Preliminary results in the determination of meat and bone meal in feedingstuffs

S. Termes,[a] R. Tauler[b] and A. Puigdomènech[c]

[a]*Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia i Pesca (DARP), Generalitat de Catalunya, Ctra. Vilassar a Cabrils, s/n, Cabrils, Barcelona, Spain*

[b]*Departament de Química Analítica, Universitat de Barcelona, Av. Diagonal 647, Barcelona, Spain*

[c]*Departament d'Universitats, Recerca i Societat de la Informació (DURSI), Gabinet Tècnic de Recerca,. Generalitat de Catalunya, Via Laietana 33, Barcelona, Spain*

Introduction

Since 2000, the presence of any processed animal tissue proteins in feedingstuffs destined for ruminants has been banned within the European Union because of its relationship with the propagation of bovine spongiform encephalopathy (or mad cow disease). The standard method of classical microscopy has some limitations like slow speed and difficulties in quantitation. In 2001, a research project called STRATFEED[1] was initiated to improve this classical method and develop new methods for the determination of meat and bone meal in feedingstuffs. The new methods are molecular biology, near infrared microscopy and near infrared spectroscopy. In this preliminary study, some of the results obtained with near infrared (NIR) spectroscopy in the STRATFEED project will be shown.

Feedingstuffs have a complex matrix with a composition changing depending on the type of animal fed and the price and geographical availability of ingredients. Meat and bone meal (MBM) also differ depending on the input material and manufacturing process. For these reasons, establishment of a global calibration equation becomes difficult. Previous works have been published on the determination of MBM using NIR spectroscopy.[2,3] In this study Near infrared spectroscopy is attempted for the quantitation of meat and bone meal in feedingstuffs samples. Several aspects are specially considered: (a) using whole NIR spectra or only a selected wavelength spectral range; (b) estimation of the quantitation errors; (c) using different concentration levels of MBM adulteration.

Experimental procedure

Fifty different types of feedingstuffs (among all the received at the Laboratori Agroalimentari de Cabrils, Spain) were first selected. MBM absence of these feedingstuffs was ensured using the classical microscopy.

Using these feedingstuffs, a group of new samples were prepared with different percentage of MBM (coming from rendering Spanish plants) at predefined adulteration levels: 0, 0.1, 0.5, 1, 3, 5, 7, 9 and 27%.

Samples were scanned in a NIRSystems™ 6500 visible-NIR monochromator instrument (Foss, UK). A total number of 642 spectra were obtained.

A subgroup of samples coming from the same type of feedingstuff was selected to evaluate if NIR can be used to control the presence of MBM. Theses samples will not include changes among

different type of feedingstuff samples and they will only reflect changes on MBM concentration levels.

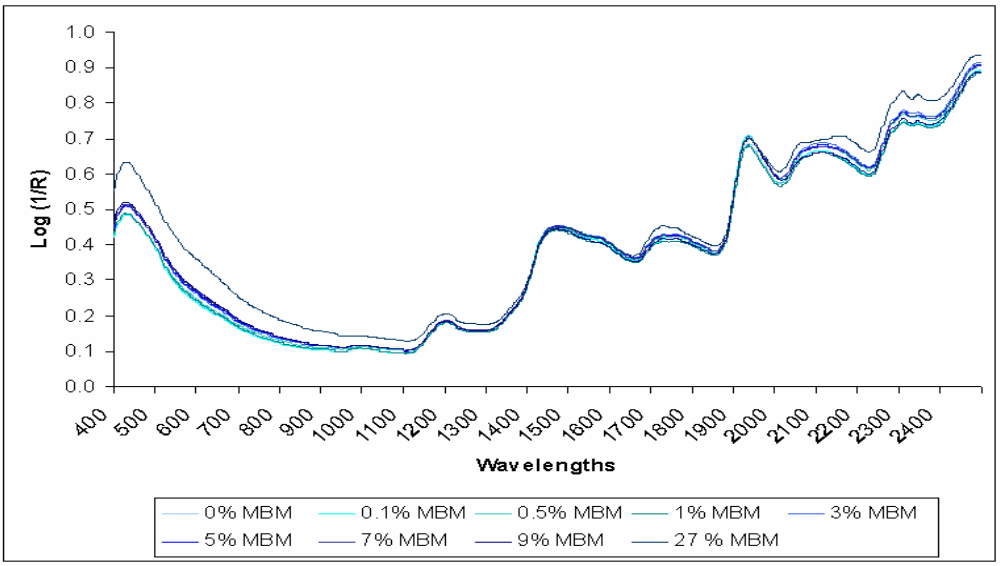

Figure 1. Spectra from same type of feedingstuff subgroup.

Calibrations equations considered: (a) all the adulteration range (0–27% MBM) and (b) a medium-low percentage of MBM adulteration (0–9%).

Additionally, three different spectral ranges of the spectra were tested: (a) 400–2500 nm; (b) 1100–2500 nm and (c) 2288–2314 nm (chosen after a study of the regression coefficients obtained from a previous calibration of the same feedingstuff samples).

The calibrations were done either using all samples or using only with the subgroup of same feedingstuff type samples.

Chemometric methods

- Second derivative and mean centring calculation of the NIR spectra
- Spectral outlier elimination using PCA using Win ISI software (Infrasoft International, USA)[4] (three outlier elimination steps)
- Multivariate calibration and prediction of MBM was performed using PLS with The Unscrambler software (CAMO)[5]

Results

Adulteration percentage range: 0–9% MBM

Subgroup of same feedingstuff type samples

Calibration equations were obtained using full cross validation. The calibration and validation sets had 33 spectra.

Table 1. Results of the calibration of the same feedingstuff samples group (Range 0–9% of MBM)

Spectra range	Nº PC		Predicted vs reference plot							
			Slope	Offset	Correlation	Bias				
400–2500 nm	5	calibration	1.00	4.E-07	0.995	-2.E-07	*RMSEC*	0.31	*SEC*	0.31
		validation	1.02	–0.07	0.982	0.01	*RMSEP*	0.57	*SEP*	0.58
1100–2500 nm	5	calibration	1.00	2.E-07	0.9989	-2.E-07	*RMSEC*	0.14	*SEC*	0.15
		validation	1.00	0.01	0.993	-0.003	*RMSEP*	0.37	*SEP*	0.38
2288–2314 nm	5	calibration	1.00	1.E-06	0.98	-1E-06	*RMSEC*	0.55	*SEC*	0.56
		validation	0.99	0.04	0.97	0.04	*RMSEP*	0.69	*SEP*	0.70

All the samples

In this case validation was done using an external test group. The calibration set had 421 (400–2500nm), 400 (1100–2500 nm) and 436 (2288–2314 nm) spectra and the validation set had 141 (400–2500nm), 139 (1100–2500 nm) and 145 (2288–2314 nm) spectra.

Table 2. Results of the calibration of all the samples (Range 0 – 9 % of MBM).

Spectra range	Nº PC		Predicted vs reference plot							
			Slope	Offset	Correlation	Bias				
400–2500 nm	18	calibration	1.00	2.E-07	0.97	–2.E-07	*RMSEC*	0.84	*SEC*	0.84
		validation	1.01	–0.08	0.95	0.06	*RMSEP*	1.12	*SEP*	1.12
1100–2500 nm	16	calibration	1.000	–2E-07	0.98	–2.E-07	*RMSEC*	0.71	*SEC*	0.71
		validation	1.010	–0.04	0.97	–0.06	*RMSEP*	0.90	*SEP*	0.90
2288–2314 nm	4	calibration	1.000	1E-06	0.38	–1.E-06	*RMSEC*	3.3	*SEC*	3.3
		validation	0.88	–0.6	0.36	–0.3	*RMSEP*	3.4	*SEP*	3.4

Adulteration percentage range: 0–27% MBM

Subgroup of same feedingstuff type samples

Calibrations equations were obtained using full cross validation. The calibration and validation sets had 33 spectra.

Table 3. Results of the calibration of the same feedingstuff samples group (Range 0 – 27 % of MBM).

Spectra range	Nº PC		Predicted vs. reference plot							
			Slope	Offset	Correlation	Bias				
400–2500 nm	5	calibration	1.000	3.E-07	0.9994	–3.E-07	*RMSEC*	0.33	*SEC*	0.33
		validation	1.002	–0.01	0.998	0.01	*RMSEP*	0.50	*SEP*	0.51
1100–2500 nm	5	calibration	1.000	–9.E-07	0.9995	–2.E-07	*RMSEC*	0.28	*SEC*	0.28
		validation	1.002	–0.01	0.9990	0.01	*RMSEP*	0.41	*SEP*	0.41
2288–2314 nm	5	calibration	1.000	–6E-07	0.994	–1.E-06	*RMSEC*	1.01	*SEC*	1.02
		validation	0.993	–0.01	0.991	0.01	*RMSEP*	1.25	*SEP*	1.27

All the samples

In this case, validation was done using an external test group. The calibration set had 436 (400–2500nm), 430 (1100–2500 nm) and 431 (2288–2314 nm) spectra and the validation set had 145 (400–2500 nm), 143 (1100–2500 nm) and 145 (2288–2314 nm) spectra.

Table 4. Results of the calibration of all the samples (Range 0 – 27 % of MBM).

Spectra range	Nº PC		Predicted vs reference plot							
			Slope	Offset	Correlation	Bias				
400–2500 nm	18	Calibration	1.000	4.E-05	0.985	–4.E-05	*RMSEC*	1.14	*SEC*	1.14
		Validation	1.002	–0.09	0.97	0.08	*RMSEP*	1.28	*SEP*	1.14
1100–2500 nm	16	Calibration	1.000	–3.E-07	0.994	3.E-07	*RMSEC*	0.70	*SEC*	0.70
		Validation	0.985	0.12	0.987	–0.06	*RMSEP*	0.88	*SEP*	0.88
2288–2314 nm	4	Calibration	1.000	–1E-06	0.68	1.E-06	*RMSEC*	4.6	*SEC*	4.6
		Validation	1.189	–0.78	0.73	–0.05	*RMSEP*	4.5	*SEP*	4.5

Conclusions

- Calibration and validation in the 0–9% range of adulteration gave better results than in the 0–27% range
- Optimal spectral range for calibration and validation was 1100–2500 nm (*RMSEP* < 0.90 in all cases)
- The 2288–2314 nm spectral range gave only good results when the same feedingstuff group was considered. This suggests that interferences could not be properly modelled using this short wavelength range when all the samples were considered.
- Results show that NIR Spectroscopy is useful for the determination of MBM adulteration in feedingstuff, especially when only samples that proceed from the same feedingstuff type were considered

References

1. www.stratfeed.cra.wallonie.be
2. I. Murray, L.S. Aucott and I.H. Pike, *J. Near Infrared Spectrosc.* **9,** 297 (2001).
3. A. Garrido-Varo and V. Fernández-Cabanás, in *Workshop on "Identification of animal feeds ingredients". Final Report Annex 10*. Ed by J.S. Jφrgensen .Lyngby, Denmark (1998).
4. *WINISI II Manual*. Foss-Tecator Infrasoft International, Silver Spring, MD, USA (1998).
5. K. Esbensen, D. Guyot and F. Westad, *Multivariate Data Analysis in Practice*. CAMO, Oslo, Norway (2000).