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Development and full assessment of a Real-Time PCR method for the specific detection of ruminant DNA in processed animal meal in feed

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As a consequence of the BSE crisis, a complete ban for the use of processed animal proteins in feed has been instituted through Commission Regulation 1234/2003. Recently, the European Commission considered a possible lifting of the ban on the use of PAP from non-ruminants in non-ruminant feed. Implementation of this adapted strategy needs reliable and validated analytical testing procedures in order to determine the species-origin of the relevant PAP.

Besides microscopic- and protein- based methods, analytical procedures enabling the detection of DNA promised to be the most powerful. Within TNO Triskelion a Real-Time PCR method detecting a very abundant target for the specific detection of ruminant DNA has been developed. At first, this method has been internally validated within the TNO Triskelion organization with respect to specificity and sensitivity. Further, in order to exclude the

possibility of negative matrix effects, the method was validated with a wide variety of samples including various types of bone meal derived from various sources. Also the applicability of the Real-Time PCR method for the traceability of ruminant meal in feed was shown.

In a next phase, this method was further assessed in close cooperation with the EURL-AP (European Union Reference Laboratory for Animal Protein in feeding stuffs) in Gembloux (Belgium). Besides a deep evaluation of the method performed with respect to specificity and sensitivity towards DNA from a wide variety of animals including ruminants, terrestrial mammalians, birds, fish species and sea mammals, the method was tested for its robustness, also including the use of various types of thermocycler equipment and reagents. By studying the efficiency and the limit of detection (LOD) of the ruminant specific Real-Time PCR method, the usefulness of setting an accurate cut-off that limits the occurrence of false positive results was emphasized. Lastly, the transferability of the method to other PCR platforms was also optimised.. The final conclusion of this assessment was that the ruminant specific Real-Time PCR method developed by TNO Triskelion was fit for a further validation through an inter-laboratory study.

Keywords Real Time PCR;processed animal protein;specificity;sensitivity;validation