Real time PCR of Nor~1 (aflD) gene of aflatoxin producing fungi and its correlative quantization to aflatoxin levels in South African compound feeds

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Abstract

Aflatoxins (AFs) are naturally occurring secondary metabolites. This toxin is principally produced by Aspergillus flavus and Aspergillus parasiticus in compound feeds worldwide. Compound feeds are feeds blended from various raw materials and additives. Contaminations of these feeds by AFs and its possible transmission into edible materials like milk, egg and organs of the body, are a serious problem. Expression of the Nor~1 (aflD) gene is the main factor responsible for AFs production. For this reason, a study was carried out to establish a correlation between levels of AFs and determinant gene (Nor~1) in South African compound feeds. To achieve this, compound feeds (n = 30) were analyzed for Nor~1 gene using real time polymerase chain reaction (RT-PCR), while AFs levels in similar samples were estimated using high-performance liquid chromatography (HPLC) after an immune-affinity clean-up extraction procedure. Results indicated that AFs levels in positive samples ranged from 0.7 to 33.0 ppb. These levels generally did not correlate (R2 = 0.093) with those of Nor~1 gene in similar samples. Consequently, Nor~1 gene levels established via RT-PCR cannot be used as a predicting model for AFs in compound feeds. Only four of the feeds analyzed, specifically poultry feeds, contained levels of AFs above the regulatory limits of 10 ppb established in South Africa (S.A.). This should be considered unsafe when consumed on a continuous basis and may pose some health related problems especially when AFs are found together with other significant mycotoxins such as ochratoxins (OTs) and/or fumonisins (FBs).