

P.25.- PCR analysis can help microscopist to identify animal species present in the sediment fraction of compound feed

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Nowadays, the official method for the detection of meat and bone meal (MBM) in animal feed is optical microscopy¹. Successfully validated, the method is limited to the differentiation of large taxonomic groups e.g. terrestrial animal or fish. In some cases, it is possible to distinguish mammalian bones from poultry bones. Nevertheless, it is experimentally observed that the detection of 0.5% of mammalian MBM (MMBM) in a feed containing 5% of poultry meal is very hard even in the sediment fraction which concentrates bone particles. During the STRATFEED project² Real Time PCR protocols able to detect ruminants as well as bovine or pig material were developed and successfully tested on samples such as pure MBMs or feedingstuffs containing MBM. The combination of the two techniques (microscopy and PCR) could answer to the need of control methods allowing for species differentiation required for the ban of intra-species recycling³.

The present work describes the first PCR results obtained with cattle and ruminant targets on DNA extracted from sediment portions of 6 feed samples containing respectively 5% of fish meal, 5% of poultry meal, 5% of poultry meal and 0.5% of MMBM (2 different samples), 0.1% of MMBM and a blank feed. With this limited sample set, some observations can be stated : a) despite sample manipulation with an organic solvent (tetrachloroethylene - Cl₂C=CCl₂) during sedimentation step, DNA can still be extracted and amplified by PCR ; b) several dilutions have to be tested as PCR inhibition can occur ; c) the presence of mammalian material in problematic samples for microscopy (feedingstuffs with 0.5% of MMBM and 5% of poultry meal) can be detected by PCR ; d) even on the sediment portion, the detection at a level of 0.1% of MMBM remains difficult but is possible.

From these preliminary promising results, we can conclude that PCR could be a useful complementary tool to microscopy to determine whether mammalian material is present in a sample containing poultry meal. The PCR analysis of the sediment portion as a way to increase its sensitivity is not evident. Therefore before applying this protocol in routine analyses, it should first be checked on a wider set of representative samples and may probably need some improvements.

References

¹ Gizzi G., van Raamsdonk LWD., Baeten V., Murray I., Berben G., Brambilla G., von Holst C. (2003). An overview of tests for animal tissues in feeds applied in response to public health concerns regarding bovine spongiform encephalopathy. *Rev. Sci. Tech. Off. Int. Epiz.* **22** (1), 311-331.

¹ UE STRATFEED project G6RD-2000-CT-00414, Strategies and Methods to detect and quantify mammalian tissues in feedingstuffs, www.stratfeed.cra.wallonie.be (2001-2004).

¹ Regulation (EC) N°1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. *Off. J. Eur. Comm.* L273, 10/10/2002, 1-95.

Keywords

PCR, microscopy, sediment, species, MBM