P.24.- Detection of animal material in feeding stuff with PCR

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The first case of Bovine Spongiphorme Encephalopathy (BSE) in Germany in the year 2000 led to a total ban of animal material derived from land living endotherms and fishes in feed for farm animals. (Exception: Fish derived protein products and fats in feed for non ruminants). The ban caused a demand for analytical methods for the detection of animal material in feed. Microscopy constitutes a well established and official method in that field. With microscopy characteristic structures such as bone fragments, muscle fibres, feathers, scales etc. are distinguishable. Limitations of this method are met with material which shows no visible morphological structures like bowels, fat etc. The allocation of microscopically detectable muscle fibres to an animal species is not possible by this technique. Molecular biological methods can expand and support microscopy as DNA based analytical methods are independent from visible structures. The development of a PCR-based method for the detection of animal material in feed is presented. Most important for the specificity of a PCR assay is the choice of appropriate primers for DNA amplification. With universal primers ubiquitous animal DNA sequences like parts of the mito-chondrial cytochrome b gene can be amplified. Animal meal is usually heated under pressure (3 bar) to 133 °C. DNA is degraded by this process. To detect even traces of heavily degraded DNA in a sample, it is crucial to chose primers that will amplify short (< 300 bp) DNA fragments. For primer design sequence homology studies of the cytb gene of 54 different animal species (including all relevant farm animals and domestic animals) were applied. Six newely designed primer pairs were tested in detail, whereof two primer pairs showed best results. With DNA from 22 different animal species these primer pairs amplified a 263 bp and 165 bp fragment. With eleven tested plants including plants common in feeding stuffs no amplicons were generated. With both primer pairs animal derived DNA was detected in different animal meal samples (meal from poultry, fish and different animals). Various tests with spiked feeding stuff samples which are typically investigated in official feed control were successfully done. The two primer pairs were tested in two ring trails. Samples investigated were pure animal meal (100% fish, 100 % poultry, 100 % different animals) and defined mixed feed samples with an animal meal content of 0,1 %, 0,5 % and 3 %. In total some 130 feeding stuff samples with different contents of defined produced animal material from cattle, pig, chicken and sheep were tested. Additionally animal DNA was detected in mixed samples with 0,1 % of the mentioned species. Certain species like cattle were identified by enzymatic cleavage of the amplicons generated with both primer pairs in RFLP-analysis. The designated primer pairs HM15149/HM9 und K12-2/K13 are applicative for the universal detection of animal material in feed admitting the detection of even animal DNA-traces in highly heated samples. The method is part of the „Molecularbiological determination of animal ingredients - PCR-method“ that was validated by 10 German laboratories and now is deposited in the method collection of the German VDLUFA.

Keywords

DNA technology (PCR), universal primer pairs for the detection of animal ingredients