P.30.- Methodology to detect animal tissues in feedingstuffs by QRT-PCR

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The current procedures of meat and bone meal (MBM) and species identification are carried out mainly using microscopic methods. This is based on the analysis of animal bone fragments, which are cumbersome, need a lot of effort and specially trained experts, being susceptible to subjective bias.

With the development of molecular biology, new approaches emerged, based on genetic differences between species. In particular, molecular techniques based on the Polymerase Chain Reaction (PCR) offer advantages in that they have the possibility to detect even minute amounts or traces of animal tissues and thus identify the different species included in MBM samples (raw and processed).

The quality of the results are depending on primers design, specially to avoid problems of cross-hybridization between species more closely related (eg., sheep-goat and chicken-turkey). We have developed species-specific oligonucleotide primers, designed from sequence information available in the GenBank database, which hybridize to short sequences of mtDNA and allow the amplification and detect of animal groups (eg., birds, mammals and fishes) and different species during the same analysis.

As a laboratory for the official control of feedingstuffs in the Andalusia region (Southern of Spain), we use methods based on the PCR technique to confirm the results that we obtained by an accredited microscopic method and thus obtain additional information.

The overall aim of our work is to develop and validate a method which could be successfully applied to detect the presence of meat and bone meal (MBM) in feeding stuffs in order to confirm the results obtained by microscopic observation. The establishment of this protocol will be included in the accreditation system in which we are involved.

Following our protocol, we have identified the presence of three animal groups (birds, mammals and fishes) and seven main species (5 mammals and 2 birds) in feedingstuffs. We are also carrying out procedures to detect another 17 extra species (6 birds, 6 mammals and 5 fishes).

Keywords

Species-specific identification, PCR, microscopic methods, MBM