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Validation of immunoassays for the detection of processed ruminant proteins in feed materials

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The consumption of food products of animal origin is an inevitable part of our daily diet. As a result of the production of meat, milk and egg products approximately 17 Million Ton of waste animal by-products are produced in the European Union each year. These by-products could be a highly valuable source of nutrients, especially proteins, except for the situation that the consumption by farmed animals is generally prohibited for avoiding mad cow disease (extended feed ban in the European Union, Regulation (EC) No 999/2001). Due to a growing aquaculture industry the demand for high quality proteins for aquatic feeds is increasing. Non-ruminant processed animal proteins (PAPs) have shown great potential for this purpose. A 2% tolerance limit for the presence of ruminant PAP in non-ruminant PAP is shown to have negligible impact on the risk of additional BSE cases. Therefore, for a safe re-introduction of non-ruminant PAPs in aqua feed methods are needed that are able to discriminate between

ruminant and non-ruminant PAPs at this tolerance level. Classical microscopy is the official EU method for detection of processed animal proteins in compound feeds or in their ingredients. However, this method lacks species-specificity. In search for alternative methods the application of immunoassays has the advantage that authorised products such as milk derived products are not detected due the specificity of the target (i.e. troponin I in muscle fibres).

The performance of MELISA-TEK™ Ruminant, a commercially available enzyme-linked immunosorbent assay (ELISA), in combination with the MELISA-TEK high Sensitivity Sample Extraction kit was evaluated. Various non-ruminant PAP samples, with ruminant PAPs (processed at 133°C, 137°C, 141°C and 145°C) added at different concentrations were analysed. The results showed an overall specificity of 99%, which indicates no cross-reaction with non-ruminant PAPs. The sensitivity of the test strongly depended on the processing temperature and the proportion of muscle fibres of the ruminant PAPs. The average sensitivity of the assay at a level of 1% and 2% of ruminant PAP in non-ruminant PAP was 100% for the muscle tissues at both levels and 85% and 100%, respectively, for the carcase tissues. The overall accuracies are 100% for the 1% and 2% ruminant spikes (processed at temperatures between 133°C and 137°C included) respectively. In conclusion, MELISA-TEK™ Ruminant combined with the concentration procedure showed very promising results, which makes it a suitable candidate method to enable a safe re-introduction of non-ruminant PAPs in aqua feed.

An intralaboratory study of two different assays is currently carried out.

M.G.E.G. Bremer, J. Vaessen, R.J.C.F. Margry, A. van Doremalen, J. van der Palen, R. Van Kaathoven, A.E.M. Kemmers-Vonken, L.W.D. van Raamsdonk. Evaluation of an Enzyme-Linked Immunosorbent Assay for Detection of Ruminant material in Non-Ruminant Processed animal Proteins. J. AOAC, submitted.