Detection and quantification of mammalian meat and bone meal in compound feedingstuffs using NIR

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Introduction

To contribute to the development of new tools and methods for the detection and the quantification of meat and bone meal in the feedingstuffs, the European Project STRATFEED (GRD1-2000-25002) has been launched since the 1st January 2001 in the frame of the Measurement and Testing generic activity of the GROWTH programme (http://stratfeed.cra.wallonie.be). The present work is being undertaken in the frame of this European project.

The objective is the implementation of near infrared (NIR) spectroscopy for detecting and quantifying the illegal addition of mammalian meat and bone meal (MBM) to compound feedingstuffs. To achieve this goal, two complementary calibration strategies have been evaluated. First, the development of a qualitative discriminant model for detecting if a feedingstuff is adulterated or not, and second the development of a quantitative model to estimate the percentage of meat meal in feedingstuffs.

Material and methods

Spectra analysis

A FOSS NIRSystems model 6500 SY-I scanning monochromator (Foss NIRSystems, Silver Spring, MD, USA), equipped with a spinning module was used to measure reflectance spectra from 400 to 2498 nm every 2 nm. The samples were previously ground in a Cyclotec Mill to 1 mm particle size and analysed in the standard ring cups (3.75 cm diameter). Spectra were recorded with the ISI NIRS 3 software ver.3.11 (Infrasoft International, Port Matilda, PA, USA). The samples used are real process feedingstuff specimens (not mixtures prepared in laboratory) provided by feed manufacturers before the prohibition of the use of MBM for production animals in December 2000. These specimens are kept at the Sample Bank of the STRATFEED Project.

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Qualitative strategy

Calibration sets

In the performance of the first strategy for developing a qualitative discriminant model, two different calibration sets have been tested to obtain the prediction equations:

- Set 1, with 1018 samples. The reference data of 560 of these samples were obtained from the formulation declared by the feed company and the other 458 data were estimated from optical microscopy
- Set 2, with 560 samples. The reference data of sample Set 2 were obtained all of them from the feed formulation.

Data treatment

MPLS regression equations were developed using the two calibration sets described. A score of two was used to identify samples adulterated with MBM and a score of one was used for samples free of MBM. The software Win ISI ver. 1.05 was used to perform the models.[3] The statistics used to select the best equations were the coefficient of determination (r^2) and the standard error of cross validation (*SECV*).[3]

Quantitative strategy

For the second strategy, in which the purpose is the development of quantitative models to predict the percentage of MBM added to compound feeds, two calibration sets with two different variation ranges for the constituent "% MBM" were also studied.

- Set 3, with 630 samples, using the total range of the available samples (Range = 0.0–31.9%).
- Set 4, with 531 samples, using a reduced variation range (Range = 0.0–8.0%).

The percentage of meat and bone meal declared by the feed company was used as reference data.

Data treatment

MPLS regression equations were developed using the two calibration sets described for the quantitative strategy. Several derivative treatments were tested. In all cases, the NIR spectral range (1100–2500 nm) and the *SNV* and Detrend methods for scatter correction were selected for the performance of the calibrations. The software Win ISI ver. 1.05 was used.[3] The statistics used to select the best equations were the coefficient of determination (r^2), the standard error of cross-validation (*SECV*), the *RPD* and the *RER* values.[3,4]

Validation set

The two best models obtained for both strategies have been validated with a blind test set of nine real process compound feedingstuffs, belonging to the Stratfeed Sample Bank. The percentage of MBM in this set ranged from 0% to 6%.

Results and discussion

Strategy 1: detection of MBM

Table 1 shows the calibration statistics corresponding to the qualitative models developed to detect if a specimen have been adulterated with MBM. The best results were obtained using the calibration Set 2, that is the one in which all the reference data were obtained from the feed

formulation. Thus, for Set 1 the standard error of cross validation (*SECV*) almost doubled (0.32%) the one obtained with the Set 2 (0.18%), with only a 57% of the variance in the reference data explained, while for Set 2 the coefficient of determination (r^2) was 0.87.

Table 1. Calibration statistics obtained with Sets 1 and 2 to detect MBM in compound feeds (qualitative strategy).

	Set 1	Set 2
N	1018	560
Mean	1.45	1.54
Range	1.00–2.00	1.00–2.00
SD	0.50	0.50
SECV	**0.32**	**0.18**
r^2	**0.57**	**0.87**

The better precision and accuracy show by the model developed with Set 2 were confirmed with the validation procedure that was carried out. The NIR predicted values (adulterated or free of MBM) of the validation set are shown in Figure 1. Two types of misclassification errors can be found. False positives, that mean specimens free of MBM but classified as adulterated. False negatives, that are specimens adulterated but classified as free. It can be observed that when the model developed with Set 1 is validated with the 9 blind compound feedingstuffs, two misclassified samples were obtained: sample 103 as false positive and sample 114 as false negative. For the model performed with Set 2, only the sample 114 is misclassified (false negative).

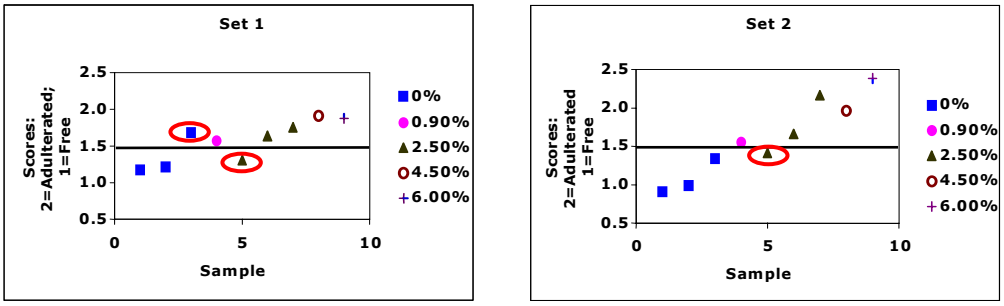

Figure 1. NIR Values predicted for the validation set using the models developed with the Sets 1 and 2 to detect MBM in compound feeds (qualitative strategy).

Strategy 2: quantification of %MBM

The MPLS calibrations statistics for predicting %MBM in compound feedingstuffs are shown in Table 2. The statistics *SECV*, r^2, *RPD* and *RER*, confirm an excellent precision and accuracy of the calibrations developed for the screening of large collection of samples. However, the predictive ability of the equations may be improved by better covering the range with samples of low MBM percentages. Therefore, it can be observed that with the available samples is more adequate to use the equation developed with the calibration Set 3 (see RPD and RER values), which uses the total available range of % MBM.

Table 2. Calibration statistics obtained with Sets 3 and 4 to predict % MBM in compound feeds (quantitative strategy).

%MBM	Total range Set 3	Reduced range Set 4
Range	0.00–31.90	0.00–8.00
Mean	3.31	1.68
SD	5.30	2.33
SECV	0.84	0.72
r^2	0.97	0.91
RPD	6.30	3.24
RER	37.98	11.11

These results were confirmed with the external validation carried out (Table 3). The best prediction values were obtained with the calibration Set 3 too. It can be observed that the *SEP* value obtained with the calibration developed with the Set 3 is half of the *SEP* obtained with Set 4.

Most of the samples in the calibration Sets 3 and 4 were from Spanish feed companies, while the validation samples 102, 103 and 111 were from Belgium. As indicate the *H* statistic values obtained ($H < 3$), all the validation samples could be predicted without any extrapolation of the models, with the exception of the sample number 103. That sample has an spectrum rather different to the others with a high *H* values (Table 3). However, both quantitative models produce negative values for that sample, declared as "free of MBM". Sample 114 which was classified as "free of MBM" by the qualitative models (Figure 1), is very well predicted by the quantitative models. However, the confidence intervals for the predictions obtained by qualitative models should be further studied.

Table 3. % MBM NIR predicted values for the validation set using the calibrations developed with Sets 3 and 4 (quantitative strategy).

Sample	Ref. data	Eqa Set 3 (total range)	Eqa Set 4 (reduced range)	$GH_{Set\ 3}$	$GH_{Set\ 4}$
102	**0.9**	**1.3**	**2.3**	1.573	1.464
103	**0.0**	**–0.4**	**–0.1**	**6.083***	**5.745***
111	**2.5**	**3.1**	**2.0**	1.724	1.462
112	**2.5**	**3.5**	**3.3**	0.307	0.347
113	**4.5**	**5.2**	**2.8**	1.280	1.324
114	**2.5**	**2.9**	**1.9**	0.697	0.750
115	**0.0**	**0.4**	**0.4**	0.784	0.722
116	**0.0**	**–0.7**	**–1.9**	1.485	1.514
120	**6.0**	**6.3**	**4.4**	1.629	2.124
SEP		**0.59**	**1.18**		
Bias		**-0.31**	**0.42**		

Conclusions

The calibration and validation results confirm that NIRS could provide the industry and inspection bodies with a fast screening procedure for detection and quantification of MBM in the vast volume of feedingstuffs traded globally. The predictive ability of the equations could be improved by better covering the range with samples of low MBM percentages, and also by a better design of the calibration set in balancing the proportion of adulterated and unadulterated specimens. On-going research within the STRATFEED project is addressing these issues by producing chemometric models with differently structured calibration sets.

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