. Neutral detergent fibre (NDF) was determined by using sodium sulphite and sodium lauryl sulphate as a neutral detergent extracting solution as described by Van Soest *et al*. (1991). Acid detergent fibre (ADF) was determined using

Table 1. Values for chemical analysis in 72 broiler excreta samples used for the near-infrared reflectance equation .and validation.

Variable	Mean	SD	Range	SEC	SECV	R^2	1-VR
Dry matter (%)	31.46	7.65	19.14-44.51	1.18	1.27	0.97	0.97
Nitrogen (%)	5.85	2.88	4.85-7.00	0.64	0.72	0.95	0.93
Ether extract (%)	1.38	0.25	0.88-1.99	0.07	0.07	0.92	0.91
Acid detergent fibre (%)	16.72	1.99	12.11-19.97	0.71	0.78	0.87	0.85
Neutral detergent fibre (%)	26.26	1.63	22.03-30.21	0.55	0.72	0.88	0.81
Gross energy (MJ/kg)	15.27	0.33	14.52-16.11	0.22	0.24	0.89	0.47
Calcium (%)	2.57	0.22	2.16-3.01	0.04	0.06	0.96	0.92
Phosphorous (%)	1.79	0.15	1.41-2.11	0.08	0.09	0.93	0.61

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 transmission calibration and validation statistics for broiler excreta.

Variable	Mean	SD	Range	SEP	Bias	R^2	Slope
Dry matter (%)	31.20	7.44	20.93-42.81	1.56	0.188	0.96	1.01
Nitrogen (%)	5.97	2.59	5.22-6.80	0.77	0.125	0.93	1.04
Ether extract (%)	1.38	0.27	0.86-1.91	0.10	0	0.88	1.01
Acid detergent fibre (%)	17.20	1.41	14.06-19.32	1.07	0.053	0.65	1.05
Neutral detergent fibre (%)	26.50	1.47	22.37-29.51	0.83	0.048	0.73	0.94
Gross energy (MJ/kg)	15.20	0.22	14.77-15.63	0.31	0.043	0.85	0.99
Calcium (%)	2.57	0.19	2.22-2.97	0.07	0.004	0.89	0.97
Phosphorous (%)	1.79	0.11	1.52-2.01	0.11	0.023	0.91	0.82

SEP: Standard Error of Prediction.

cetyl trimethyl ammonium bromide and 1N H_2SO_4 as described by Roberston and Van Soest (1981). Calcium (Ca) and phosphorus (P) were measured with an atomic absorption spectrophotometer (PHILIPS model PU9100, single beam) according the procedures of AOAC (1990). Gross energy (GE) was measured using a bomb calorimeter. Analysis for all items was done in triplicate on a dry matter basis.

Near-infrared reflectance spectroscopy analysis

Samples were divided into calibration and prediction sets, which consisted of 72 and 40 samples, respectively. Prediction samples were randomly selected from the calibration samples. All calibration samples were analysed by conventional chemical methods in the laboratory and then scanned in the NIRS for comparison.

A 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 by linear regression. 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 72 broiler excreta samples were collected in the NIRS region (800-2800 nm). Calibration equations were computed for DM, N, EE, ADF, NDF, GE, Ca and P 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 then calculates the cross validation errors for each component, and the modified partial least square statistics was determined. The program repeats this process for each component and upon completion, the NIRS equation was developed to predict the constituents in the product.

Statistically, the equation was evaluated using the monitor results program, which performs a statistical comparison between chemical reference values and NIRS predicted values for the data set. It also provided tabular 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 different constituents of broiler excreta are summarized in Tables 1 and 2. There was a considerable variation in the composition,

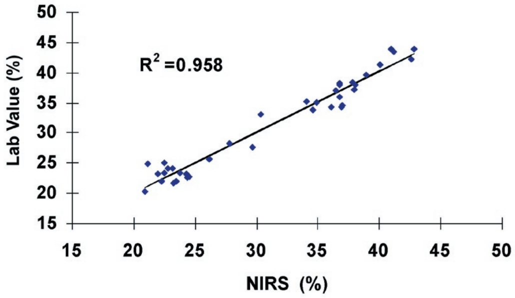

Figure 1. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined percentage dry matter values of 72 broiler excreta samples.

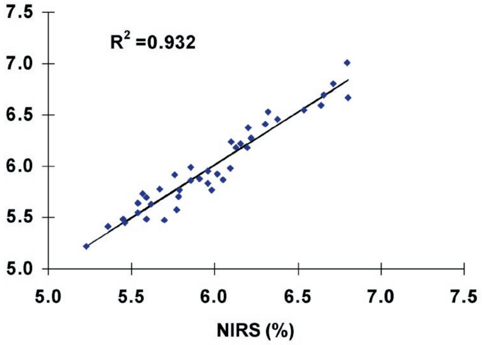

Figure 2. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined nitrogen values (%) of 72 broiler excreta samples.

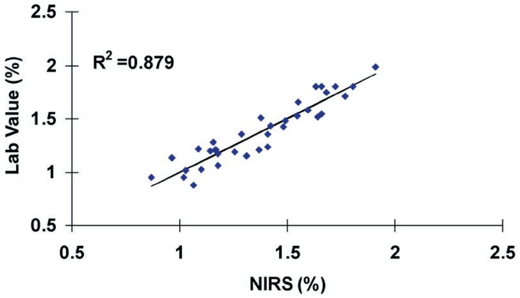

Figure 3. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined fat values (%) of 72 broiler excreta samples.

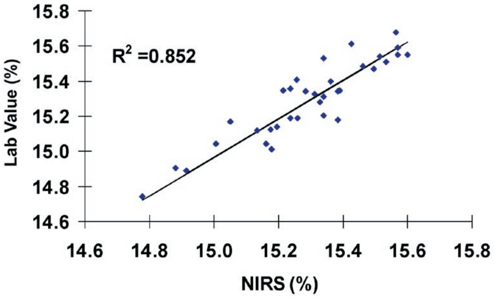

Figure 4. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined gross energy values (%) of 72 broiler excreta samples.

as shown by the wide range of values. It is essential for any calibration procedure to ensure that a range of spectral variation found in the whole population is represented in the samples selected for calibration development (Foley *et al*., 1998). Calibration and validation statistics are shown in Tables 1 and 2, respectively. The range of excreta parameters of DM, N, EE, NDF, ADF, GE, Ca and P in the calibration set are similar to the corresponding ranges in the prediction set.

Limited studies have been conducted utilizing NIRS to analyse the chemical composition of poultry manure or excreta (Reeves, 2001; Smith *et al*., 2001). Contradictory conclusions have been reported by these authors on the suitability of the NIRS to estimate the mineral contents. The findings of Reeves (2001) indicated that NIRS was not suitable for the determination of minerals (P, Ca, etc.) in poultry manure. This may be attributed to the nature of the samples being used. However, Smith *et al*. (2001) attempted to calibrate an NIRS to predict the Ca and P contents of chicken excreta. They obtained an R^2 of 0.86, an SEP of 0.14 for Ca and R^2 0.93 and an SEP of 0.13 for P. They concluded that NIRS was a good predictor for Ca and total P content of chick excreta. There are no absorption bands for minerals in the near-infrared region, but organic complexes and chelates may still be detected (De Boever *et al*., 1994). Studies have shown that Ca and P 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 P in forages were

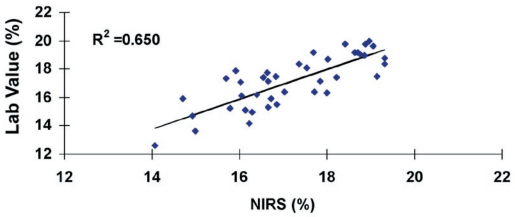

Figure 5. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined acid detergent fibre values (%) of 72 broiler excreta samples.

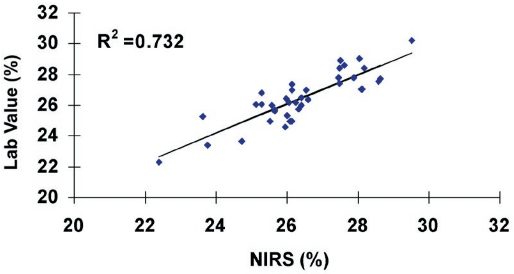

Figure 6. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined neutral detergent fibre values (%) of 72 broiler excreta samples.

found to be acceptable by Clark *et al*. (1987) but were not acceptable in similar studies by Vasquez Aldana *et al*. (1995). De Boever *et al*. (1994) reported that P of feedstuffs could be predicted by NIRS with an R^2 of 0.94 to 0.96 and SEP of 0.08. In grass samples, Dealdana *et al*. (1995) found that Ca could be predicted with an R^2 0.73 to 0.92 and an SEP 0.15 to 0.22. The estimation of mineral elements by NIRS is generally dependent on the occurrence of these elements in organic or hydrated molecules (Clark *et al*., 1987; Vasquez de Aldana *et al*., 1995), or mineral levels may simply be correlated to some organic material that the NIRS can easily measure. The findings of the present study confirm previous reports that NIRS is a good predictor for Ca and P with an R^2 of 0.89 and 0.91, respectively.

Similarly, high coefficients of determination and relatively low SEP were obtained in the present study for DM (R^2, 0.96, SEP, 1.56) (Figure 1), N (R^2, 0.93, SEP, 0.77) (Figure 2), fat (R^2, 0.88, SEP, 0.10) (Figure 3), GE (R^2, 0.85, SEP, 0.31) (Figure 4), ADF (R^2, 0.65, SEP, 1.07) (Figure 5), NDF (R^2, 0.73, SEP, 0.83) (Figure 6), Ca (R^2, 0.89, SEP, 0.07) (Figure 7) and P (R^2, 0.91, SEP, 0.11) (Figure 8).

There have been some studies utilizing NIRS to analyse the moisture and N content of excreta samples. Smith *et al*. (1999) reported an R^2 of 0.97 with an SEP of 0.91 for determining the N content of rooster excreta and an R^2 of 0.92 with an SEP of 0.13 for GE determination. Smith *et al*. (2001) reported an R^2 of 0.93 with an SEP of 0.34 for moisture and an R^2

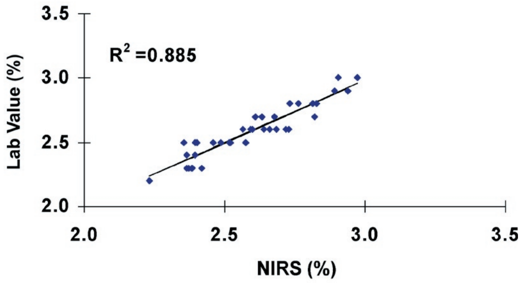

Figure 7. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined calcium values (%) of 72 broiler excreta samples.

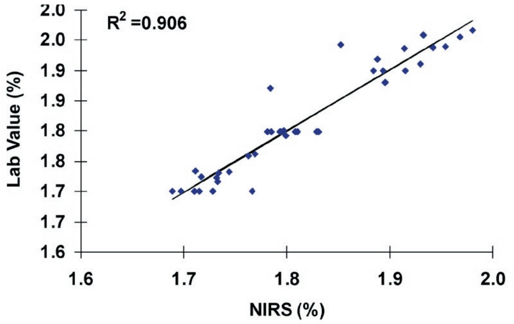

Figure 8. The relationship between near-infrared reflectance spectroscopy (NIRS) predictions and determined phosphorous values (%) of 72 broiler excreta samples.

of 0.99 with an SEP of 0.19 for N contents of chicken excreta, thus supporting the findings of the present study.

Conclusions

The current study has indicated that determination of the composition of broiler excreta by NIRS is feasible with high accuracy. Therefore, NIRS may be routinely used to predict excreta constituents. It may also be useful in monitoring environmental contamination by the poultry industry. With the inclusion of more samples in the calibration set to cover a broader range of constituents as well as some more refinement in the sampling technique, it would likely seem that more robust calibrations could be developed.

NIRS requires no consumables, it is environment friendly, has no operational cost, needs no sample preparation (only drying and grinding to uniform size) and is cost effective and reproducible. Once the initial calibration and equation model for the excreta sample has been established, NIRS may be used to replace wet chemistry in quantifying many compositional parameters of poultry excreta samples to an acceptable level of accuracy,.

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