

Taking NIR Calibrations of Feed Compounds from the Laboratory to the Process: Calibration Transfer between Predispersive and Postdispersive Instruments

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In the context of current demands in the animal feed industry for controls and analyses, the use of instruments that may be applied on the process line has acquired a significant interest. A key aspect is that the calibrations developed for quality control with instruments sited in the laboratory (at-line) must be transferred to instruments that will be used in the plant itself (online). This study evaluates the standardization and the calibration transfer between a grating monochromator instrument (predispersive) designed for laboratory analysis and a diode array instrument (postdispersive) more adapted to process conditions. Two procedures that correct differences between spectra of two instruments were tested: the patented algorithm by Shenk and Westerhaus and piecewise direct standardization (PDS). Although results were slightly better with PDS, both methods achieved good spectral matching between the two instruments, with levels of repeatability similar to that of the grating instrument itself. The calibration transfer was evaluated in terms of the standard error of prediction (SEP), which was considerably reduced after standardization. However, final calibration models to be used in the diode array instrument must contain spectra from both types of instruments to give acceptable prediction accuracy.

KEYWORDS: Instrument standardization; calibration transfer; NIRS; compound feeds; diode array instruments; grating monochromator

INTRODUCTION

Nowadays, the manufacturing process of animal feed compounds is governed by extensive legislation and internal control frameworks. Techniques and instrumentation able to carry out control and assessments during the process have become of decisive importance.

Worldwide, the animal feed industry has been one of the first industries to implement near-infrared reflectance spectroscopy (NIRS) in the laboratory (1). Large and small feed companies utilize NIRS in their laboratories for the analysis of incoming materials and finished product. This technology offers a number of advantages such as speed of response and nondestruction of the sample, which make it well-suited for meeting current demands of online feed analysis. NIRS has also demonstrated its ability to analyze a range of samples from finely milled products to pellets and granules of different size and shape (2).

Recent works have shown the feasibility of this technology for carrying out measurement at the mixing and the final stage when diode array instruments, more adapted to process conditions, are used (3, 4). However, before NIRS can be widely used for feed process control, a number of issues must be tackled.

A key issue concerning the implementation of NIRS for feed process control is to demonstrate that the robust, expensive, and large calibration sets already existing for laboratory feed analysis, which were developed during many years of investment by feed industrials, may be used for online analysis. Moving to the process should not involve starting again with the calibration procedure for data collected online as though nothing had been done before.

Although there has been much progress in NIR instruments, making them more alike, there still exist differences that make impossible the direct transfer of calibration models from one instrument to other instruments (5, 6). There is clearly a need for procedures to standardize traditional laboratory instruments (i.e., predispersive) with instruments more adapted for online analysis (i.e., diode array).

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As stated by Fearn (6) there are three broad types of approach to the transfer problem: (i) make robust calibrations that are not sensitive to spectral differences between instruments, (ii) adjust spectra from one instrument so that they look like spectra from the other one, and (iii) adjust calibrations developed in one instrument so that they can be used in the other one.

Robust calibration models that are not sensitive to instrumental responses can be made by using appropriate pretreatment techniques (7), selecting wavelengths (8), or including several instruments in the calibration set (9, 10). However, pretreatment is likely to be adequate only when the problem is related to absorbance shifts, the instruments involved are very similar, and the instrumental differences are small; selecting only those wavelengths that are robust against instrumental differences is not appropriate to correct intensity differences or peak broadening in spectra, and the method of slope and bias correction to adjust calibrations works well when one is correcting wavelength shifts or linear intensity differences in spectra, but it cannot cope with peak broadening (11). To correct more complicated effects when quite different NIR spectrophotometers are involved, standardization procedures are needed. In this study we have focused on two algorithms that correct the differences between spectra of two instruments: the patented algorithm by Shenk and Westerhaus (12) and piecewise direct standardization (PDS) introduced by Wang et al. (13).

The patented algorithm has been widely used with agricultural products to standardize predispersive grating instruments not only of the same model (14–17) but also of different models (18) or two diode array instruments (19). With regard to feed compounds, this algorithm was used for transferring equations predicting the ingredient composition of feeds (20). The PDS method has also been extensively applied with successful results in equation transfers between diode array instruments (11, 21, 22), between grating and FT-NIR instruments (23), and between diode array and FT-NIR instruments (11). However, there are no scientific papers reporting standardization and transfer of equations between grating monochromator and diode array instruments. Therefore, there is a need to know if compound feed calibrations may be transferred from predispersive instruments to postdispersive ones.

In feed process control a number of critical parameters must be monitored, including not only major analytical parameters (i.e., crude protein) but also physical parameters such as pellet durability. High pellet durability is required to support repeated transfers and movements along feed handling systems and to avoid dusting potential, explosion risks, habitats preferred for germs, and feed losses by fines because some animals refuse the intake of fines (24).

The objective of the present paper is to study the reliability of two standardization algorithms for spectral matching between a predispersive instrument and a postdispersive diode array instrument and for the transfer from one instrument to another of equations predicting critical process parameters of intact feed samples.

MATERIALS AND METHODS

NIRS Instruments. *Instrument 1.* A FOSS NIRSystems model 6500 SY-II scanning grating monochromator (Silver Spring, MD) equipped with a transport module was used to measure reflectance spectra from 400 to 2498 nm, with data recorded every 2 nm. The transport module is a device that allows the use of rectangular cells larger than the traditional ring cups. In this study, the analysis was carried out using the natural product transport cell, which is a rectangular cell with a depth of 4.3 cm and a quartz viewing window of dimensions 4.7 cm × 20 cm. Spectra were recorded using WINISI

II software version 1.5 (Infrasoft International, Port Matilda, PA). Absorbance values were recorded as $\log(1/R)$, where R is the sample reflectance. The number of scans averaged per measurement was 32. For each sample the cell was filled three times with subsamples of the same sample, and the mean spectrum was used for calculations.

Instrument 2. A CORONA 45 VIS+NIR (Carl Zeiss, Inc.) diode array spectrometer was used to measure reflectance spectra from 400 to 1690 nm, with data recorded every 2 nm. The instrument was equipped with the Turnstep module (revolving plate) and a Petri dish of 7 cm diameter to contain the samples (38.5 cm² irradiated). Absorbance values were recorded as $\log(1/R)$, where R is the sample reflectance. White referencing and dark current measurement was carried out manually. All spectra were recorded using CORA software version 3.2.2 (Carl Zeiss, Inc.). With an integration time of 100 ms, 20 scans were averaged for each measurement. For each sample the cell was filled five times with subsamples of the same sample, and the mean spectrum was used for calculations.

Calibration Development. Compound feed samples covering the variability encountered in the real production process were provided by a Spanish feed plant, over an extended period of time (2001–2005). The spectra of a total of 623 nonmilled samples were recorded using instrument 1. This set of samples is referred to as set 1 and was used for calibration development.

Mathematical procedures were performed with WINISI II software version 1.5 (Infrasoft International). The linear regression method was based on a modification of the partial least-squares (PLS) algorithm (25), where the X and Y residuals are standardized at each iteration. Calibrations were developed with the following settings: 400–1690 nm by 2 nm and four cross-validation segments.

Different combinations of derivatives [1,5,5,1; 2,5,5,1; 1,10,5,1; and 2,10,5,1, where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing, and the fourth is the second smoothing (26)] and pretreatments [standard normal variate and detrending methods for scatter correction (27)] were tried; and the optimum number of PLS factors was determined by cross-validation.

A set of 150 nonmilled samples (set 2) scanned in instrument 1 served to validate calibrations developed on instrument 1 using samples of set 1.

Prediction performances were expressed in terms of the standard error of prediction (SEP) and the standard error of prediction corrected for the bias [SEP(c)].

Wet Chemistry. Chemical reference data for crude protein (CP) and crude fiber (CF) were determined by Association of Official Analytical Chemists (AOAC) methods 976.06 and 978.10, respectively (28). A pellet tester (24) was used for determining reference data regarding durability (%) of each compound feedstuff. Pellets fed into a cylindrical test unit were set in motion by a rotor and thus exposed to mechanical action. After the test, fines were separated and related to the originally weighed material. The pellet durability index (PDI) was calculated as follows: $PDI = (m_{\text{after}}/m_{\text{before}}) \times 100$, where m_{before} = initial weight and m_{after} = initial weight – weight of fines.

Standardization Procedure. To transfer calibration models developed on instrument 1 to instrument 2, standardization was necessary. Two data sets were used to compare and standardize instruments.

The first set (set 3) consisted of 40 unground feeds, measured at the same time on both instruments placed side by side in the laboratory. These 40 samples covered variability related to type of feed, form of presentation, and particle size and hence cover a range of levels of absorbance. Different combinations of these samples were used to develop several standardization files, that is, mathematical matrices designed to correct spectral differences between the two instruments. The selection attempted to include the range of variation in the absorbance levels. The numbers of samples selected for the matrices varied between 3 and 10. Samples not used for matrices were used for validation of the spectral matching. The methods used for creation of different standardization files are explained below.

The second set consisted of exactly the same 150 samples of set 2 that were also recorded using instrument 2. In this case, set 2 was used to test the impact of combining spectra from two instruments in one database. The 150 samples were split randomly into two groups.

Seventy-five samples were used to update the original 623 sample database of set 1 and the other 75 were used to create a validation file. This strategy has been already successfully used by Welle et al. (19) for the standardization of two diode array instruments.

The results of standardization were evaluated by means of two tests. First, the RMS(c) statistic was used to test the spectral matching of the standardized instruments. This statistic is the averaged root-mean-square of differences corrected for the bias between spectra obtained in two instruments, at n wavelengths

$$\text{RMS(c)} = 10^6 \times \sqrt{\frac{\sum_{i=1}^n D_i^2 - \frac{\left[\sum_{i=1}^n D_i\right]^2}{n}}{n-1}} \quad (1)$$

where $D_i = y_{i1} - y_{i2}$ and y_{i1} and y_{i2} are the log ($1/R$) values of two spectra of a single sample scanned in instruments 1 and 2 at a given wavelength.

The second test evaluates equation transfer between the two instruments by means of the standard error of prediction (SEP) obtained when the standardized spectra of instrument 2 were used in the original models developed on instrument 1. As a rule of thumb to judge the success of the transfer, the standard error of prediction corrected for bias or SEP(c), which should not exceed 1.30 times the standard error of calibration (SEC) (29), was used.

Another statistic used to evaluate the successfulness of the cloning is the H distance (30). This statistic, analogous to the Mahalanobis distance, measures, in a space defined by principal components of the calibration samples, the distance between one spectrum and the center of a group of spectra or between the centers of two groups of spectra. In this case, the H distance provided information about how far the samples scanned on instrument 2 are from the samples scanned on instrument 1.

Two H distances were calculated: the global H (GH) tells how far a sample is from the average of the calibration file and the neighborhood H (NH) tells how far a sample is from the most similar samples in the calibration file (25).

The H distances are standardized using averages calculated from the calibration samples, so that it is possible to interpret their values on a standard scale. Values of GH higher than 3–4 and NH values higher than 0.6–1.2 mean that the samples scanned on instrument 2 are judged to be far from the calibration samples scanned on instrument 1 (30).

Standardization Methods. *Shenk and Westerhaus Patented Algorithm.* This patented algorithm performs a univariate full-spectrum correction in two main steps, namely, wavelength index correction followed by spectral intensity correction. First, the method adjusts for wavelength shifts, if necessary. The simplest version takes each wavelength on the first instrument and matches it to the wavelength on the second instrument, where the first-derivative spectral data correlate best. Later versions allow interpolation between wavelengths. Then, with the wavelength matched, simple linear regression is used, now using log($1/R$) data, one wavelength at a time, to estimate an offset and slope to convert the response of the second instrument to that of the first instrument.

A very good detailed description of this method is given by Bouveresse et al. (31).

Computations for this method were developed with WINISI II software version 1.5 (Infrasoft International).

Piecewise Direct Standardization (PDS). In PDS, a transformation matrix is constructed on the basis of the spectra of the standardization samples or transfer set measured with both devices. This transformation matrix can then be used to transform an arbitrary spectrum measured on one instrument to the other instrument. The calibration models available for the first instrument can then be used to predict the CP, CF, and durability from this spectrum. To construct the transformation matrix, a local PC or PLS regression model is built between each wavelength of the first instrument spectrum and a corresponding spectral window in the second instrument. The window size and the number of principal components (PC) or latent variables (LV) used in each PC or

Table 1. Reference Chemistry and Calibration Statistics for Unground Compound Feeds Measured with Instrument 1 for 623 Samples over the Period from 2001 to 2005

| | reference chemistry (%) | | | calibration statistics | | | |
|---------------|-------------------------|------|-----------|------------------------|------|------------|------|
| | mean | SD | range | R^2_c | SEC | R^2_{cv} | SECv |
| crude protein | 18.2 | 3.91 | 8.4–33.8 | 0.96 | 0.70 | 0.96 | 0.75 |
| crude fiber | 7.5 | 4.60 | 1.7–23.9 | 0.98 | 0.68 | 0.98 | 0.74 |
| durability | 97.9 | 1.27 | 93.0–99.5 | 0.75 | 0.50 | 0.75 | 0.54 |

Table 2. Validation Statistics of 150 Unground Compound Feeds Manufactured in 2006 and Measured with Instrument 1, Predicted with Calibrations Developed in Table 1^a

| | reference chemistry (%) | | | validation statistics | | | |
|---------------|-------------------------|------|-----------|-----------------------|------|--------|------|
| | mean | SD | range | R^2_p | SEP | SEP(c) | RPD |
| crude protein | 17.7 | 3.14 | 12.6–30.6 | 0.93 | 0.88 | 0.88 | 3.57 |
| crude fiber | 9.8 | 5.49 | 2.1–20.6 | 0.98 | 0.85 | 0.85 | 6.70 |
| durability | 98.2 | 1.16 | 93.0–99.7 | 0.70 | 0.64 | 0.63 | 1.87 |

^aSEP(c)limit = $1.3 \times \text{SEC}$; SEP(c)limit CP = 0.91; SEP(c)limit CF = 0.88; SEP(c)limit durability = 0.65.

PLS model need to be optimized. The standardization samples must be representative enough to describe the differences between the instruments and should cover the entire experimental domain.

Computations for this method were developed with MATLAB vs 7.01 (The Mathworks, Natick, MA) using PLS_Toolbox (eigenvector Research, Inc., Manson, WA).

Each local model of the PDS was constructed with three principal components according to the recommendations by Bouveresse and Massart (5) and Feudale et al. (32). The window sizes investigated varied between 3 and 21 wavelength points.

Because instrument 1 scans up to 2498 nm, but instrument 2 scans up to only 1690 nm, the effective wavelength after standardization was that of instrument 2: 400–1690 nm.

RESULTS AND DISCUSSION

Calibration and Validation. Wet chemistry reference values for samples used in calibration are given in Table 1. Protein and fiber were selected as representative of the analytical parameters because these are considered to be the main constituents that must be controlled in most feed ingredients (33).

The wide ranges and the standard deviations for crude protein and crude fiber confirm the wide diversity of compound feedstuffs included in the population selected for the study. The range for durability was not as wide, varying only between high values of that parameter (34).

Table 1 presents the results for calibration and cross-validation. Results obtained when calibrations derived on instrument 1 were applied on validation samples measured with the same instrument are shown in Table 2. This table also reveals the similarity between the descriptive statistics of the calibration and validation sets.

Crude protein and crude fiber were determined with good accuracy and precision as indicated by high coefficients of determination and RPD values higher than the minimum recommended (RPD = 3) for quantitative analyses (35). Comparing these results with those of other research works dealing with intact feed compounds, we found that RPD values of this work were slightly lower than those obtained by Pérez-Marín et al. (2) and Fernández-Ahumada et al. (3) with the same grating instrument. This can be explained by the fact that a more stringent test set was used in this study. The RPD values reported by Pérez-Marín et al. (2) and Fernández-Ahumada et al. (3)

were referred to a cross-validation set, whereas the values presented here corresponded to a validation set that was really an independent set with samples manufactured in a different year from those included in the calibration.

The lower ability to estimate durability may be due to the physical nature of this parameter on which different factors have influence. Durability may have changed in the period elapsing between reference analysis and analysis by NIRS. Reference data were obtained in the factory, and afterward the samples were packed and sent to the laboratory of the university. The transportation may have caused a decrease in the durability values of the samples. The correct way to develop a calibration for this parameter would consist of performing the reference analysis and taking the NIR scan in parallel. However, this is not always possible for calibrations developed in the framework of research projects. The need for an online measurement then becomes evident. Determining durability more precisely requires an improved calibration, but results may be viewed as a first approach to measuring physical parameters that also need to be under control.

Standardization. *Optimization of Transfer Set and Window Size for PDS.* According to Fearn (6), who indicated that, ideally, the best option is the use of real samples, analogous to calibration samples, all matrices were designed with these kinds of samples. Furthermore, a previous work (20) aimed at standardizing two grating monochromators for transferring calibrations of unground feedstuffs evaluated the spectral adjustment with different kinds of samples and concluded that the best results were obtained with real samples.

The choice of samples for transfer matrices was done trying to cover an absorbance range similar to that of calibration samples and with highly varied samples representative of the total data set. As a result, six different matrices were developed combining 3, 5, and 10 samples and the two algorithms evaluated: (1) STD1, transfer matrix with 10 samples and patented algorithm; (2) STD2, transfer matrix with 5 samples and patented algorithm; (3) STD3, transfer matrix with 3 samples and patented algorithm; (4) STD4, transfer matrix with 10 samples and PDS; (5) STD5, transfer matrix with 5 samples and PDS; (6) STD6, transfer matrix with 3 samples and PDS.

With the PDS method, the window size was optimized to compute the transfer matrix. The window size that resulted in the minimal RMS(c) value was selected. On the basis of these results (data not shown) a window size of 19 was chosen. Other works (21, 22) dealing with the standardization of instruments with similar optical design suggest values for the window size lower than 19, between 3 and 9, but when the standardization takes place between two more different instruments, research works (11) show that the optimum window size reaches values similar to that obtained in this study.

Values above 19 for the window size gave poorer results for the RMS(c) statistic, suggesting the occurrence of overfitting when too many nonrelevant channels are included, which is the main problem of direct standardization when the full spectral region is included (13).

Spectral Matching. A clear difference between the spectra measured on the diode array and the grating monochromator spectrophotometers can be observed (Figure 1). Basically, this difference is mainly an absorbance shift and a subtle wavelength shift that might be attributed to the differences in measurement and working principle between the instruments.

Results for the RMS(c) statistic with the different matrices and algorithms used are shown in Table 3. Although differences between the spectra of instruments 1 and 2 before standardiza-

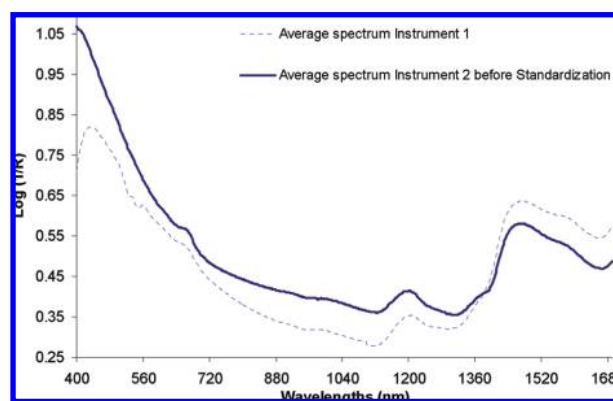


Figure 1. Average spectra of the samples used for validation of spectral matching, analyzed in instruments 1 and 2 before standardization.

Table 3. Average RMS(c) Values Obtained with the Samples of Set 3 Not Used for the Transfer Matrices, Comparing Spectra from Instrument 1 with Those from Instrument 2 before and after Standardization^a

| | RMS(c) |
|------------------------------|--------|
| instrument 1 | 6766 |
| instrument 2 before | 70582 |
| STD1 (patented) $N = 10$ | 7524 |
| STD2 (patented) $N = 5$ | 7148 |
| STD3 (patented) $N = 3$ | 7500 |
| STD4 (PDS, $w=19$) $N = 10$ | 7368 |
| STD5 (PDS, $w=19$) $N = 5$ | 6923 |
| STD6 (PDS, $w=19$) $N = 3$ | 6953 |

^a The value reported in the first line for instrument 1 is based on the comparison of subsamples measured on this instrument. w = window size; N = number of samples.

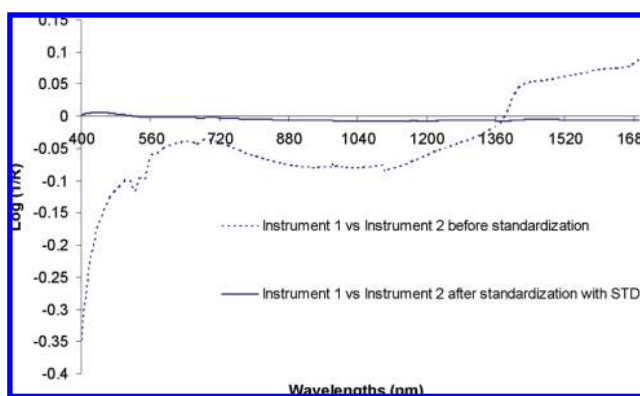


Figure 2. Difference between average spectra of the samples used for validation of spectral matching analyzed in instruments 1 and 2 before standardization and after standardization with matrix STD5 (best standardization).

tion were considerable, they were quite well corrected with the standardization (Figure 2), yielding RMS(c) values for spectra of instruments 1 and 2 of the same order as those obtained for two subsamples analyzed on instrument 1 (6766). This is the level of adjustment required for adequate standardization of instruments, because the limit to spectral correction between instruments is imposed by the repeatability of the instrument itself (36). According to that, an important improvement was achieved with the standardization procedure.

Results were very similar with all methods and matrices evaluated, being slightly better with the PDS method. Because PDS is a multivariate standardization procedure, it seems to work better than the univariate correction of the patented algorithm and be able to cope with more complicated effects

Table 4. Global and Neighborhood *H* Distances (GH and NH) Corresponding to the Validation Set Analyzed on Instruments 1 and 2 before and after Application of STD2 and STD5 Standardization Matrices

| | instrument 1 (<i>N</i> = 150) | without standardization (<i>N</i> = 150) | after standardization (<i>N</i> = 150) | | after standardization and update (<i>N</i> = 75) | |
|----|-----------------------------------|---|---|------|---|------|
| | | | STD2 | STD5 | STD2 | STD5 |
| | | | | | | |
| GH | 1.16 | 29.97 | 3.95 | 3.88 | 1.98 | 1.71 |
| NH | 0.55 | 24.33 | 2.73 | 2.57 | 0.69 | 0.50 |

Table 5. Validation Statistics of 150 Unground Compound Feeds Manufactured in 2006 Predicted with Calibrations Developed in Instrument 1 before and after Standardization with the Patented Algorithm (STD2) and the PDS Method (STD5)^a

| | without standardization | | | after standardization with STD2 | | | after standardization with STD5 | | |
|---------------|------------------------------------|------|--------|------------------------------------|------|--------|------------------------------------|------|--------|
| | <i>R</i> _p ² | SEP | SEP(c) | <i>R</i> _p ² | SEP | SEP(c) | <i>R</i> _p ² | SEP | SEP(c) |
| | | | | | | | | | |
| crude protein | 0.36 | 5.17 | 2.78 | 0.73 | 1.96 | 1.79 | 0.75 | 1.85 | 1.85 |
| crude fiber | 0.61 | 3.93 | 3.94 | 0.82 | 2.85 | 2.31 | 0.82 | 3.16 | 2.40 |
| durability | 0.38 | 1.53 | 1.52 | 0.25 | 1.46 | 1.21 | 0.42 | 0.96 | 0.91 |

^a SEP(c)limit = 1.3 × SEC; SEP(c)limit CP = 0.91; SEP(c)limit CF = 0.88; SEP(c)limit durability = 0.65.

and differences. Concerning the number of samples, the transfer matrix of five samples gave the best results, in accord with Bouveresse and Massart (5), who recommended selecting more than three standardization samples to avoid any kind of artifacts in the transfer parameters.

Performance of Calibration Transfer. The transfer matrices that yielded the best spectral matching with each algorithm (STD2 with the patented algorithm and STD5 with PDS method) were applied to the 150 samples scanned on instrument 2. These standardized samples were then projected onto the principal components computed with set 1 and *H* distances, global (GH) and neighborhood (NH), were calculated. **Table 4** shows these values before standardization and after standardization with each matrix.

The standardization matrices reduced GH and NH values considerably, confirming a good spectral matching, as seen above. However, although the reduction was significant, GH and NH values after standardization were slightly higher than the limits suggested (GH = 3.0–4.0, NH = 0.6–1.2) for considering the predicted values accurate (30) and quite far from the values obtained with instrument 1 (**Table 4**).

When the strategy of combining spectra of the two instruments was applied, the results became better. As explained before, the 150 samples scanned in instrument 2 were standardized by STD2 and STD5 matrices. Once standardized, the file was split into two groups; 75 samples were used to update the calibration, and the remaining 75 samples were utilized for validation. With this procedure, GH and NH values were reduced to within reasonable limits, remaining very similar to those of instrument 1 (**Table 4**).

Validation statistics (**Table 5**) demonstrate that standardizing the spectra improved considerably the predictions on instrument 2, which were quite unacceptable without standardization. The original calibration models became useless for spectra of instrument 2 unless some adjustment was made. The standardization improved these results, and an important reduction of SEP values was achieved with the patented algorithm (CP, 62%; CF, 27%; durability, 4.6%) and the PDS method (CP, 64%; CF, 20%; durability, 37%).

Table 6. Reference Data for Updating (*N* = 75) and Validating (*N* = 75) Samples

| | reference chemistry of updating samples (%) | | | reference chemistry of validating samples (%) after update | | |
|---------------|---|------|-----------|--|------|-----------|
| | mean | SD | range | mean | SD | range |
| | | | | | | |
| crude protein | 18.5 | 3.88 | 13.4–30.6 | 17.3 | 3.20 | 12.6–28.8 |
| crude fiber | 8.1 | 5.29 | 2.1–20.6 | 10.9 | 5.31 | 2.6–18.7 |
| durability | 97.9 | 1.42 | 93.0–99.7 | 98.4 | 1.29 | 96.0–99.4 |

Table 7. Calibration and Validation Statistics after Standardization with the Patented Algorithm and Update^a

| | calibration statistics with 75 updating samples | | | | validation statistics of 75 remaining samples | | |
|---------------|---|------|-------------------------------------|------|---|------|--------|
| | <i>R</i> _c ² | SEC | <i>R</i> _{cv} ² | SECV | <i>R</i> _p ² | SEP | SEP(c) |
| | | | | | | | |
| crude protein | 0.96 | 0.74 | 0.96 | 0.80 | 0.88 | 0.98 | 0.98 |
| crude fiber | 0.97 | 0.73 | 0.97 | 0.80 | 0.97 | 0.96 | 0.96 |
| durability | 0.75 | 0.54 | 0.75 | 0.57 | 0.67 | 0.64 | 0.64 |

^a SEP(c)limit = 1.3 × SEC; SEP(c)limit CP = 0.96; SEP(c)limit CF = 0.95; SEP(c)limit durability = 0.70.

Table 8. Calibration and Validation Statistics after Standardization with PDS Method and Update^a

| | calibration statistics with 75 updating samples | | | | validation statistics of 75 remaining samples | | |
|---------------|---|------|-------------------------------------|------|---|------|--------|
| | <i>R</i> _c ² | SEC | <i>R</i> _{cv} ² | SECV | <i>R</i> _p ² | SEP | SEP(c) |
| | | | | | | | |
| crude protein | 0.96 | 0.72 | 0.96 | 0.81 | 0.90 | 0.95 | 0.96 |
| crude fiber | 0.96 | 0.78 | 0.96 | 0.83 | 0.97 | 0.94 | 0.95 |
| durability | 0.74 | 0.48 | 0.74 | 0.52 | 0.67 | 0.61 | 0.62 |

^a SEP(c)limit = 1.3 × SEC; SEP(c)limit CP = 0.94; SEP(c)limit CF = 1.01; SEP(c)limit durability = 0.62.

Although the reductions in SEP were considerable, these results were not in compliance with the SEP(c) control limits (29).

Analogous to GH and NH values, results for SEP became acceptable when spectrally matched instrument 2 samples were used to update the instrument 1 calibration. **Table 6** shows the descriptive statistics for CP, CF, and durability for the updating and validation sets. **Tables 7** and **8** show the calibration and validation statistics after standardization and update with the patented algorithm and PDS method, respectively. In both cases, the SEP values were close to those obtained in the validation study with samples measured on instrument 1. All values approached the SECVs found during calibration and were within the SEP(c) control limits.

The standardization together with the updating worked well for the three parameters studied, yielding RPD values (3.27 for CP, 5.59 for CF, and 1.93 for durability, with the patented algorithm; 3.33 for CP, 5.64 for CF, and 2.15 for durability, with PDS method) close to those obtained with validation samples scanned on instrument 1 (**Table 2**). Results were slightly better with the PDS algorithm, which was consistent with the results obtained during spectral matching.

The Shenk and Westerhaus patented algorithm and PDS method have shown their ability to perform good spectral matching between spectra of grating and diode array instruments working on intact compound feed samples. Transfer matrices with five samples gave the best results with both procedures. Final calibration models to be used in the diode array instrument

must contain spectra from both types of instruments to give acceptable prediction values.

The results described in this paper represent an important advance in the process of online implementation of NIRS in feed manufacturing plants. The paper demonstrates for first time that calibration equations already available for laboratory or at-line analysis can be transferred to online diode array instruments to be located in different key points at the animal feed plant to produce real-time process information of important parameters in feed manufacturing.

ABBREVIATIONS USED

NIRS, near-infrared reflectance spectroscopy; PDS, piecewise direct standardization; FT, Fourier transform; R, reflectance; vis, visible; PLS, partial least-squares; CP, crude protein; CF, crude fiber; PDI, pellet durability index; PC, principal component; LV, latent variable; GH, global H distance; NH, neighborhood H distance; SD, standard deviation; R^2 , coefficient of determination, fraction of explained variance for cross-validation; SEC, standard error of calibration; SECV, standard error of cross-validation; SEP, standard error of prediction; SEP(c), standard error of prediction corrected for the bias; RPD, ratio of the standard deviation divided by the SEP; STD, standardization or transfer matrix.

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