L.13.- PCR as tool to identify taxon-specific processed animal proteins

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There is the consolidated evidence that the use of feeds containing specific TSE risk materials in animal nutrition contributed to the spread of BSE among cattle. Because the difficulty to detect directly the causative agents in feeds, the preventive measures have been diverted to the detection and the identification of those animal constituent from those species that could harbour the causative agent.

To this respect, methods based on DNA identification are generally proved to be effective in tracing the origin of specimens. The PCR techniques contribute to amplify the target DNA sequence, thus allowing the detection of small copies of DNA, as in the case of that from heat and pressure stressed animal proteins, the so called PAPs (Processed Animal Proteins). In this work we illustrate the results achieved within the STRATFEED project in developing PCR techniques for a reliable taxon-specific detection of PAPs. The first attention has been focused on the suitability of mitochondrial vs nuclear DNA, and on the length of target amplicons; then, a comparison between classical vs real time PCRs have been made, in order to achieve lower detection limits. To this respect, rather than the total amount of DNA extracted from MBM and feeds, plays a key role its purity, to reduce possible matrix-induced inhibitory effects. In this light, the addition of plasmids as quality control could help the setting of sharper cut-off values for a better discrimination between compliant/non compliant samples. At <1.0% >0.5% MBM contamination level, it seems mandatory to perform different replicates from the same extract, to draw any conclusion about compliance. The PCR approach can allow a taxonomic modulation of the research, at level of ruminants as well as level of single species identification. Reinforcements in the forensic evidence could consist in performing PCRs using two independent and not DNA overlapping target sequences primers, thus achieving more than one information (identification point) on the same sample tested. Nevertheless, the technique requests a strict control of the environmental requirements, to avoid possible cross-contamination and carry-over phenomena during all the procedure, from samples handling to PCR runs. Dedicated rooms and facilities are required, as well Real Time PCR devices.

Keywords :
Real Time PCR, MBM, PAP