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► To cite this version:

Hamid Boudra, Markus Godejohann, Diego Morgavi. Uncovering metabolic changes using a combined LC-MS/NMR approach: Ochratoxin's effect in sheep. 3. Journées Scientifiques du Réseau Français de Métabolomique et Fluxomique, Feb 2008, Bordeaux, France. 1 p., 2008. hal-02751102

HAL Id: hal-02751102

<https://hal.inrae.fr/hal-02751102>

Submitted on 3 Jun 2020

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Uncovering metabolic changes using a combined LC-MS/NMR approach: Ochratoxin's effect in sheep



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Introduction

Ochratoxins, a group of highly toxic secondary metabolites produced by some species of *Aspergillus* and *Penicillium*, are commonly found in foods and feeds. Ochratoxin A (OTA), the most important toxin of this family, is nephrotoxic, hepatotoxic, teratogenic and carcinogenic in animals and was recently classified by the International Agency of Research on Cancer as a class 2B, possible human carcinogen. Ruminants are considered to be less sensible because OTA are hydrolyzed by rumen microorganisms into a less toxic compound. However, it was recently found that the rumen detoxification action may be less important than previously reported as OTA was detected in blood and milk of sheep receiving a contaminated feed. In this study the effect of a chronic OTA ingestion on urinary metabolites excretion was examined in dairy ewes.

Methods

1- Animals:

- Six lactating dairy ewes were randomly divided in 2 lots. They were fed at libitum a diet consisting of 28 % wheat-based concentrate and 72 % hay twice a day. Animals received 30 µg OTA/kg bw/day for 4 weeks.
- Urines were collected on animals placed in individual cages fitted with a mesh to separate faeces. Four sampling periods were done during the experiment: U0 (Day -3 to 0, no toxin); U1 (day 1 to 4); U2 (day 15 to 18); U3 (day 29 to 31).

2- Urines analysis

The measurements of urine samples were done by combined LC-MS/NMR system.

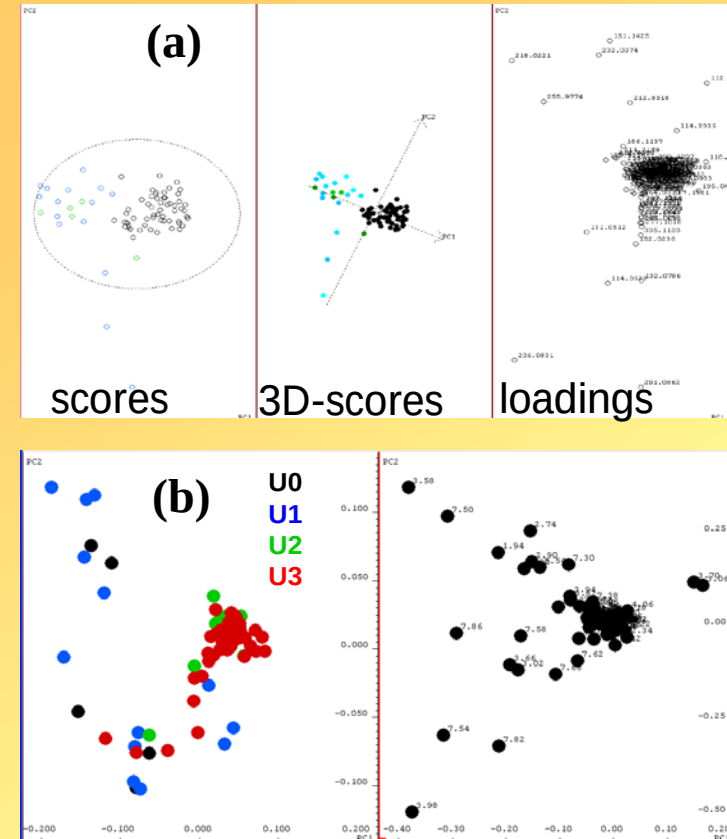
LC-MS analysis was performed on a Bruker micrOTOF in ESI/ positive mode.

NMR analysis was performed in a 600 MHz ¹H NMR spectroscopy.

Results

- OTA administration did not affect milk production, live weight or animal behavior. However, the high dose tended to decrease intake.
- Principal component analysis (PCA) of both data (MS and NMR) uncovered differences in the metabolic profiles of animals before and following OTA exposition. The exact masses of possible markers of OTA exposition can be directly obtained from the loadings plot of Figure (a). Further investigations, e.g. by direct LC-NMR coupling, would be needed to characterize these markers unambiguously.

Figure: PCA overview of (a) LC-MS data and (b) NMR data



Conclusion

Metabolic profiling of low MW molecules by LC-MS/NMR can detect early subtle metabolic changes in animals due to a mycotoxin exposition.