VALIDATED LC-MS/MS MULTI-METHODS TO DETECT 25 MYCOTOXINS AND *IN VIVO* PHASE I METABOLITES IN BIOLOGICAL FLUIDS OF PIGS AND BROILER CHICKENS AND APPLICATION TO SCREENING STUDIES FOR MYCOTOXIN EXPOSURE

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ABSTRACT

Worldwide surveys show that mycotoxins occur in more than 70% of the tested feed samples. In 38% of these samples multiple mycotoxins were found [1]. Therefore, methods that can simultaneously detect multiple mycotoxins are preferred. Until now, mycotoxin exposure in animals is mainly investigated by feed analysis. However, it is well known that so called 'hot spots' are responsible for an uneven distribution and non-proportional spread of mycotoxins in feed, hampering representative sample collection and evaluation of mycotoxin exposure in animals. This can be overcome by the analysis of biological matrices (e.g. plasma, urine, faeces), since the mycotoxin concentration may be more constant and information about the exposure of the animal on an individual level is possible.

The presented methods aim to detect mycotoxins and their *in vivo* phase I metabolites in plasma and excreta of broiler chickens and in plasma, urine and faeces of pigs. The targeted mycotoxins belong to the regulated groups, i.e. aflatoxins, ochratoxin A, *Fusarium* mycotoxins (T2-toxin, zearalenone, deoxynivalenol, fumonisins) and to two groups of emerging mycotoxins, i.e. *Alternaria* mycotoxins and enniatins.

Sample preparation of pig plasma was accomplished by deproteinization with acetonitrile. An additional clean-up step using an Ostro®-plate was required for chicken plasma to remove lipophilic substances. The sample preparation for all other matrices was achieved with a pH-dependent liquid-liquid extraction using different extraction solvents, depending on the matrix. The liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was done using a HSS-T3 UPLC column with appropriate pre-column on an Acquity UPLC system coupled to a Xevo TQ-S mass spectrometer operating in positive and negative electrospray ionisation mode. All methods were in-house validated according to European and international guidelines [2,3].

Subsequently, these methods were applied to screen the occurrence of mycotoxins and *in vivo* phase I metabolites in samples collected from different broiler chicken and swine farms in Europe, where problems with mycotoxins were suspected. Besides, their phase II *in vivo* metabolites were determined using HR-MS.

[1] Kovalsky P, Kos G, Nährer K, Schwab C, Jenkins T, Schatzmayr G, Sulyok M, Krska R. Toxins. 2016;8(12), [2] VICH GL49, FDA, 2015, [3] Volume 8, EC, 2005.