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A real-time PCR method for the detection of black soldier fly (*Hermetia illucens*) in feedstuff

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Abstract

A real-time PCR method was developed for the detection of black soldier fly (*Hermetia illucens*) in feed. The method amplifies a sequence of 89 bp size within the mitochondrial barcode region (cytochrome c oxidase gene, COI). The PCR efficiency of the system achieved ~ 96 % in a background of commercial fish feed DNA. The Limit of Detection (LOD_6) of the method with pure *Hermetia illucens* DNA diluted in compound feed DNA is 0.1 genome copies, corresponding to an absolute amount of 0.13 pg DNA. In addition, the sensitivity was investigated with differently processed material. It could be shown that the method reliably detects delipidated *Hermetia* protein in mixtures down to 0.01 % (w/w) in aquaculture feed. Supplementary experiments exploring the impact of drying, heating and defatting revealed no negative impact on the sensitivity even for hot solvent-extracted dried larvae pre-treated at 140 ^{o}C/20 min. The specificity of the PCR system was confirmed with a broad range of terrestrial and aqueous species. The selection included most relevant arthropods, comprising the seven insect species, which are allowed in aquaculture feeding in Europe. It is concluded that the method is robust and fit for purpose, enabling the sensitive and reliable detection of raw or processed *Hermetia illucens* products as an upcoming new component in feed but also food. Since a standard time/temperature program is used the approach is well suited for the development of future multiplex or relative quantitation methods.