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New developments in the detection and identification of processed animal proteins in feeds

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Abstract

It is generally accepted that the most likely route of infection of cattle with bovine spongiform encephalopathy (BSE) is by consumption of feeds containing low levels of processed animal proteins (PAPs). This likely route of infection resulted in feed bans, which were primarily aimed at ruminant feeds, and were later extended to all feeds for farmed animals. The feed bans were expected to develop into a future enforcement of the "species-to-species" ban, which prohibits only the feeding of animal-specific proteins to the same species. The species-to-species ban requires support of species-specific identification methods.

In the European Union, microscopic evaluation is currently the only accepted method for the detection and characterization of PAPs in feeds, since it is possible to detect contaminations at the requested level of 1 g/kg with hardly any false negative nor positive results. This method is predominantly focused on the presence and characteristics of bone fragments, although other structures, *e.g.* muscle fibres, may provide circumstantial evidence of the respective animal types. Recent developments are the identification of bone fragments at the level of classes (mammal *versus* bird *versus* fish), supported by image analysis of bone characteristics.

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Detection of DNA and specific proteins are additional methods that can be applied for the identification of PAPs in feeds. DNA is known to be very specific for animal species and breeds, whereas proteins can also indicate the type of tissue. The latter aspect is important to differentiate between proteins that are authorised in animal nutrition from banned proteins. Improvements can be noted in recent years for both methods. For a proper application of polymerase chain reaction (PCR) to detect specific sequences of DNA, primer sets have been developed which amplify a DNA sequence shorter than approximately 100 nucleotides. Specific antibodies have been developed for protein detection of ruminant or bovine material. Recent results of various studies indicate that specific DNA and protein detection methods can detect PAPs at a contamination level of 1 g/kg. However, full validation of these methods still needs to be carried out.

Other methods such as near-infrared spectroscopy (NIRS), near-infrared microscopy (NIRM), near-infrared imaging, liquid chromatography (LC) and olfactometry techniques can and will be applied for the detection of PAPs. NIRS is a non-destructive method that can be applied on-line in feed production plants. Generally, the detection limit is still too high to be applied in official control laboratories. Nevertheless, industrial application is feasible. NIRM and near-infrared imaging are techniques that allow collection of near-infrared spectra from individual particles. The level of detection is lower than 1 g/kg since it is based on the microscopic technique, in combination with the option of identification of the individual particles. LC is based on the detection and, if present, the ratio of different polypeptides. For example, carnosine is mainly present in mammals and anserine mainly found in birds. Olfactometry is based on detection of volatile non-specific agents. It is a non-destructive and fast technique. For both LC and olfactometry it appears that the presence of fish material masks the detection of proteins of land animals, even at a contamination level of 5 g/kg.

Since 2003 five different proficiency studies and ring trials have been organized. The first proficiency study, allowing the participants to apply their own protocol, revealed that correct microscopic detection of 1 g/kg of mammalian PAP in the presence of 50 g fish meal/kg was realised in 0.44 of the cases. However, a bench mark study organized in the same year showed that a microscopic detection of 0.98 can be reached provided the application of an optimal protocol and a sufficient level of expertise. More recent studies showed that training, the application of a decision support system and use of an improved microscopy protocol resulted in a higher sensitivity.

An attractive approach is the combination of the very low detection level of microscopy with identification by other methods. Several strategies for a combination of screening and confirmation methods are discussed in the present paper. The new developments in methodology will support current or new legislation (*e.g.* species-to-species ban, general application of fish meal).

Keywords: BSE; Feed ban; Animal proteins; Microscopy; PCR; Immunoassay; NIR; HPLC; Protein analysis

1. Introduction

In the history of development of feeds with a high nutritional value, materials of animal origin were considered appropriate as ingredients in compound feeds. This inclusion was based on natural feeding patterns of carnivorous (*e.g.* fur animals) or omnivorous (*e.g.* pigs) animals. However, the same strategy appeared to be profitable for herbivorous animals as well. Animal by-products can be readily compared to soy bean hulls, but provide a higher amount of fat as energy source, higher levels of protein and minerals (Ca and P), and supply some essential vitamins. For these reasons formulations including significant