

## **P.46.- Technological Development for Detection of Animal Materials in Feed in Japan**

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We have initiated a research project to develop/upgrade a detection method of animal materials in feed towards an eradication of BSE, which involves in NIRS, ELISA, PCR and DNA chip, while authorized agencies in Japan are conducting examination of contamination of animal materials by the combination of microscopic examination, PCR and ELISA. NIRS: Analytical accuracy of NIRS to detect MBM in formula feeds for swine, poultry and cattle was examined using samples containing MBM from 0.15 to 6.0 %. The results indicated that NIRS could be used to distinguish MBM material included in formula feed within 0.5% level. It was also shown that MBM included in fishmeal could be clearly distinguished (Amari et al. 2003). PCR: The PCR primers for the detection of materials derived from ruminants, pigs and chickens were newly designed on the basis of sequences of the Art2 short interspersed repetitive element (SINE), PRE-1 SINE, and CR1 long interspersed repetitive element, respectively. With the primers, detection of Art2, PRE-1, or CR1 in test feed at concentrations of 0.01% MBM or less was possible. This method was suitable for the detection of micro-contamination of feed by animal materials (Tajima et al., 2002). The PCR method using primers for mitochondrial DNA (mtDNA, Kusama et al., 2004), which is applied for practical examination by authorized agencies in Japan, was modified by adding an isolation procedure of bone particles (Toyoda et al., 2004). Bone particles were isolated from feed containing bovine MBM and milk products as precipitates in chloroform solution, treated with sodium hypochlorite solution and EDTA/proteinase K solution, and then subjected to PCR examination. As bovine DNA derived from milk products can be eliminated by this procedure, this method is suitable for the selective detection of MBM in feed. It allowed detection of the presence of bovine mtDNA in feed containing 0.1% of bovine MBM. When the treatment with sodium hypochlorite was excluded, the detection limit was improved up to 0.0001% at the expense of specificity. ELISA: Sandwich ELISA system, constructed by the combination of rabbit polyclonal antibody with mouse monoclonal antibody detectable cattle-origin antigen, showed very high reaction to cattle-origin antigen with low reaction to sheep- and goat-origin antigen and without any reaction to swine- and poultry- origin antigen. References: Amari, M. et al., 2003, Proc. 11th Int. Conf. on Near Infrared Spectroscopy 5, 116; Kusama T. et al., 2004, J. Food Prot. vol. 67 (in press); Tajima, K. et al., 2002, Biosci. Biotechnol. Biochem. 66, 2247-2250; Toyoda, A. et al., 2004, J. Food Prot. (in press).

### **Keywords**

*NIRS, PCR, ELISA, meat and bone meal, BSE*