## VALIDATION OF IMMUNOASSAYS FOR THE DETECTION OF PROCESSED RUMINANT PROTEINS IN NON-RUMINANT PROTEINS

Leo W.D. van Raamsdonk<sup>1</sup>, Rob J.C.F. Margry<sup>2</sup>, Robert G.C. Van Kaathoven<sup>2</sup>, Monique G.E.G. Bremer<sup>1</sup>

<sup>1</sup>RIKILT Wageningen UR (University & Research centre), P.O. Box 230, 6700 AE Wageningen, The Netherlands

<sup>2</sup>CCL Nutricontrol, Feed to Food Quality Services, P.O. Box 107, 5460 AC Veghel, The Netherlands

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

Due to a growing aquaculture industry the demand for high quality proteins for aquafeeds 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.

The performance of MELISA-TEK™ Ruminant in combination with the MELISA-TEK high Sensitivity Sample Extraction kit was evaluated in an intralaboratory study. The results showed an overall specificity of 99%, which indicates no cross-reaction with non-ruminant PAPs. The sensitivity was sufficient from a contamination level of 0.5% up, although depending on the processing temperature. These results were sufficient to start a large interlaboratory validation study, in which Melisa-Tek was compared with Reveal.

The study comprised a training phase, an entrance test and the final validation experiment. Samples were spiked at 0.5%, 1.0% and 2.0%. Fourteen participants passed the test and investigated the samples. For both Melisa-Tek and Reveal specificity and sensitivity were at 97% or higher. Concordance and accordance were also at good levels. The study complied with AOAC guidelines as far as possible for a qualitative study.

- Immunoassays for the detection of ruminant PAP (Melisa-Tek and Reveal) are validated at 0.5% and higher with non-ruminant PAP as matrix.
- The design of the study can be used as guideline for future studies with qualitative results.

**Keywords**: feed ingredients, former food products, bakery products, packaging materials, quantification