JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Feasibility of Diode-Array Instruments To Carry Near-Infrared Spectroscopy from Laboratory to Feed Process Control

ELVIRA FERNÁNDEZ-AHUMADA,* ANA GARRIDO-VARO, AND JOSÉ EMILIO GUERRERO-GINEL

Department of Animal Production, E.T.S.I.A.M., University of Córdoba, N-IV, Km. 396, Campus Rabanales, Edif. Producción Animal, 14014, Córdoba, Spain

Near-infrared calibrations were developed for the instantaneous prediction of the chemical and ingredient composition of intact compound feeds. Two rather different instruments were compared (diode array vs grating monochromator). The grating monochromator was used in a static mode in the laboratory, whereas the diode-array instrument—better adapted to online analysis—was placed on a conveyor belt to simulate measurements at a feed mill plant. Modified partial least squares (MPLS) equations were developed using the same set of samples analyzed in the two instruments. Sample set 1 ($N = 398$) was used to predict crude protein (CP) and crude fiber (CF), while sample set 2 ($N = 393$) was used for the prediction of one macroingredient (sunflower meal, SFM) and one microingredient (mineral−vitamin premix, MVP). The standard error of cross-validation (SECV) and the coefficient of determination (R^2) values for CF were better using the monochromator instrument. However, results obtained for CP, SFM, and MVP using the samples analyzed in the diode-array instrument showed similar or even greater accuracy than those obtained using samples analyzed in the grating monochromator. The excellent predictive ability [$R^2 > 0.95$; RPD (ratio of standard deviation to SECV) > 3] obtained for CP, CF, and SFM opens the way for the online use of NIRS diode-array instruments for surveillance and monitoring in the manufacture, processing, and marketing of compound feeds. R^2, RPD, and SECV values for MVP showed similar performance for both instruments. Although RPD values did not reach the minimum recommended for quantitative analysis, results are encouraging for an ingredient present in feed compounds in such very low amounts.

KEYWORDS: Compound feeds; NIRS; diode-array instruments; grating monochromators; on-site analysis; chemical composition; ingredient composition

INTRODUCTION

Since the publication of the White Paper on Food Safety in 2000 (*1*), the manufacture of industrially produced compound feeds has increasingly been regulated by legislation in a number of areas (ingredients declaration, official inspection and controls, labeling, traceability, undesirable substances, prohibited ingredients) (*2*). Formal and informal official inspection programs and self-control requirements applied to the manufacture of compound feeds include not only checks on incoming raw materials and finished products but also the testing of in-process materials (i.e., homogeneity, formulation errors, labeling errors, etc.). The implementation of these checks on the huge amount of compound feeds circulating across European frontiers is being hampered by the lack of affordable analytical methods that can be applied at different key points in the feed industry.

Traditional quality controls in the feed processing industry have relied heavily on manual sampling followed by physical/chemical measurement. These methods often give rise to a time-lag between product processing and the actual result, culminating in a product possibly having to be downgraded later (*3*), with an increasing number of consumer complaints and product recalls (*4*).

Recently, many feed manufacturers have started to use near infrared reflectance spectroscopy (NIRS) for the analysis of raw materials and finished compound feeds. This technology is now widely recognized as an affordable, fast, nondestructive, and nonpolluting technique for the analysis of compound feedstuffs (*5, 6*). However, European Authorised Officers and compound feed manufacturers are displaying a growing interest in spot, on-site measurements that can be used not only for regulatory official analysis but also for surveillance and monitoring purposes (*4*).

Traditional and modern NIRS laboratory instruments have a number of constraints (e.g., lack of robustness leading to poor adaptability to harsh environments, low scanning speed, high cost, nonportability) that hinder their use in industrial plants or in general outside the laboratory. However, recently developed spectrometers based on array detectors offer a range of

* Corresponding author. E-mail: g82feahe@uco.es.

10.1021/jf073534t CCC: $40.75
Published on Web 04/12/2008

advantages, including ability to record a full spectrum at high speed, lack of moving parts, wavelength repeatability and compatibility with fiber optics for flexible process interfacing, and lower price (*7*). The extension of diode-array technology to the long-wavelength NIR (beyond 1100 nm) was until recently hampered by the relatively high cost of detector materials (*8*). However, falling costs have increasingly facilitated the use of diode-array spectrometers at wavelengths formerly accessible only by scanning NIR instruments. Array detectors operating in the near-infrared region are potentially useful for process measurement because they are more rugged and better-suited to online applications, even under aggressive conditions (*9*).

Although diode-array spectrometers appeared in the 1990s, scientific information regarding their performance in the analysis of animal feeds is very limited as compared to the data available on grating monochromators. Some early studies addressed the use of this technology on harvesters for analysis of grains and forages (*10–14*) and also for mixed rations (*15*). However, no scientific information has hitherto been available regarding the viability of diode-array spectrometers for on-site quality control in the feed compound industry.

In this industry not only the analytical control of traditional parameters such as moisture, crude protein, crude fiber, crude fat, etc. is required. The prediction of ingredient composition is also necessary for compliance with current legislation (*16*) and with the manufacturers' interest. Although they are no longer bound to list all ingredients used for the consumer in general, this information must be available if required by an official inspection.

The aim of the present paper is to carry out a pilot study to compare the performance of an NIR diode-array instrument working on a conveyor belt with that of a traditional laboratory monochromator, for the online monitoring of chemical and ingredient composition in intact compound feeds.

MATERIAL AND METHODS

Samples and Reference Data. Compound feedstuff samples were collected from a Spanish feed plant, over an extended period of time (2001–2005), thus representing the variability encountered in the real production process. A set of finished compound feed samples was used to develop equations predicting chemical (set 1, $N = 398$) and ingredient composition (set 2, $N = 393$). Feed samples were manufactured for different animal species (cattle, sheep, goat, pig, poultry, rabbit, and pet), using different forms of presentation (meals, crumbs, pellets of varying sizes, extruded, etc.).

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 (*17*). Reference data regarding ingredient composition (%) of each compound feedstuff were provided by the feed manufacturer.

NIRS Analysis. For comparative purposes, all samples were analyzed with two different instruments and devices: a diode-array spectrometer placed on a conveyor belt and a scanning grating monochromator.

Diode-Array Spectrometer: CORONA−Haldrup. The Haldrup stationary module attached to the CORONA 45 VIS+NIR (Carl Zeiss, Inc.) diode-array spectrometer (**Figure 1**) was used to simulate "on-line" measurements. The samples passed along the conveyor belt underneath the spectrometer, which is a postdispersive optical device. This instrument passes all specified wavelengths through the sample at the same time, and records all absorbances simultaneously (*18*). The diode array consists of very small diodes arranged in a row. Each diode records absorbance for a 400−1690 nm wavelength range. Every 2 nm, absorbance values were recorded as log (1/*R*), where *R* is the sample reflectance. White referencing and dark current measurement was carried out manually by tilting the spectrometer in the opposite direction from the conveyor belt. All spectra were recorded using CORA software version 3.2.2 (Carl Zeiss, Inc.).

Figure 1. CORONA 45 VIS+NIR instrument on the conveyor belt of the Haldrup stationary module.

Figure 2. Foss NIRSystems 6500 instrument equipped with transport module and natural cell for unground feedstuffs.

Measurement parameters, such as focal distance, conveyor belt speed, and sample layer thickness, had previously been optimized (*19*). The parameters used here were as follows: focal distance = 13 mm, speed of conveyor belt = 8 m min^{-1}, thickness of layer = 1 cm.

The CORA software was programmed to capture one spectrum per second; 10 spectra were captured per sample, and the average was used in calculations.

Scanning Monochromator: FNS-6500. A FOSS NIRSystems model 6500 SY-II scanning grating monochromator (Silver Spring, MD) was used to measure reflectance spectra from 400 to 2498 nm, every 2 nm (**Figure 2a**). This spectrometer typically comprises a dispersive medium, entrance and exit slits, and imaging components that produce a parallel beam path. To record a spectrum, a detector located behind the exit slit must sequentially record the incident light while the dispersive component or the exit slit is moved (*18*).

Absorbance values were recorded as log (1/*R*), where *R* is the sample reflectance. The number of scans per sample was 32. Each sample was measured twice and the mean was calculated. Spectra were recorded using WINISI II software version 1.5 (Infrasoft International, Port Matilda, PA). To enable comparison of the performance of the two instruments, the FNS-6500 range was trimmed to 400–1690 nm.

The FNS-6500 instrument was equipped with a transport module, which 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 internal dimensions of 4.7 cm wide, 20 cm long, and 4.3 cm deep (**Figure 2b**). The quartz viewing window (4.7 cm × 20 cm) allows 94 cm^2 of the sample surface area to be irradiated.

Development of NIR Calibrations. Calibrations for spectra captured by the two instruments were developed using WINISI II software version 1.5 (Infrasoft International, Port Matilda, PA). The modified partial least-squares (MPLS) regression method was used to obtain NIR equations for all the studied parameters. MPLS is a variant of partial least-squares (PLS) regression, which is similar to principal component regression (PCR) but uses both reference data (chemical, physical, etc.)

Table 1. Descriptive Statistics for Set 1 (%) and Set 2 (%)

constituent	N	mean	SD	range	set
crude protein	398	18.46	3.63	12.40–33.10	1
crude fiber	398	7.91	5.17	1.70–45.63	1
sunflower meal	393	5.82	8.10	0–30.00	2
mineral−vitamin premix	393	0.28	0.22	0–3.00	2

and spectral information to form the factors useful for fitting purposes (*20*). In MPLS, the NIR residuals at each wavelength, obtained after each factor is calculated, are standardized (divided by the standard deviations of the residuals at a wavelength) before calculating the next factor.

When developing MPLS equations, cross-validation is recommended in order to select the optimal number of factors and avoid overfitting (*21*). In all cases, cross-validation was performed by splitting the population into eight groups.

All multivariate regression equations were obtained using the standard normal variate and detrending methods for scatter correction (*22*). Moreover, four derivative mathematical treatments were tested in the development of NIRS calibrations—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 (*23*).

The statistics used to select the best equations were the coefficient of multiple determination (R^2) and the standard error of cross-validation (SECV). Another statistic used was RPD, i.e. the ratio of the standard deviation to the SECV of the reference data (SD) (*24*). The RPD should ideally be at least three for quantitative purposes (*25*).

Before performing calibrations, a spectral outlier detection routine was followed for the elimination of samples with atypical spectra. The CENTER algorithm (*26*) used starts by performing principal component analysis, which reduces the original spectral information (log 1/*R* values) to a few linearly independent variables, thus facilitating the calculation of spectral distances. Having obtained these new variables, the center of the spectral population is calculated, together with the distance (expressed as Mahalanobis distance *H* and called global *H*) from each sample of the initial set to that center. The algorithm ranks samples according to their distance from the center of the group. Experience has shown that samples with distances over three can be considered as potential spectral outliers (*27, 28*). This limit (global $H > 3$) was used here.

When performing PLS regressions, two outlier tests are also recommended. One of these tests uses the Mahalanobis distance and calculates the global *H*. The *H* calculation during calibration is constituent-dependent, since it is based on the PLS scores for each regression equation. To minimize the risk of removing distant but valuable spectra, only samples with *H* values greater than 10.0 were considered as outliers (*29, 30*). The second test is the Student *t* test, which provides criteria for assessing the variation between a predicted value and its primary chemical value. To avoid having to refer to the *t* tables, a rule of thumb is that *t* values of greater than 2.5 are considered significant, and those predicted analyses having such large *t* values may possibly be outliers (*31, 32*).

RESULTS AND DISCUSSION

The mean, standard deviation (SD), and range values for chemical and ingredient composition are given in **Table 1**. The wide range and the standard deviation for crude protein and fiber confirm the wide diversity of compound feedstuffs in the calibration set. Protein and fiber were selected because both are considered as the main constituents that must be controlled in most feed ingredients and, with greater frequency, either weekly or in every load (*33, 34*). There is therefore considerable interest in measuring these parameters online, in a first approach to quality assurance. Sunflower meal and mineral−vitamin premix were selected as being representative of materials usually included in feed formulation in varying amounts.

Spectral Outlier Detection and Interpretation. Database structure and spectral quality are important aspects of NIR calibration development (*24, 29*). Therefore, prior to calibration, the CENTER algorithm was applied to both sets of samples studied (sets 1 and 2). The *H* values should be used to identify the most extreme spectra. Then a determination needs to be made as to which spectra are mistakes and which are legitimate, knowing that samples with the most extreme reference values will also have extreme spectra (*30*).

When CENTER algorithm was applied, samples selected as outliers were rescanned in order to determine whether they were actually spectral outliers, poorly represented samples, or misscanned samples. After that, the CENTER algorithm was again applied and spectral outliers ($H > 3$) were detected. No misscanned samples were detected.

A detailed inspection of the spectral outliers of set 1 showed that they included extruded compound feeds of varying colors; extruded feeds are intended for pets and are usually presented in brown colors. However, these outlier feeds were the only extruded samples presented in different colors within the population (**Table 2**). Traditional chemical analyses of these samples detected no abnormal values, so color seemed to account for this outlier identification. Other samples considered as outliers by both instruments were poultry feeds presented in crumb form; these were not numerous and were poorly represented in the population. In addition, several samples were considered spectral outliers only when scanned with the monochromator. These samples (pellets with a diameter of 2 mm, intended for milk-fed lambs, and beef cubes to be delivered on the ground) were also poorly represented in the sample set as a whole. The monochromator may have classified more samples as outliers (**Table 2**) due to lower sampling intensity resulting from the smaller window size. All set 1 samples considered as spectral outliers were removed from the calibration set but not discarded. In all cases, they constituted a small proportion of the sample set; in the future, as the number of samples of this kind increases, they will be added and used for recalibration.

Set 2 samples classed as spectral outliers using both the CORONA−Haldrup device and the Foss NIRSystems 6500 were also studied. Again, both instruments classed extruded pet food samples of different colors and poultry-feed samples in crumb form as spectral outliers. The sample presented in pellet form, diameter 2 mm, and the cattle-feed samples for delivery on the ground were also detected as outliers by both instruments (**Table 3**). As for set 1, spectral outliers in set 2 were eliminated but not discarded for the future.

Means, standard deviation, and ranges for CP, CF, SFM, and MVP in the new calibration sets obtained after outlier elimination are shown in **Table 4**. It should be noted that, despite outlier removal, chemical diversity was maintained in the calibration sets, as demonstrated by the broad range of analytical values obtained.

NIRS Calibrations for Prediction of Chemical Composition. Once the initial population was free of spectral outliers, MPLS regression equations were obtained. During calibration development, the calibration set was further refined in terms of both spectral ($H > 10$) and chemical outliers ($t > 2.5$).

Crude protein calibrations developed with the monochromator instrument displayed only two samples with *H* values greater than 10. For crude fiber, only one sample was detected with $H > 10$. In both cases, it was confirmed that these samples were quite different and distant from the rest of the population. With the diode-array instrument only one sample had an *H* value above 10. This was the only sample with a pellet diameter

Table 2. Spectral Outliers of Set 1

FNS-6500			CORONA−Haldrup		
samples	global *H*	species and physical features of samples	samples	global *H*	species and physical features of samples
13931	3.085	extr diff colors pets	13931	4.499	extr diff colors pets
13934	3.484	extr diff colors pets	13934	4.180	extr diff colors pets
14443	3.018	extr diff colors pets	14443	5.116	extr diff colors pets
14927	4.406	extr diff colors pets	14927	3.605	extr diff colors pets
14929	3.520	extr diff colors pets	14929	3.419	extr diff colors pets
14933	4.258	extr diff colors pets	14933	3.843	extr diff colors pets
14936	3.854	extr diff colors pets	14936	3.522	extr diff colors pets
10386	3.179	crumbs for poultry	10656	4.203	crumbs for poultry
10656	3.303	crumbs for poultry	10798	3.865	crumbs for poultry
10798	3.545	crumbs for poultry	14403	8.588	crumbs for poultry
10947	3.392	crumbs for poultry	14631	5.304	crumbs for poultry
14404	3.460	crumbs for poultry			
14631	5.701	crumbs for poultry			
14410	3.671	pellet < 2 mm for lambs			
14943	3.010	beef cubes			

Table 3. Spectral Outliers of Set 2

FNS-6500			CORONA−Haldrup		
samples	global *H*	species and physical features of samples	samples	global *H*	species and physical features of samples
13571	4.545	extr diff colors pets	13571	3.872	extr diff colors pets
13572	4.870	extr diff colors pets	13572	4.616	extr diff colors pets
13489	3.429	extr diff colors pets	13489	4.135	extr diff colors pets
13931	3.827	extr diff colors pets	13931	3.953	extr diff colors pets
13934	3.470	extr diff colors pets	13934	4.827	extr diff colors pets
14443	3.544	extr diff colors pets	14443	4.020	extr diff colors pets
9946	3.122	crumbs for poultry	10304	3.059	crumbs for poultry
10299	3.658	crumbs for poultry	10656	8.794	crumbs for poultry
10656	3.479	crumbs for poultry	10798	3.305	crumbs for poultry
10798	3.024	crumbs for poultry	10874	3.638	crumbs for poultry
14552	3.520	crumbs for poultry	14403	8.020	crumbs for poultry
14631	4.960	crumbs for poultry	14631	5.184	crumbs for poultry
14643	4.152	crumbs for poultry	14657	3.158	crumbs for poultry
14410	3.413	pellet < 2 mm for lambs	14410	4.023	pellet < 2 mm for lambs
10950	3.087	beef cubes	14570	3.231	beef cubes
14570	3.721	beef cubes			

Table 4. Descriptive Statistics after Spectral Outlier Elimination for Set 1 (%) and Set 2 (%)

constituent	instrument	*N*	mean	SD	range	set
crude protein	grating	383	18.20	3.32	12.40–33.10	1
crude protein	diode array	387	18.24	3.34	12.40–33.10	1
crude fiber	grating	383	8.07	5.19	1.70–45.63	1
crude fiber	diode array	387	8.04	5.18	1.70–45.63	1
sunflower meal	grating	377	5.98	8.16	0–30.00	2
sunflower meal	diode array	378	5.87	8.10	0–30.00	2
mineral-vitamin premix	grating	377	0.27	0.18	0–3.00	2
mineral-vitamin premix	diode array	378	0.28	0.22	0–3.00	2

smaller than 2 mm, and had been classed as a spectral outlier during the first step ($H > 3$) when analyzed with the monochromator, thus confirming that it was not well-represented in the calibration set. For crude fiber, no samples with $H > 10$ were detected with the diode-array instrument.

The second outlier test during calibration development used *t* values. The Studentized residuals from regression models fitted using least-squares is a very common approach to identifying discordant observations in linear regression problems (*35*). Most often, high *t* test values here indicate poor laboratory results or a problem with sample presentation (*31, 32*). The final frequency histograms after outlier elimination for crude protein are shown in parts **a1** and **a2** of **Figure 3** for the monochromator and the diode-array instrument, respectively. It was found that there were more *t* outlier samples with the diode-array instrument, especially around the mean and in the range from 22.5% to the end. This suggested that a better coverage of this range is needed in order to improve predictions for samples in this range. With regard to chemical outliers for crude fiber, the grating monochromator detected more samples, but most were situated in ranges of the constituent where there were more similar samples, possibly indicating that they were redundant. However, fewer samples were detected as outliers by the diode-array instrument, and most belonged to the range 11–13%, which was not well covered (**Figure 3b1,2**).

Differences between the two instruments in outlier detection can be found in the technical differences such as measurement principle. A hypothesis for that could be that each type of instrument may need a different number of samples in the calibration set in order to be able to model the intrinsic chemical and physical characteristics of a given sample.

The performance statistics of NIRS equations, after outlier removal, for predicting the composition of crude protein and crude fiber using calibration set 1 for the two different instruments are provided in **Table 5**.

Results in **Table 5**, and also in **Table 6**, were derived from the cross-validation, considering this method as an internal validation. Shenk and Westerhaus (*27*) report that the SECV is the best single estimate of the prediction capability of the equation and that this statistic is similar to the average standard error of prediction (SEP) from 10 randomly chosen prediction sets. The SECV value has the advantage over use of one

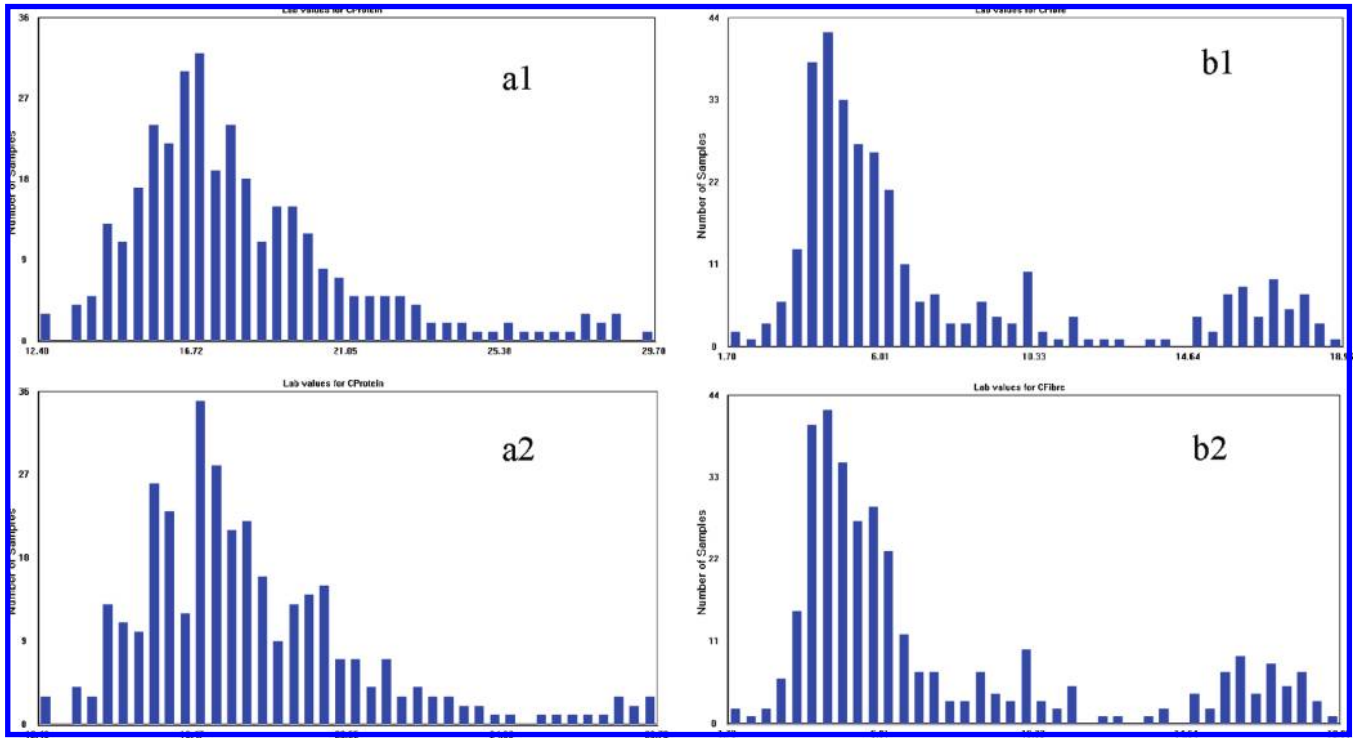

Figure 3. Frequency histograms after chemical outlier elimination for crude protein with monochromator (**a1**) and diode-array instrument (**a2**) and crude fiber with monochromator (**b1**) and diode-array instrument (**b2**).

Table 5. Cross-Validation Statistics for Predicting Chemical Composition (%)

		calibration set				cross-validation statistics			
constituent	instrument	*N*	mean	SD	range	SECV	R^2	RPD	LV
crude protein	grating	337	18.06	3.14	12.40–29.70	0.65	0.96	4.83	14
crude protein	diode array	335	17.94	3.02	12.40–28.70	0.70	0.95	4.31	10
crude protein	grating full range	319	17.99	3.16	12.40–28.96	0.47	0.98	6.72	14
crude fiber	grating	327	7.41	4.43	1.70–18.96	0.58	0.98	7.67	9
crude fiber	diode array	343	7.38	4.35	1.70–18.96	0.85	0.96	5.12	11
crude fiber	grating full range	333	7.76	4.59	1.70–18.96	0.51	0.97	8.83	12

Table 6. Cross-Validation Statistics for Predicting Ingredient Composition (%)

		calibration set				cross-validation statistics			
ingredient	instrument	*N*	mean	SD	range	SECV	R^2	RPD	LV
sunflower meal	grating	333	5.06	7.46	0–30.00	1.27	0.97	5.88	12
sunflower meal	diode array	309	4.02	6.43	0–25.10	0.98	0.98	6.52	15
sunflower meal	grating full range	303	4.58	6.99	0–30.00	0.94	0.98	7.44	15
mineral-vitamin premix	grating	332	0.26	0.14	0–0.50	0.06	0.77	2.06	11
mineral-vitamin premix	diode array	330	0.25	0.14	0–0.50	0.06	0.76	2.03	8
mineral-vitamin premix	grating full range	314	0.26	0.14	0–0.50	0.05	0.83	2.46	12

single validation (prediction set) to produce a SEP value that all the samples representing a given population are contributing to the SECV value. In our case, the use of blind samples to evaluate the prediction capacity of the calibrations should be done once the calibrations have a large number of samples and they become robust enough.

Calibrations for crude protein developed with the diode-array instrument displayed RPD values higher than the recommended minimum (*25*) and very close to those obtained with the monochromator. The crude protein model developed using the diode-array instrument accounted for 95% of the variation existing in the calibration set, showing a very good capacity for quality control of this parameter. Although the SECV was somewhat higher (0.70%) with the diode-array instrument than with the grating monochromator (0.65%), the number of latent variables (LV) used was lower, which yielded a simpler model. As indicated earlier, the literature provides no data regarding the prediction of chemical composition of feedstuffs using diode-array instruments, so the results obtained may only be compared with those reported by other authors for grating monochromators. Results for crude protein obtained in this study compare quite well with the accuracy of equations reported by Verheggen et al. (*36*) with a monochromator and 150 commercial and ground samples. However, for crude protein De Boever et al. (*37*) and Aufrère et al. (*38*) obtained higher SEP values (1.4% and 0.96%, respectively) and slightly lower RPD values (3.43 and 4.02, respectively). This may be due to the smaller number of calibration samples and also to the nature of the samples; most samples used by De Boever et al. (*37*) were experimental feeds made in the laboratory. Other authors (*39, 40*) developed

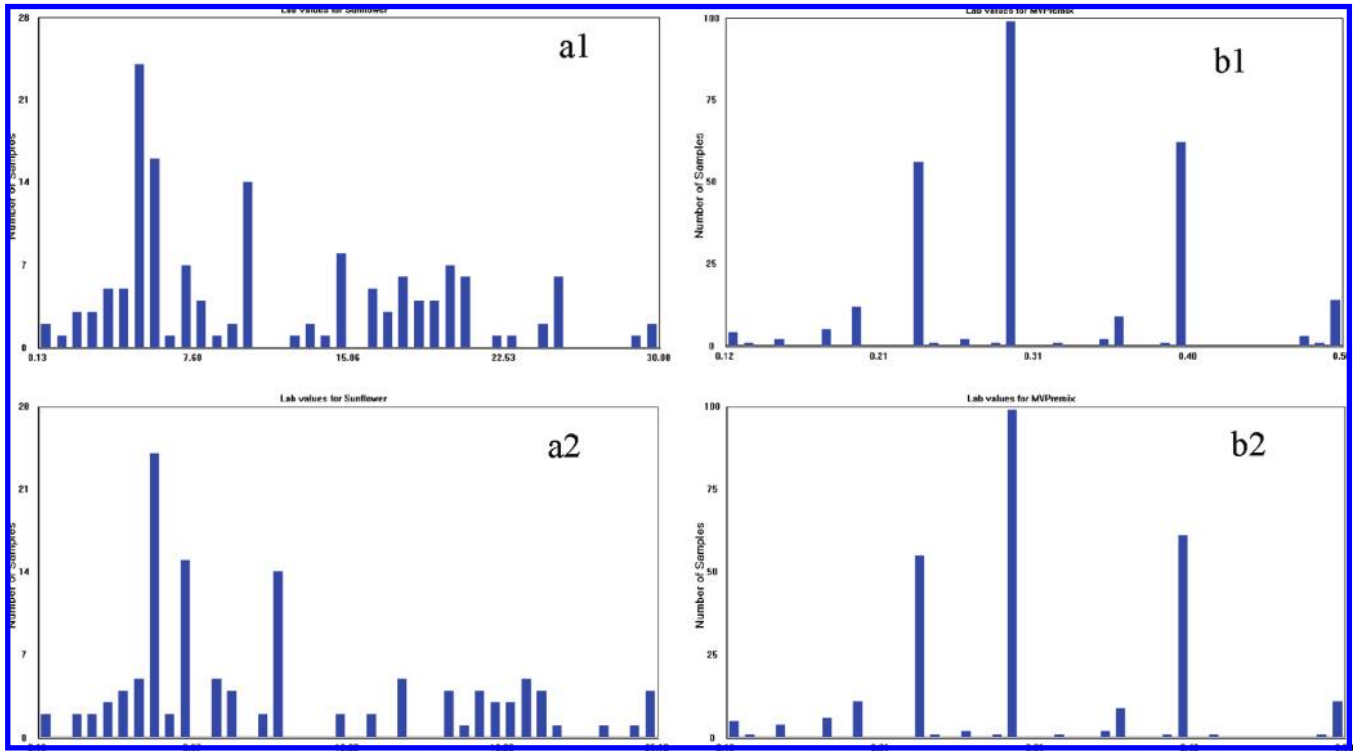

Figure 4. Frequency histograms after chemical outlier elimination for sunflower meal with monochromator (**a1**) and with diode-array instrument (**a2**) and mineral−vitamin premix with monochromator (**b1**) and with diode-array instrument (**b2**).

equations for only one animal species and obtained better SECV values (0.62% and 0.56%, respectively) than those obtained here with the diode-array instrument. Nevertheless, White and Rouvinen-Watt (*41*) evaluated feeds only intended for mink and recorded quite high SECV values (1.75%) for crude protein. However, when using the RPD statistic in order to better compare studies using populations with different mean and SD, it was observed that Xicatto et al. (*40*) reported RPD values of 2.67, not reaching the recommended minimum, while White and Rouvinen-Watt (*41*), whose calibration set had a higher SD, obtained RPD values (3.97) higher than 3 and close to that obtained here. Cizmar et al. (*42*), using a larger number of samples ($N = 650$) in calibration sets, obtained good SECV values (0.5%), but the RPD was slightly below 3 (2.68). Pérez-Marín et al. (*34*) using a monochromator instrument and analyzing the sample in a static environment reported the lowest SECV values and the highest RPD values for crude protein (0.55% and 5.84, respectively). The improvement in results in this case was probably due to the wider wavelength range used for calibrations (400–2500 nm).

Equations for crude fiber developed with the diode-array instrument showed R^2 values of 96% which, together with RPD values of 5.12, indicated very good accuracy and precision for this parameter. However, results obtained with the monochromator were better, and differences between instruments were slightly higher than those observed for crude protein. The diode-array instrument seems to have encountered more difficulties when predicting this constituent. This was also evident in the larger number of latent variables used by the model developed with the diode-array instrument. Comparison with the results reported by other authors using grating monochromators shows that the R^2 and SECV values obtained here with the diode-array instrument compare quite well with those recorded by Verheggen et al. (*36*), Büchman et al. (*39*), and Aufrère et al. (*38*), all of whom used ground samples. However, De Boever et al. (*37*), Xicatto et al. (*40*), and White and Rouvinen-Watt (*41*) obtained poorer results with RPD values lower than 3. This is attributable to the use of calibration sets with more reduced SD, due to the inclusion of feeds intended for a single species. Cizmar et al. (*42*) recorded a lower SD (0.6%) but RPD failed to attain the value of three, also due to a reduced SD, despite a larger number of calibration samples. Pérez-Marín et al. (*34*) obtained RPD and SECV values (7.06 and 0.57%, respectively) similar to those obtained here with the monochromator, confirming that these instruments performed better for the prediction of crude fiber. This better performance can be related to the sensitivity for chemical outlier detection. The monochromator instrument removed more samples as chemical outliers and probably those samples were real chemical outliers because the SECV was lower than with the diode-array instrument, although the mean, SD, and range of calibration set were similar with both instruments. It seems that moving from current table-top-sized NIRS instruments (i.e., FNS-6500) to portable diode-array instruments may compromise resolution, signal-to-noise ratio, sensitivity to outlier detection, and therefore performance.

Most used NIR diode-array instruments cover the range 900–1700 nm and have a price that is half of the conventional scanning monochromators. However, modern NIR diode-array instruments extend the ranges to 2500 nm, but the cost of the instrument rises as the spectral range increases (*43*). From **Table 5**, which shows the equations obtained using the grating instrument and the full spectral range (400–2500 nm), it can be derived that, upon enlarging the spectral range up to 1700 nm, a reduction in the SECV values for the prediction of CP and CF is obtained.

NIRS Calibration Development for Prediction of Ingredient Composition. The European rules and measures relating to the circulation of feed materials and compound feedstuffs highlight the importance of a detailed description of the ingredients used in the manufacture of feedstuffs. For this comparative study, sunflower meal was selected as representative of ingredients present in high amounts in the feed samples

used here and mineral−vitamin premix as representative of ingredients present in low amounts.

A larger number of spectral outliers was detected before calibration using set 2 ($H > 3$), but no samples displayed $H > 10$ either with the monochromator or with the diode-array instrument. However, there were several t outlier samples, especially with the diode-array instrument for sunflower meal. Generally speaking, as **Figure 4** shows, histograms differed from the ideal rectangular-shaped distribution (*24*), but this is a constraint that must be accepted when dealing with real-process samples.

Table 6 shows performance statistics of NIRS equations developed for predicting the percentage of sunflower meal and mineral−vitamin premix included in compound feeds, using set 2 as calibration set, scanned with the two instruments.

R^2 and RPD values showed an excellent predictive ability for determining inclusion percentage of sunflower meal using the diode-array instrument. The accuracy and precision obtained were even slightly better than those recorded with the grating monochromator, although the number of latent variables used by the model was higher.

Results obtained here compare quite well with those ($R^2 = 0.98$, SECV = 0.94) reported by Pérez-Marín et al. (*34*) with intact samples, using a grating monochromator and a wider wavelength range (400–2500 nm). However, Xicatto et al. (*40*) developed a calibration for compound rabbit feeds and recorded RPD values of 1.76 in validation for sunflower meal. The fact that results were less good than those obtained here may be linked to the lower SD of the populations used.

R^2, RPD, and SECV values showed similar degrees of accuracy and precision for determining the percentage inclusion of mineral−vitamin premix in feed compounds with both instruments studied. **Figures 4b1** and **4b2** confirm the similar performance of the two instruments. Although RPD values failed to attain the minimum recommended for quantitative analysis, results are encouraging for a minor ingredient present in feed compound in very low amounts, particularly given that added minerals do not absorb in the near infrared region. Pérez-Marín et al. (*34*) reported higher values of RPD (3.51) for this ingredient, using a wider wavelength range (400–2500 nm) and also a population with a better coverage of the ingredient's range.

Calibration equations for the full spectral range (400–2500 nm) using the grating monochromator instrument were also developed (**Table 6**) and showed a reduction in the SECV for sunflower meal and mineral−vitamin premix.

Results for all parameters studied suggest that calibrations developed with the low-cost diode-array instrument were remarkably accurate, even in dynamic conditions. This good performance may be due in part to the larger scanning area: roughly 150 cm^2 for the diode array on the conveyor belt (94 cm^2 for the grating). This is higher than the scanning area recommended by Brimmer and Hall (*44*) (60 cm^2 or more) in order to improve reproducibility of collected NIR spectra when pellets, tablets, or flakes with highly heterogeneous presentation are being analyzed.

Further studies are needed to evaluate if diode-array instruments with extended spectral range, up to 1700 nm, may bring significant improvement for quality control of other constituents and ingredients in compound feeds.

The results obtained, together with the speed of response and the lower price of diode-array instruments, suggest their viability for use in real online industrial environments. On-site NIRS controls at different stages in the feed industry would make official inspections easier for the authorities and also allow manufacturers to exercise real-time process control, thus ensuring final product quality and minimizing complaints and product recalls.

ABBREVIATIONS USED

NIRS, near-infrared reflectance spectroscopy; VIS, visible; R, reflectance; CP, crude protein; CF, crude fiber; SFM, sunflower meal; MVP, mineral−vitamin premix; SD, standard deviation; N, number of samples of the equation; PLS, partial least-squares; MPLS, modified partial least-squares; PCR, principal component regression; R^2, coefficient of determination, fraction of explained variance for cross validation; SECV, standard error of cross-validation; RPD, ratio of the standard deviation divided by the SECV; SNV, standard normal variate; DT, detrending; LV, latent variables.

ACKNOWLEDGMENT

This work was carried out using NIRS hardware and software from the NIR/MIR Unit of the SCAI and the Animal Production Department (University of Córdoba, Spain). We are grateful to the feed company SAPROGAL for providing samples and reference data.

LITERATURE CITED

(1) Commission of the European Communities. White Book on Food Safety. 12/01/2000. COM (1999) 719 final, Brussels.

(2) Commission of the European Communities. Report from the Commission on the feasibility of a positive list of feed materials. 24/04/2003, COM (2003) 178 final, Brussels, p 31.

(3) Brennan, D.; Alderman, J.; Sattler, L.; O'Connor, B.; O'Mathuma, C. Issues in development of NIR micro spectrometer system for on-line process monitoring of milk product. *Measurement* **2003**, *33*, 67–74.

(4) Federation Europeenne des Fabricants d'Aliments Composes pour animaux (FEFAC). Feed Safety Management System. In European Feed Manufacturers Guide (EFMC), Brussels, Belgium, 2007; version 1.0.

(5) Roberts, C. A.; Stuth, J.; Flinn, P. Analysis of Forages and Feedstuffs. In *Near-Infrared Spectroscopy in Agriculture*; Roberts, C. A., Workman, J., Reeves, J. B., III, Eds.; ASA, CSSA y SSSA, Inc.: Madison, WI, 2004, pp 231–268.

(6) Garrido, A. La spectroscopie proche infrarouge: Une technologie d'appui pour un service intégral en alimentation animale. In *La spectroscopie infrarouge et ses applications analytiques*, 2nd ed.; Bertrand, D., Dufour, E., Eds.; TEC & DOC: Paris, France, 2006; pp 473–495.

(7) Lindström, H.; Malinen, J.; Marbach, R. Performance evaluation of standard and extended InGaAs detector array spectrometers. In *Near Infrared Spectroscopy: Stretching the NIR Spectrum to the Limit*; Davies, A. M. C., Garrido-Varo, A., Eds.; NIR Publications: Chichester, West Sussex, UK, 2004, pp 99–104.

(8) Brownrigg, J. T. Diode-array spectrometers: Potential use in process monitoring. *Process Contr. Qual.* **1992**, *2*, 1–9.

(9) Bertrand, D.; Baeten, V. Instrumentation. In *La spectroscopie infrarouge et ses applications analytiques*, 2nd ed.; Bertrand, D., Dufour, E., Eds.; TEC & DOC: Paris, France, 2006; pp 247–305.

(10) Dardenne, P.; Femenias, N. Diode array NIR instruments to analyse fresh forages. In *Near Infrared Spectroscopy: Proceedings of the 9th International Conference*; Davies, A. M. C., Giangiacomo, R. Eds.; NIR Publications: Chichester, West Sussex, UK, 1999; pp 121–124.

(11) Von Rosenberg, C.; Abbate, A.; Drake, J.; Mayes, D. A rugged near-infrared spectrometer for real-time measurements of grains during harvest. *Spectroscopy* **2000**, *15*, 34–38.

(12) Welle, R.; Greten, W.; Rietmann, B.; Almley, S.; Sinnaeve, G.; Dardenne, P. Near-infrared spectroscopy on chopper to measure

maize forage quality parameters online. *Crop Sci.* **2003**, *43*, 1407–1413.
(13) Sinnaeve, G.; Herman, J. L.; Baeten, V.; Sadaoui, Y.; Frankinet, M.; Dardenne, P. Quality assessment of wheat and forage using diode array NIR instrument on the harvester. In *Near Infrared Spectroscopy: Stretching the NIR Spectrum to the Limit*; Davies, A. M. C., Garrido-Varo, A., Eds.; NIR Publications: Chichester, West Sussex, UK, 2004; pp 319–325.
(14) Paul, C.; Pfitzner, C. Analytical use of NIR diode array spectrometers on forage harvesters. In *Near Infrared Spectroscopy: Stretching the NIR Spectrum to the Limit*; Davies, A. M. C., Garrido-Varo, A., Eds.; NIR Publications: Chichester, West Sussex, UK, 2004; pp 333–338.
(15) Berzaghi, P.; Serva, L.; Ghiraldi, A.; Vicenzi, M. Use of NIR on a feed-mixer for dairy cow feeding. In *Contents of 2nd International Conference on Embedded Near Infrared Spectroscopy*; Gembloux, Belgium, 18th and 19th November, 2004.
(16) Directive 2002/2/EC of the European Parliament and of the Council of 28 January 2002 amending Council Directive 79/373/EEC on the circulation of compound feedstuffs and repealing Commission Directive 91/357/EEC.
(17) AOAC. *Official Methods of Analysis*; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, 2000; Vol. I.
(18) Mouazen, A. M.; Saeys, W.; Xing, J.; De Baerdemaeker, J.; Ramon, H. Near Infrared spectroscopy for agricultural materials: an instrument comparison. *J. Near Infrared Spectrosc.* **2005**, *13*, 87–97.
(19) Fernández-Ahumada, E.; Garrido-Varo, A.; Guerrero-Ginel, J. E.; Pérez-Marín; D.; Gómez-Cabrera, A. Static versus dynamic NIR analysis of compound feedstuffs. In *Near Infrared Spectroscopy: Proceedings of the 12th International Conference*; Burling-Claridge, G. R., Holroyd, S. E., Sumner, R. M. W., Eds.; Near Infrared Spectroscopy Society Inc.: New Zealand, 2006, pp 651–654.
(20) Martens, H.; Naes, T. Partial Least Squares Regression (PLSR). In *Multivariate Calibration*; John Wiley and Sons, Ltd; Wiley, Chichester, 2001, pp 116–165.
(21) Shenk, J. S.; Westerhaus, M. O. *Analysis of Agriculture and Food Products by Near Infrared Reflectance Spectroscopy*; Foss NIR-Systems: Silver Spring, MD, 1995.
(22) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. *Appl. Spectrosc.* **1989**, *43*, 772–777.
(23) ISI. The complete software solution using a single screen for routine analysis, robust calibrations, and networking. In *Manual*; FOSS NIRSystems/TECATOR Infrasoft International, LLC: Silver Spring, MD, 2000.
(24) Williams, P. C. Implementation of near infrared technology. In *Near-Infrared Technology in the Agricultural and Food Industries*, 2nd ed.; Williams, P. C., Norris, K., Eds.; American Association of Cereal Chemists Inc.: St. Paul, MN, 2001; pp 145–170.
(25) Williams, P. C.; Sobering, D. How do we do it: A brief summary of the methods we use in developing near infrared calibrations. In *Near Infrared Spectroscopy: The Future Waves*; Davies, A. M. C., Williams, P. C., Eds.; NIR Publications: Chichester, West Sussex, UK, 1996; pp 185–188.
(26) Shenk, J. S.; Westerhaus, M. O. Population definition sample selection and calibration procedures for near infrared spectra and modified partial least squares regression. *Crop Sci.* **1991**, *31*, 469–474.
(27) Shenk, J. S.; Westerhaus, M. O. Calibration the ISI way. In *Near Infrared Spectroscopy: The Future Waves*; Davies, A. M. C., Williams, P. C., Eds.; NIR Publications: Chichester, West Sussex, UK, 1996; pp 198–202.
(28) Shenk, J. S.; Workman, J. J.; Westerhaus, M. O. Application of NIR Spectroscopy to Agricultural Products. In *Handbook of Near Infrared Analysis*, 2nd ed.; Burns, D. A., Ciurczak, E. W., Eds.; Practical Spectroscopy Series: Marcel Dekker: New York, 2001; pp 419–474.
(29) Shenk, J. S.; Westerhaus, M. O. Population structuring of near infrared spectra and modified partial least squares regression. *Crop Sci.* **1991**, *31*, 1548–1555.
(30) Westerhaus, M., Workman, J., Jr., Reeves, J. B., III; Mark, H. Standardization and Calibration Transfer. In *Near-Infrared Spectroscopy in Agriculture*; Roberts, C. A., Workman, J., Reeves, J. B., III, Eds.; ASA, CSSA y SSSA, Inc.: Madison, WI, 2004; pp 133–174.
(31) Mark, H.; Workman, J. Calibration: Auxiliary statistics for the calibration model. In *Statistics in Spectroscopy*; Academic Press, New York, 1991; pp 293–302.
(32) Workman, J. J. NIR Spectroscopy Calibration Basics. In *Handbook of Near Infrared Analysis*, 2nd ed.; Burns, D. A., Ciurczak, E. W., Eds.; Practical Spectroscopy Series, Marcel Dekker: New York, 2001; pp 91–128.
(33) Lenser, G. W.; McEllhiney, R. R. *Feed Manufacturing Technology IV*; McEllhiney, R. R., Ed.; American Feed Industry Association, Inc. Arlington, VA, 1994; p 282.
(34) Pérez-Marín, D.; Garrido-Varo, A.; Guerrero-Ginel, J. E.; Gómez-Cabrera, A. Near-infrared reflectance spectroscopy (NIRS) for the mandatory labelling of compound feedstuffs: chemical composition and open-declaration. *Anim. Feed Sci. Technol.* **2004**, *116*, 333–349.
(35) Martin, M.; Roberts, S. An evaluation of bootstrap methods for outlier detection in least squares regression. *J. Appl. Stat.* **2006**, *33*, 703–720.
(36) Verheggen, J., Dardenne, P., Théwis, A., Biston, R. Application of near infrared reflectance spectroscopy (NIRS) for predicting chemical composition, cellulase digestibility of organic matter and energy values of compound feedstuffs for ruminants. In *Proceedings of the Third International Conference on NIRS*; Biston, R., Bartiaux-Thill, N., Eds.; Agricultural Centre, Gembloux, Belgium, 1990; pp 287–290.
(37) De Boever, J. L.; Cottyn, B. G.; Vanacker, J. M.; Boucqué, Ch. V. The use of NIRS to predict the chemical composition and the energy value of compound feeds for cattle. *Anim. Feed Sci. Technol.* **1995**, *51*, 243–253.
(38) Aufrère, J.; Graviou, D.; Demarquilly, C.; Pérez, J. M.; Andrieu, J. Near infrared reflectance spectroscopy to predict energy value of compound feeds for swine and ruminants. *Anim. Feed Sci. Technol.* **1996**, *62*, 77–90.
(39) Büchman, N. B. The use of a new near infrared transmission (NIT) analyser from Tecator for determining protein and fat in animal feeds. In *Leaping Ahead with Near Infrared Spectroscopy*; Batten, G. D., Flinn, P., Welsh, L. A., Blakeney, A. B., Eds.; NIR Spectroscopy Group, Royal Australian Chemical Institute, Victoria, Australia, 1995, pp 248–251.
(40) Xiccato, G.; Trocino, A.; De Boever, J. L.; Maertens, L.; Carabaño, R.; Pascual, J. J.; Pérez, J. M.; Gidenne, T.; Falcao-E-Cunha, L. Prediction of chemical composition, nutritive value and ingredient composition of European compound feeds for rabbits by near infrared reflectance spectroscopy (NIRS). *Anim. Feed Sci. Technol.* **2003**, *104*, 153–168.
(41) White, M.; Rouvinen-Watt, K. Near-infrared evaluation of wet mink diets. *Anim. Feed Sci. Technol.* **2004**, *111*, 239–246.
(42) Cizmar, D.; Stana, J.; Pospichaloba, M. Feed analysis using NIR spectroscopy. ALVA-Jahrestagung, 29–31 May 2001, der Bundesanstalt für Milchwirstschaft Wolfpassing, pp 133–134.
(43) Shenk, J. S. Are we there yet? In *Contents of 2nd International Conference on Embedded Near Infrared Spectroscopy*; Gembloux, Belgium, 18th and 19th November, 2004.
(44) Brimmer, P. J.; Hall, J. W. Method development and implementation of near-infrared spectroscopy in industrial manufacturing support laboratories. In *Near-Infrared Technology in the Agricultural and Food Industries*, 2nd ed.; Williams, P. C., Norris, K., Eds.; American Association of Cereal Chemists Inc.: St. Paul, MN, 2001; pp 187–198.

Received for review December 4, 2007. Revised manuscript received February 21, 2008. Accepted February 26, 2008. We are grateful to the Spanish MCYT for providing financial support for this study as part of Project INIA RTA2005-00212-C02-00.

JF073534T