P.26.- An original strategy coupling NIRM and PCR for detection and species identification of MBM particles

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The potential of near-infrared microscopy (NIRM) for the detection of meat and bone meals in feed was demonstrated by researchers of CRA-W. Wide spectral libraries including thousands spectra from particles of animal, vegetal and mineral ingredients used for the preparation of compound feeds were built and discriminant models have been developed to predict the origin of unknown particles. Five discriminant equations (between 2 and 4 used simultaneously) decide about the group assignation of the particles with a success rate of more than 90 %. Defined groups are : 1) vegetal ; 2) animal ; 3) terrestrial animal ; 4) fish ; 5) bovine & pig ; 6) poultry[1]. Up to now, discrimination between bovine and pig particles remains impossible by NIRM. Moreover, a positive detection obtained by NIRM always needs confirmation with a forensic method. As the NIRM methodology is a non destructive analysis, the particles classified as animal origin can be selected and analysed by another technique like PCR. Certified pure species animal meals were used to test the potential of PCR to amplify DNA targets directly from singel particles identified as belonging to terrestrial animal.

In the analytical procedure, animal meat and bone particles were spread on a spectralon plate and presented to the NIR microscope for spectrometric analysis. Then some particles were recovered and put each separately into the wells of the PCR plate. Real Time PCR protocol developed at CRA-W targetting short mitochondrial DNA fragments[2] was used to confirm the species origin of these particles. The results obtained showed that a single particle can be used directly as 'template' for PCR with an interesting rate of success. In that case, great care should be taken to the environment during the preparation of the PCR plate to avoid any air contamination. Efforts will be made to improve again the rate of successful amplification and to develop a system allowing to test more than one species on the same particle. Tracing back the species origin of the particle by PCR will also allow the building of species specific spectral databases. Based on such libraries, it should be possible to develop models of discrimination between bovine and pig particles. On the other hand, as NIRM is able to discriminate some authorised animal components (e.g. blood and milk powder) from MBM particules, it can help PCR to determine which is the source of the detected DNA.

1 Baeten V., Michotte Renier A., Sinnaeve G. & Dardenne P. (2001). Analyse of feedingstuffs by near-infrared microscopy (NIRM) : detection and quantification of meat and bone meal (MBM). In *Proc. of the sixth International Symposium on food authenticity and safety*, 28-30 November 2000, Nantes, 1-11.
2 Dubois M., Fumière O., von Holst C. & Berben G. (2002). - Meat and bone meal detection in feed by search of specific animal DNA segments. *181st meeting of the Belgian Society of Biochemistry and Molecular Biology*, 4th of May, Katholieke Universiteit Leuven (KUL), Heverlee (Belgium) abstract nr. 7, (*http://www.biochemistry.be/4may2002/abstracts_1_13.htm#dubois*).

Keywords
NIRM, PCR, particles, MBM