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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Measurement of Lipid Supplements in Poultry Feed by Infrared Spectroscopy

Ronald A. Holser and Samantha A. Hawkins

Quality and Safety Assessment Research Unit, United States Department of Agriculture,  
950 College Station Road, Athens, GA 30605, USA

**Abstract:** Rapid measurement of a fatty acid supplement in poultry feed formulations was performed using near Infrared (NIR) spectroscopy with chemometric analysis. A standard feed formulation was amended with up to 10 wt% fatty acid supplement containing Docosahexaenoic Acid (DHA) and scanned from 10,000  $\text{cm}^{-1}$  to 4000  $\text{cm}^{-1}$ . Spectra were evaluated by Principal Component Analysis (PCA) to detect outliers followed by Partial Least Square (PLS) regression. Models were developed with first derivative spectra. A typical dataset of 36 spectra was subdivided with 30 spectra used for model calibration and 6 spectra for the validation set. The PLS model produced a regression coefficient of 0.98314 with an RMSEC value of 0.44772. This technique provided a rapid method to analyze the amount of fatty acids in supplemented feeds.

**Key words:** Chemometrics, poultry, rations, spectroscopy

### INTRODUCTION

Infrared spectroscopy offers particular advantages for applications where rapid non-destructive measurements of product compositions are needed. Near Infrared (NIR) technology is commonly used in the food industry to monitor products and is readily adapted for on-line instrumentation (Sato, 2002; Cozzolino *et al.*, 2008). NIR spectra records combinations and overtones of the fundamental vibrations. In practice, NIR methods are used to quickly determine the moisture, fat and protein content in food and feed samples but can also provide more basic information relevant to chemical structure. NIR spectra show strong absorbance peaks for C-H, O-H and N-H bonds which are characteristic of lipids, water and protein (Pazdernik *et al.*, 1997; Fox and Cruickshank, 2005; Tillman *et al.*, 2006). NIR is routinely used to determine these components in oil seed crops and the corresponding spectral regions have been identified (Panford and DeMan, 1990; Bhatt, 1991; Velasco and Becker, 1998). Instrumentation for infrared reflectance measurements is widely available and the sample preparation for this technique is minimal.

Collected spectra are typically analyzed with multivariate statistical methods to construct predictive models that describe the components in a formulated product. Model equations are developed and calibrated using samples of known composition with techniques such as partial least squares or principal component analysis. A validated model is then used for routine analysis. This study investigated the use of NIR spectroscopy to analyze poultry feed formulations supplemented with the highly unsaturated lipid compound docosahexaenoic acid ethyl ester (DHA). This omega-3 fatty acid has attracted interest due to reported benefits for cardiovascular and nervous system health (Harris, 1989; Leaf, 1990; Arterburn *et al.*, 2006).

### MATERIALS AND METHODS

Commercial poultry starter feed was obtained from Pilgrim's Pride Corporation (Athens, GA, USA). A lipid feed supplement, DHA Gold, containing 40% fatty acids was obtained from Novus International (St. Louis, Missouri, USA). The supplement was mixed with poultry feed at 0, 1, 3, 5, 7 and 10 wt% levels. The mixtures were conditioned in constant humidity chambers prior to infrared analysis.

Spectra were collected using a Spectrum 400 instrument equipped with NIR and MIR sources for diffuse reflectance and attenuated total reflectance measurements, respectively (Perkin Elmer, Inc., Waltham, MA USA). NIR spectra were collected from 10,000  $\text{cm}^{-1}$  - 4000  $\text{cm}^{-1}$ . Each spectrum was the sum of 32 co-added scans. Samples were prepared and analyzed in triplicate for each mixture. Chemometric analysis was performed with Unscrambler version 10.0 (CAMO Software AS, Oslo, Norway). Raw spectra were evaluated by Principal Component Analysis (PCA) to detect outliers followed by Partial Least Square (PLS) regression. Models were developed with first derivative spectra using full cross-validation. Derivatives were calculated by the method of Savitsky-Golay with 5 point smoothing. The model was calibrated using 30 spectra from the dataset of 36 spectra. The remaining 6 randomly selected spectra were used for validation.

### RESULTS AND DISCUSSION

The raw NIR spectra for mixtures of poultry feed and lipid supplement are presented in Fig. 1. The NIR spectrum of the feed supplement is shown in Fig. 2 with the prominent absorbance peaks labelled. A preliminary analysis of the spectra was performed after scaling to generate a Principal Component (PC) model that captured 99.81% of the variance with 5 components.

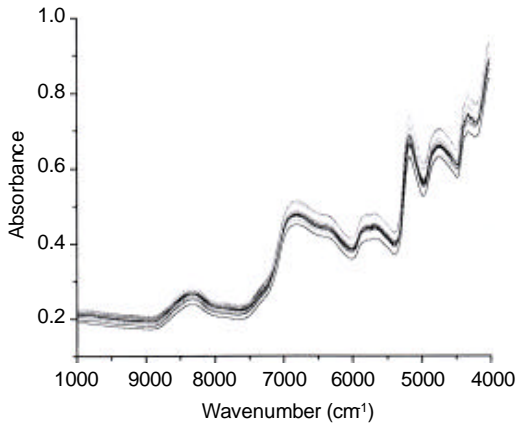


Fig. 1: NIR spectra of starter feed with increasing levels of lipid supplement

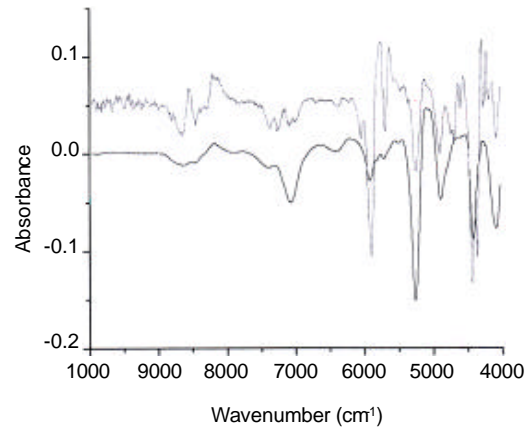


Fig. 3: Derivative spectra of supplement (upper curve) and feed used in PLS model

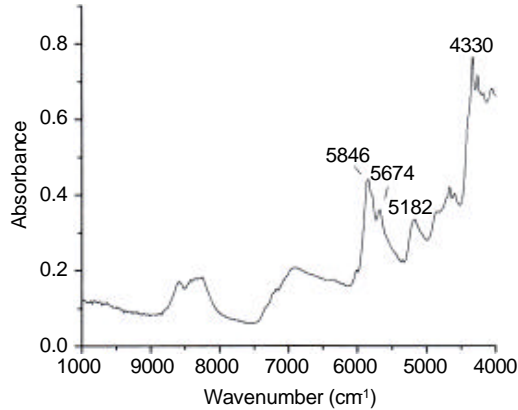


Fig. 2: NIR spectrum of feed supplement with significant lipid absorbance peaks

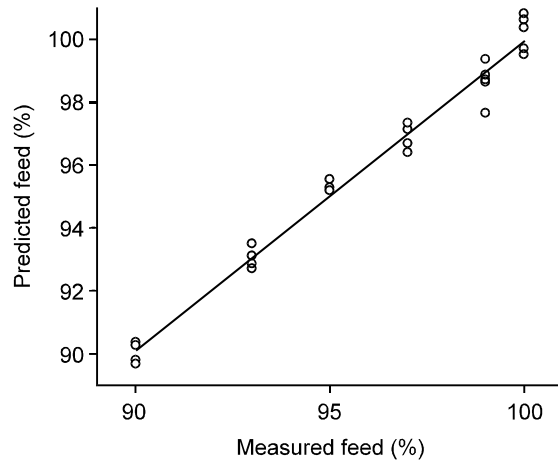


Fig. 4: Measured and predicted levels of feed and supplement mixtures calibrated from derivative spectra

Examination of the Q residuals indicated one outlier could be removed from the data set which reduced the number of principal components from 5 to 3 in the recalculated model.

The regions between  $7140\text{ cm}^{-1}$  -  $6940\text{ cm}^{-1}$  and  $5260\text{ cm}^{-1}$  -  $5130\text{ cm}^{-1}$  are typically used to quantify moisture content with absorbance between  $6000\text{ cm}^{-1}$  -  $4000\text{ cm}^{-1}$  attributed to oil components. However, analysis of the full spectrum proceeded with cross-validation to produce a model with a regression coefficient of 0.8448 and RMSEC of 1.35975% feed supplement. For comparison, the corresponding first derivative spectra were analyzed in the same manner. Figure 3 shows the first derivative spectra obtained for the fatty acid supplement (upper curve) and the poultry feed without added supplement (lower curve). The model based on first derivative spectra provided a regression coefficient of 0.98314 with an RMSEC value of 0.44772% feed supplement. A plot of predicted versus measured values for the mixtures of feed and supplement is shown in Fig. 4. These results demonstrate the utility of NIR to quickly determine

compositions of feed formulations that are supplemented with additives such as DHA. Spectroscopic methods have several advantages compared to more traditional analytical or instrumental techniques. Infrared instruments are relatively inexpensive, sample preparation is minimal and analysis of the data can be automated. Applications for NIR continue to expand for feed composition and quality measurements. Future investigations include the combination of Mid-Infrared (MIR) and NIR.

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