CITRIRISK: INCIDENCE OF CITRININ IN THE BELGIAN FOOD AND FEED CHAIN & RISK FOR HUMAN AND ANIMAL HEALTH

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INTRODUCTION AND OBJECTIVES

- **Citrinin (CIT)** is a mycotoxin produced by several *Aspergillus*, *Penicillium* and *Monascus* species. In 2012, the European Food Safety Authority (**EFSA**) published a scientific opinion on CIT whereby the **need for additional quantitative occurrence and toxicity data** was emphasized, since the CONTAM Panel concluded that the impact of **uncertainties** on the risk assessment is large, and more data regarding the toxicity and the occurrence of citrinin in food and feed are needed to enable refinement. In Belgium, recent work showed that CIT (and/or its metabolite dehydrocitrinone) can be detected in up to **90%** of human urine samples (**BIOMYCO** study)[2] which indicates that **exposure** to CIT might be more important than assumed so far.
- **The aim of the CITRIRISK project** is to gather information on the occurrence of CIT in feed and foodstuffs available on the Belgian market with the prospect of identifying all relevant sources of intake and their importance. Since CIT often co-occurs with ochratoxin A (OTA), it is interesting to investigate the presence of both mycotoxins. Furthermore, it is the intention to collect data on the toxicokinetics and absolute oral bioavailability of CIT in chickens and pigs, and carry-over to edible tissues. All results of the chemical analyses will be brought together in a databank in order to perform a risk assessment in Belgium (exposure assessment and risk characterization for both Belgian population and pig and poultry sector).

PROJECT OVERVIEW

UPLC-MS/MS methods

- Development of suitable **UPLC-MS/MS methods** to determine CIT/OTA in Feed – Food – Edible tissues of animal origin – Plasma – Urine
- **Validation** according to Commission Regulation No. 401/2006/EC and Commission Decision No. 2002/657/EC

ANALYSIS OF BELGIAN FOOD AND FEED

- **400 food samples**:
  cereal products – fruit and vegetable juices – herbs and spices – nuts and seeds – alcoholic beverages – baby food – soy and vegetarian products – food supplements – meat products
- **100 feed samples**:
  pig – broiler chickens – laying hens

KINETIC STUDIES

- **Toxicokinetic study** after 1 bolus (oral and IV) in 8 animals (pigs and broiler chickens) in a 2-way cross-over design
  → *ADME parameters, plasma protein binding and absolute oral bioavailability?*
- **Steady-state study**: 3 weeks administration of contaminated feed to pigs, broiler chickens and laying hens
  → *Tissue residues in muscles, kidneys and eggs?*
- **Post-mortem evaluation**
  → *Organ damage?*
- **MetID (HRMS)**: CIT phase I and phase II metabolites

RISK ASSESSMENT

PRELIMINARY RESULTS

Based on a previously developed QuEChERS-based method[3], optimal MS/MS-parameters for simultaneous analysis of CIT and OTA were achieved (Table 1) using a Waters Acquity UPLC system coupled to a Xevo-TQS mass spectrometer.

Table 1: Mass spectrometric parameters for the analysis of CIT and OTA and their internal standards ^{13}C-CIT and ^{13}C-OTA

Analyte	Retention time (min)	Precursor ion		Cone voltage (V)	Product ion m/z (collision energy)		
		m/z	Ion species		Quantifier	1st Qualifier	2nd Qualifier
CIT	3.6	281.0	$[M+MeOH-H]^-$	50	249.0 (15V)	205.0 (25V)	177.0 (30V)
^{13}C-CIT		294.0	$[M+MeOH-H]^-$	50	262.0 (15V)		
OTA	4.6	404.0	$[M+H]^+$	35	238.9 (20V)	221.0 (30V)	192.8 (40V)
^{13}C-OTA		424.2	$[M+H]^+$	35	249.8 (28V)		

Good chromatography of all analytes was achieved within 10 minutes using an Acquity UPLC HSS T3 column. Typical chromatograms of spiked and naturally contaminated feed samples are shown in Fig 1 and Fig 2. Table 2 summarizes the occurrence data of CIT and OTA for 19 feed samples.

The method was successfully validated achieving:

- Extraction recovery of 90% and 95% for CIT and OTA respectively
- RSD_R ranging between 0.7% and 12.6% for CIT and between 1.5% and 12.2% for OTA

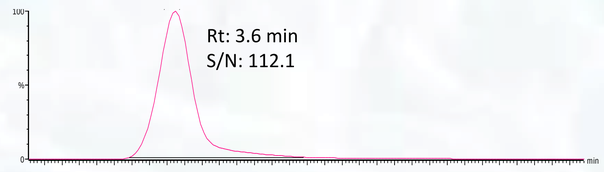

Fig 1: Chromatogram of a blank feed sample spiked with CIT (20 µg/kg)

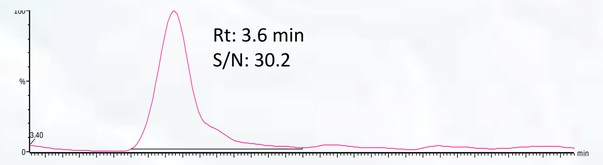

Fig 2: Chromatogram of a feed sample containing CIT (2.8 µg/kg)

Table 2: Occurrence data of CIT and OTA in Belgian feed

Mycotoxin	% positive samples (n=19)	Contamination (µg/kg)		LOD (µg/kg)	LOQ (µg/kg)
		Mean*	Max		
Citrinin	79%	1.0 ± 0.3	2.8	0.2	0.5
Ochratoxin A	68 %	0.7 ± 0.1	0.9	0.1	0.2

* Mean of samples above LOQ

CONCLUSION

- The quantitative LC-MS/MS method is applicable for determination of CIT and OTA in feed
- Validation was done for the target toxins and good values for extraction recovery and precision were obtained
- In a high percentage of the analysed samples, CIT and OTA were detected above the LOD, proving their co-occurrence in the Belgian feed chain

REFERENCES

[1] EFSA CONTAM PANEL *EFSA Journal 10(3):2605*
[2] Heyndrickx *et al.* 2015 *Environment International No 84, 82-89*
[3] Kiebooms *et al.* 2016 *World Mycotoxin Journal No 9, 343-352*

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