

Proteomics approaches for species and tissues specific differentiation of processed animal proteins in aquafeeds

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Non-ruminant processed animal proteins (PAP) are again allowed to be used in aquafeeds. Currently, light microscopy methods are used to detect PAP when the feed is not supposed to contain PAP or blood products, and an European Union Reference Laboratory (EURL) validated polymerase chain reaction (PCR) based method is used for ruminant DNA-detection when the feed is known to contain PAP or blood products, as indicated from the declaration or the labelling. However, PCR does not allow for a differentiation of cellular origin, and hence cannot be used for tissue specific (i.e. bone, feather etc.) identification. Potential need for identification of sources (tissue) of contamination have sparked the development of analytical methods complementary to PCR. Protein-based methods already are well established and the latest developments in the field of proteomics and proteomics bioinformatics have strongly facilitated the use of these tools in the study of food safety and food authenticity. We investigated different proteomics tools for the detection of species and tissue specific peptide marker candidates of prohibited PAP material of bovine and ovine origin, and allowed PAP material such as porcine (blood, bone-carass, greaves) and poultry (blood, bone-carass, feather) origin. We found that irrespective of sample preparation gel-based proteomics tools are inappropriate when working with PAP. Gel free shotgun proteomics approaches on the other hand are able to provide quality data that can successfully be mined for species specific peptide markers using peptide mass fingerprinting and machine learning. Yet, this approach still fell short when tissue specificity was the goal. Satisfying results in terms of both PAP species and tissue specificity were achieved only when gel-free shotgun proteomics in combination with spectral library based data analysis methods were used. Current work is now focused on the standardization of the protein extraction and digestion procedures for regulatory use and the creation of an extensive freely available PAP spectral library reference collection.