Near Infrared Reflectance Spectroscopy for the Determination of Free Gossypol in Cottonseed Meal[1]

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Primary Audience: Feed Manufacturers, Nutritionists, Quality Assurance Personnel, Researchers

SUMMARY

Gossypol is a toxic polyphenolic compound produced by the pigment glands of the cotton plant. The free gossypol content of cottonseed meal (CSM) is commonly determined by the American Oil Chemists' Society (AOCS) wet chemistry method. The AOCS method, however, is laboratory-intensive, time-consuming, and therefore, not practical for quick field analyses. To determine if the free gossypol content of CSM could be predicted by near infrared reflectance spectroscopy (NIRS), CSM samples were collected from all over the world. All CSM samples were ground and a portion of each analyzed for free gossypol by the AOCS procedure (reference data) and by NIRS (reflectance data). Both reflectance and reference data were combined in a calibration. The coefficient of determination (r^2) and standard error of prediction (SEP) were used to assess the calibration accuracy. The r^2 was 0.728, and the SEP was 0.034 for the initial calibration that included samples from all over the world. However, the r^2 and SEP improved to 0.921 and 0.014, respectively, if the calibration was made using CSM samples only from the United States. These results indicate that a general prediction equation can be developed to predict the free gossypol content of CSM by NIRS. From a practical standpoint, NIRS technology provides a method for quickly assessing whether a particular batch of CSM has a free gossypol content low enough to be suitable for use in poultry diets.

Key words: cottonseed meal, gossypol, near infrared reflectance spectroscopy

2008 J. Appl. Poult. Res. 17:243–248
doi:10.3382/japr.2007-00078

DESCRIPTION OF PROBLEM

Cottonseed meal can be an attractive alternative protein source for poultry diets. However, the possibility that it contains high levels of gossypol discourages its use. Gossypol is a toxic polyphenolic compound produced by, and located in, the pigment glands of the cotton plant. The gossypol found in CSM exists in both a free and bound form. Because of its overall negative

[1]This research was supported in part by grant 05-635GA from the Georgia Cotton Commission, Perry, GA.
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Table 1. Free gossypol content of cottonseed meal samples obtained from several countries

Country	Number of samples	Range of free gossypol concentration[1] (%)	Average free gossypol concentration[1] (%)
United States	51	0.057 to 0.238	0.123
China	10	0.101 to 0.036	0.064
Egypt	6	0.075 to 0.392	0.236
Tajikistan	6	0.013 to 0.106	0.057
Turkey	6	0.071 to 0.124	0.080
Peru	5	0.093 to 0.116	0.105
Iran	4	0.030 to 0.088	0.055
Uzbekistan	3	0.062 to 0.101	0.084
Burkina Faso	2	0.131 to 0.261	0.196
Brazil	1	0.207 to 0.207	0.207
Zimbabwe	1	0.037 to 0.037	0.037
Total	95	0.013 to 0.392	0.106

[1]Free gossypol concentrations determined by the official methods of the AOCS [19].

charge, gossypol tends to form complexes with positively charged molecules such as lysine in CSM [1]. In chickens, free gossypol is assumed to be more readily absorbed from the gastrointestinal tract than bound gossypol and therefore to be the primary cause of observed negative effects associated with its feeding [1–3].

In broiler production, feeding diets that contain CSM with a high level of gossypol has been attributed to poor weight gain [1, 3–6] and poor feed efficiency [6–9]. However, CSM containing low levels of gossypol can successfully be used in broiler diets that contain adequate levels of all essential nutrients, with no adverse effects on body weight or mortality [10, 11]. Furthermore, the lower nutrient density of CSM, when compared with soybean meal, makes it a desirable protein source for broiler breeder pullets [12]. In fact, utilizing CSM in broiler breeder pullet diets reduces the need for severe feed restriction, which results in better flock body weight uniformity [12].

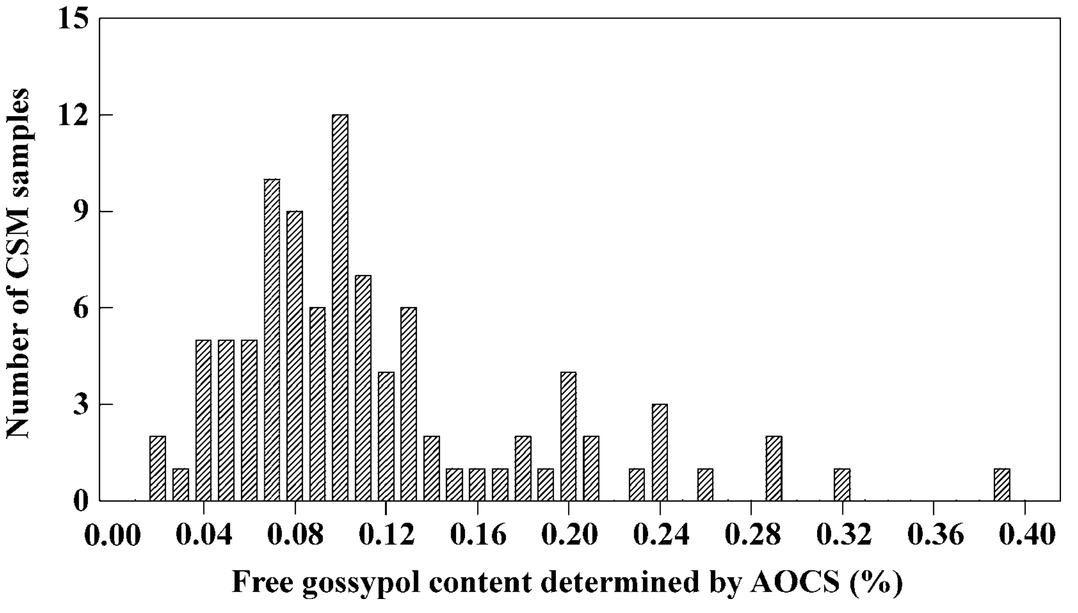

Figure 1. Distribution of the free gossypol content determined by the official methods of the American Oil Chemists' Society (AOCS) [19] in the cottonseed meal (CSM) samples analyzed from several countries.

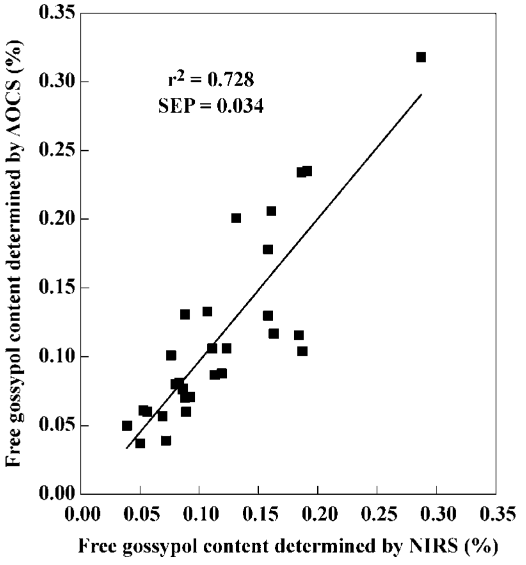

Figure 2. Correlation between the free gossypol content of the international cottonseed meal samples determined by the American Oil Chemists' Society (AOCS) [19] official method and the predicted content based on near infrared reflectance (NIRS) measurements.

Although CSM can be successfully utilized in poultry diets, it typically is not included because the gossypol content is unknown. The gossypol concentration of CSM can vary considerably based on the genetic variety of the cotton [13–15]. In addition, Pons et al. [16] reported that the gossypol content of cottonseeds was negatively correlated with the temperature and positively correlated with the rainfall that the cotton plants were exposed to while producing the seed. Furthermore, methods of cottonseed oil extraction that achieve maximum oil production yield CSM with the lowest gossypol content [1]. Finally, CSM that contains soapstock has a higher concentration of gossypol than a meal without added soapstock because soapstock, a waste by-product from the cottonseed oil extraction process, contains a high concentration of gossypol [17, 18].

For CSM to be utilized as a feed ingredient in poultry diets, a quick and reliable method for the determination of gossypol is needed. The official methods for the quantification of free and total gossypol in CSM are those of the AOCS [19, 20]. However, these procedures are time-consuming as well as laboratory- and labor-intensive. A quicker alternative method for determining the gossypol content of CSM might be near infrared reflectance spectroscopy (NIRS). As reviewed by Van Kempen [21], NIRS technology has been used for the past several years to quickly and efficiently determine moisture, protein, fat, metabolizable energy, total amino acid, and digestible amino acid contents in feed ingredients and feeds.

Previous attempts have been made to measure the gossypol content of whole cottonseeds by NIRS [22, 23]. There are no reports, however, of predicting the gossypol content of CSM by NIRS. Therefore, it is the goal of the present study to develop a NIRS calibration prediction equation to analyze the free gossypol content of CSM.

MATERIALS AND METHODS

Experiment 1

Ninety-five CSM samples were obtained from various locations around the world (Table 1). All of the CSM samples were ground through a 1-mm sieve in a Wiley Laboratory Intermediate Mill [24] to obtain a uniform particle composition across all samples. The free gossypol content of a portion of each CSM sample was then chemically determined by the AOCS method [19].

A portion of each CSM sample was also scanned for gossypol using a model 6500 monochromator NIRS system [25]. The NIRS system continuously scanned wavelengths from 1,100 to 2,500 nm. Reflectance data were recorded at 2-nm intervals. The wavelengths used for free gossypol calibration were based on the analysis of the pure gossypol sample [26] and previous reports of free gossypol determination in whole cottonseeds by NIRS [22, 23]. The NIRS spectra data were then processed using the software WinISI 11 [27]. The spectra data from 56 randomly selected samples of the original 95 samples were then combined with the AOCS reference data to generate a calibration prediction equation using a modified partial least-squares regression method [27].

Once the prediction equation was obtained it was validated with the remaining CSM samples. To validate the prediction equation, a linear regression analysis was used to compute the correlation between the gossypol content obtained for these samples by the AOCS method and the NIRS method. The validation procedure was used to estimate the r^2 and standard error of prediction (**SEP**) as parameters for calibration accuracy. The validation was also used to determine the average difference between the gossypol reference method and predicted values (bias), and the linear regression of the gossypol reference method against predicted values (slope).

Experiment 2

Many of the international samples used in experiment 1 appeared to be poorly processed based on the presence of a large amount of residual cotton fibers and the very dark appearance of the meal. Furthermore, the oil extraction procedures for the international samples were not known. Therefore, in experiment 2 only the more uniformly processed CSM samples obtained from the United States were used for the NIRS calibration. Thus, a total of 51 samples from the original 95 were used in this experiment. Because the sample population was small, the validation sample set was composed of the same 51 samples used for the calibration. The CSM samples from the United States were obtained from 15 different oil processing mills distributed across 8 states (AR, CA, GA, MS, OK, SC, TN, and TX). When CSM samples were obtained from the same oil mill, the samples were collected from different batches of meal production.

RESULTS AND DISCUSSION

Experiment 1

As determined by the AOCS method, the free gossypol content of CSM samples had a wide distribution from 0.01 to 0.39% (Figure 1). Obtaining CSM samples with this high variability in free gossypol content was critical because a NIRS prediction equation should be constructed from samples that represent the range of values that will be encountered in field testing. The CSM samples obtained from Egypt and Peru contained a high concentration of gossypol, whereas the samples from Tajikistan and Iran had lower gossypol levels (Table 1).

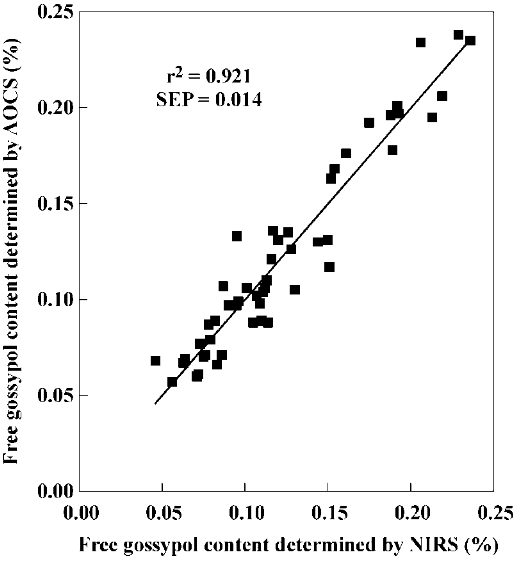

Figure 3. Correlation between the free gossypol content of the US cottonseed meal samples determined by the American Oil Chemists' Society (AOCS) [19] official method and the predicted content based on near infrared reflectance (NIRS) measurements.

The regression analysis for the validation of the reflectance values for gossypol in the international CSM samples yielded an r^2 of 0.728 and SEP of 0.034 (Figure 2). Although this coefficient of determination is statistically acceptable, a higher correlation between the reference and reflectance values was desirable.

Experiment 2

Cottonseed oil extraction in the United States is very efficient and yields a uniform high quality CSM that is devoid of excess lint and residual oil from the cottonseed [1, 28]. Therefore, it was not surprising that when only the samples obtained from the United States were utilized for the NIRS prediction equation, the regression analysis of the gossypol determinations between the AOCS and the NIRS methods yielded a higher r^2 of 0.921 and a SEP of 0.014 (Figure 3).

Because only 51 CSM samples from the United States were available, the same samples had to be used to formulate and validate the NIRS free gossypol prediction equation. Ideally, the validation would have been done as in experiment 1, with new CSM samples. For comparison purposes, we also determined that if the validation sample set consisted of the same 56

CSM samples used to formulate the prediction equation for the international samples (experiment 1), the r^2 value actually decreased to 0.652 and the SEP value remained at 0.034. Therefore, we feel confident that the NIRS free gossypol prediction equation that was constructed in experiment 2 can be utilized to accurately determine the free gossypol content of CSM produced in the United States.

To obtain an accurate free gossypol concentration in CSM samples using NIRS, the results from the 2 current experiments suggest that NIRS prediction equations for free gossypol content of CSM may have to be constructed for different geographical regions of the world. The need for different free gossypol prediction equations reflects the differences in CSM oil extraction processing in different parts of the world, which result in CSM that varies tremendously in quality and appearance. Many of the international CSM samples contained an excessive amount of residual lint compared with the CSM produced in the United States. This lint may interfere with accurate free gossypol determinations by NIRS. Wadsworth and Richard [23] reported a very high SEP when they developed an NIRS calibration for gossypol in whole fuzzy (covered in lint) cottonseed.

The main purpose of constructing a NIRS prediction equation in 1 NIRS system is to then transfer that prediction equation to other NIRS instruments. However, no 2 NIRS systems are the same, and even subtle differences between them may cause variation in the spectral data. Such differences can make the transfer of calibration equations ineffective. Fortunately, there are mathematical manipulations that use the slope and bias factors obtained when each prediction equation is constructed, which can be used to correct spectral data between NIRS analyzers. For the international samples, the slope and bias values were 1.034 and –0.003, respectively, whereas for the US samples the slope and bias were 1.0 and 0.0, respectively. Additionally, instead of using the bias and the slope correction method, a new calibration equation for free gossypol could also be quickly established for a different NIRS system by utilizing a preestablished CSM sample set in which the reference (AOCS) was already determined.

CONCLUSIONS AND APPLICATIONS

1. A NIRS calibration was successfully developed for the determination of free gossypol in CSM.
2. Due to differences in CSM processing around the world, separate prediction equations for individual regions of the world may be needed to confidently predict the free gossypol content of CSM by NIRS.
3. Cottonseed processing plants in the United States could use NIRS as a rapid means of quantifying the gossypol content of the CSM they produce. Once the gossypol content was determined, poultry nutritionists and feed mill operators could decide whether to purchase the CSM for use in poultry diets.

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