Near infrared reflectance spectroscopy as a tool to predict qualitative and quantitative meat and bone meal presence in compound feed

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Introduction

Bovine spongiform encephalopathy (BSE) belongs to the group of diseases called TSE (transmissible spongiform encephalopathys), that can be transmitted between animals and humans. It is one of the most serious problems that has affected the economy of European cattles and public safety.

It is believed that the disease is caused by a natural protein which folds in the wrong way and then causes other similar proteins to change into this shape. The wrong shape form gradually builds up and spreads. These proteins are called prions. Possible sources or vehicles of infection could be imported cattle, contact with sheep, contact with wildlife and contaminated biological products, including foodstuff. In the case of the latter, the most likely vehicle for infection is meat-and-bone meal (MBM) which was included in cattle rations as a source of valuable bypass protein until it was banned.[1,2] The prohibited use of any animal tissue in animal feed means that a fast and reliable analytical methods to identify those ingredients in compound feed is needed.[3] The ban has been extended at the moment until 1st January 2002 (Regulation 1326/02001/EC of 29/06/01). Nowadays the official method for MBM detection in compound feeds is a classic microscopy technique, although other techniques such as polymerase chain reaction (PCR), ELISA, NIR and NIR microscopy are being studied.[3]

This official methodology is a subjective tool and requires exhaustive quantitative analysis and needs to differentiate between mammalian and poultry bones. In adittion, the separation of the different fractions in a sample by density before the analysis requires the use of organochlorate products such as tetracholromethane (CCl_4), which cause serious damage in the atmosphere's ozone content.

NIR methodology is a possible way to confirm and identify animal ingredients in compound feed. The capabilities for quantitative and qualitative analysis of feed by NIR has already been demonstrated many times.[3–6]

The purpose of this preliminary study was to test NIR methodology as an alternative tool to microscopy for qualitative and quantitative analysis to detect MBM in compound feed and to test the better samples presented for analysis (ground or intact samples) using NIR.

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Materials and methods

Population

A total of 264 ground samples (population A) and 121 intact commercial samples, pellets, small pills and flour (population B), were used to create a spectral database.

Set A was built with 133 non-adultered compound feedstuffs, 113 with the addition of MBM as a cross-contaminant, adultered or made experimentally with different weight proportions to obtain high MBM levels and 18 raw MBM samples.

Set B was created using a similar procedure to set A which included 74, 41 and 6 samples, respectively for each group.

In the adultered compound feed, the MBM quantitative analysis was performed using microscopy technology as the reference method after making some modifications.[7]

NIR scanning and calibration procedures

The samples belonging to population A were milled at 0.75 mm. The rest (population B) were used in their intact form. Both populations were scanned using a Foss-NIRSystem 6500 monochromator with transport sampler, over a wavelength range from 400 to 2500 nm in steps of 2 nm.

Every sample was measured in two replicates and the average of the replicate spectra obtained as log 1/*R* (*R* = Reflectance) was used in the calibration. Population boundaries were established with a maximum standardised *H* distance from the average spectrum of 3.0.[8] The discriminant procedures and the calibration equations for ground and intac feedstuffs were obtained using WINISI II v 1.02 sofware (Infrasoft International, Port Matilda, PA, USA), using the full wavelength range.

For the discriminant analysis, each population was divided into three sets depending on the MBM percentage (0, 0.05–90 and 100) using partial least square (PLS) as the discriminant procedure. Modified partial least square (MPLS) was used as the regression method for quantitative analysis.

Standard normal variate and detrend[9] (SNVD) was used for scatter correction in population A and standard normal variate (SNV)[10,11] for population B. Spectral data were transformed using second derivative as the mathematical treatment.

Results and discussion

In Figures 1 and 2 we can see the absorption spectra of ground and intact compound feed samples according to their different MBM content. The major variation sources between spectra of each group are related to protein and oil absorption bands in relation to MBM percentages.

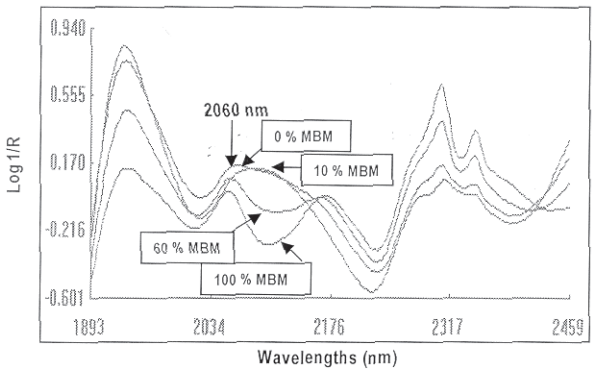

Figure 1. NIR absorption spectra of ground compound feed with different MBM percentage.

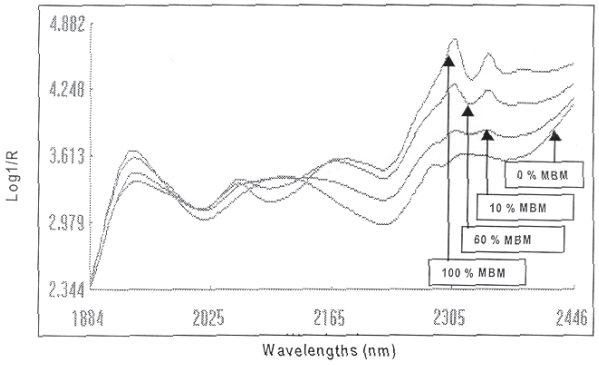

Figure 2. NIR absorption spectra of intact compound feed with different MBM percentage

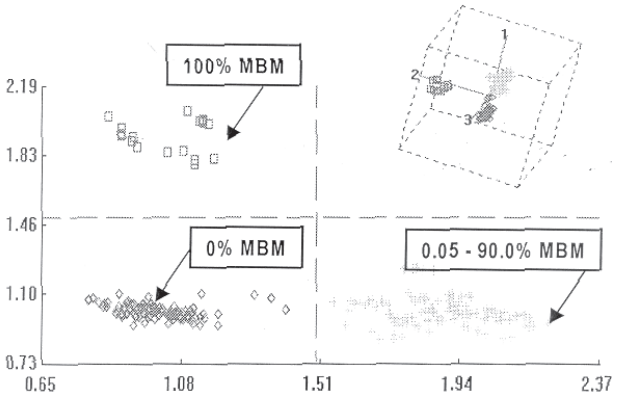

Figure 3. Spacial classification of ground compound feed according to different MBM contents after discriminant analysis.

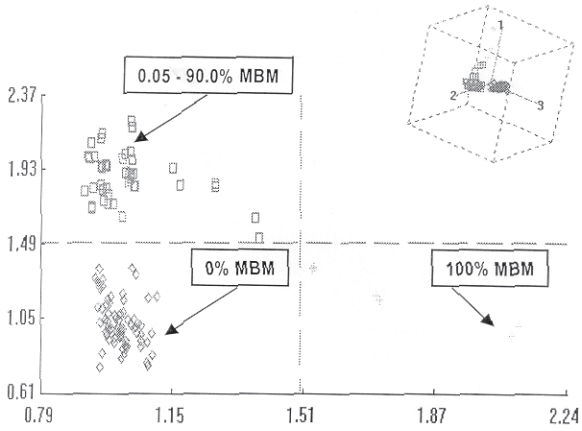

Figure 4. Spacial calssification of intact compound feed according to different MBM contents after discriminant analysis.

Table 1. Statistical results of calibrations and population characteristics for quantifying meat and bone meal in compound feed.

	SEC	R^2	*SECV*	r^2	Range	*SD*
Ground samples	0.895	0.999	0.988	0.999	0–100	31.932
Intact samples	1.008	0.999	1.328	0.998	0–100	28.571

Qualitative analysis

Discriminant PLS equations were developed to identify the presence of MBM, banned as an ingredient in compound feed composition.

The discriminant models used for ground and intact samples employed six factors for each. The general concept indicates that a predicted value of 2 is a perfect identification, 1 as not in the group and 1.5 indicates that the classification could go either way.[12]

Spacial classification for ground and intact compound feed, according to different MBM content after discriminant analysis in the calibrations sets, are show in Figures 3 and 4. The correct classification accuracy of these models was 93.25% and 91.74% for ground and intact samples, respectively. The main problem in these models is the accurate discrimination of low MBM levels, always lower than 0.2%.

A test set of six samples with known concentrations of MBM was used. These samples were a perfect match.

Quantitative analysis

Table 1 shows the characteristics of population and stastistical results for calibration and cross-validation to estimate the proportion of MBM in compound feed.

The calibrations for MBM show a good predictive capacity on ground compound feed as well as in tact compound feed, with r^2 values greater than 0.99 and low *SECV* (0.988 and 1.328, respectivily) which agree with the results reported by Garrido and Fernández.[13] Nevertheless, these error values are elevated to predict cross-contamination or low level adulteration by MBM in compound feed.

Conclusions

The preliminary results obtained showed that it is possible to achieve similar accuracy in identifying the presence of meat and bone meal using either ground or intact compound feed. To get the best results using NIR it is recommended that intact samples are used for analysis. However, it is necessary to add more samples to achieve the best possible representation of low levels of MBM, especially between 0.05 and 1% of the population.

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