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Enniatins and Beauvericin Emerging Mycotoxins Affect Pigs' Metabolome

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Overview

- Pigs exposed to beauvericin and enniatins (emerging mycotoxins) through diet
- UHPLC-ESI-HRMS metabolomics of liver and plasma
- Discrimination according to food metabolites, uric acid derivatives, purines, amino acid, lipids.
- Potential effect on the renal function

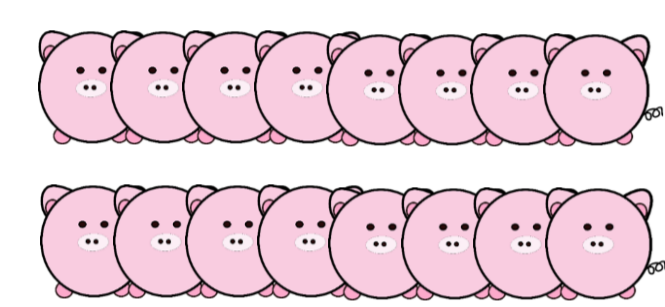
Introduction

In addition to deoxynivalenol, fumonisins and zearalenone, *Fusarium* fungi produce other toxins such as beauvericin or enniatins, which are called "emerging" mycotoxins (EMs). Occurrence data and biological effects for EMs are still limited. Recently, feed ingredients were shown to contain 66% to 98% of beauvericin, enniatin B, aurofusarin or apicidin [1]. *In vitro*, data suggest that BEA and ENN induce cytotoxicity, oxidative stress and apoptosis. *In vivo*, while they do not induce apparent acute mycotoxicosis, no conclusion can be drawn on chronic exposure. To study the impact of EMs, we conducted a LC-MS based metabolomics study of plasma and liver of piglets (5 weeks) fed with a mixture of enniatins B, B1 and beauvericin during 14 days.

Study design [2]

approval code LF1-TVG-57/015-2019

Piglets (5 weeks)
n = 8/group



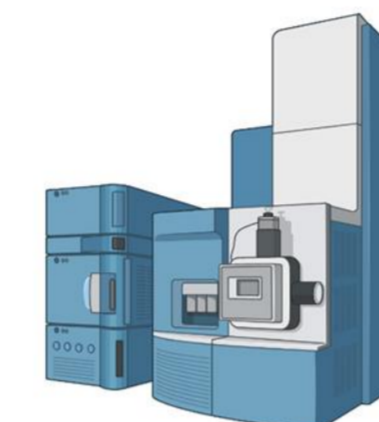
Control diet
vs
EMs enriched diet

Samples preparation



Plasma: proteins precipitation with 2 volumes of CH₃OH centrifugation Speedvac evaporation
Liver: Fastprep grinding in CH₂Cl₂/CH₃OH/H₂O (4/4/2.85 v:v:v) centrifugation Speedvac evaporation of aqueous phase
Urine: dilution in mobile phase A centrifugation

Analyses [3]



UHPLC: ACQUITY I-Class (Waters)
Hypersil Gold C18 column (100x2.1 mm, 1.9µm) (Thermo Scientific), mobile phases A: H₂O/CH₃OH/CH₃CO₂H 95/5/0.1 (v:v:v); B: CH₃OH/CH₃CO₂H 100/0.1 (v:v) flow rate of 0.3mL/min, Vinj 10µL, temperature 40°C
Untargeted HRMS: Synapt G2-SI QToF, resolution ~15000 (Leu-Enk), m/z 50-800, ESI positive and negative
Targeted HRMS: LTQ Orbitrap XL, resolution 30000 (m/z 400), m/z 50-800, ESI positive and negative
Sequence of injection: random order of samples, experimental blank and QC (pool) injected 11 times

Data processing



Peak picking and alignment with W4M [4] (XCMS)
Data curation: Sample/Blank > 3
Discriminant features with VIP >1 and Wilcoxon test <0.05
Metabolites annotation with in-house databases of RT, of HRMS data (Synapt) and of MS/MS data (LTQ Orbitrap XL)



Plasma collection

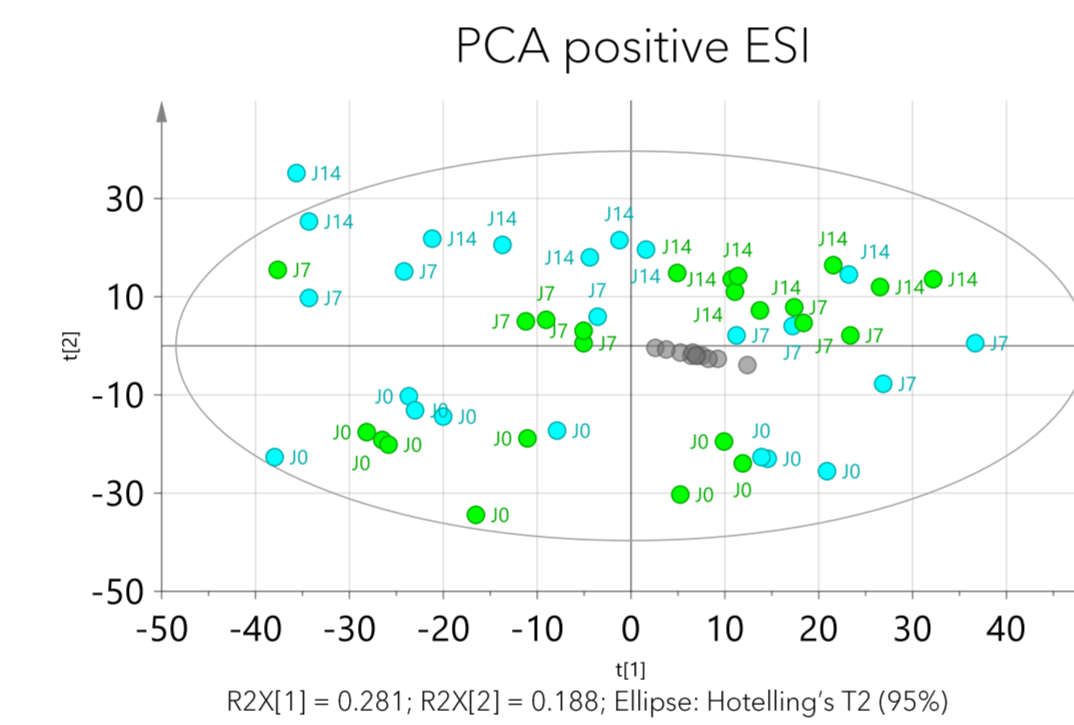
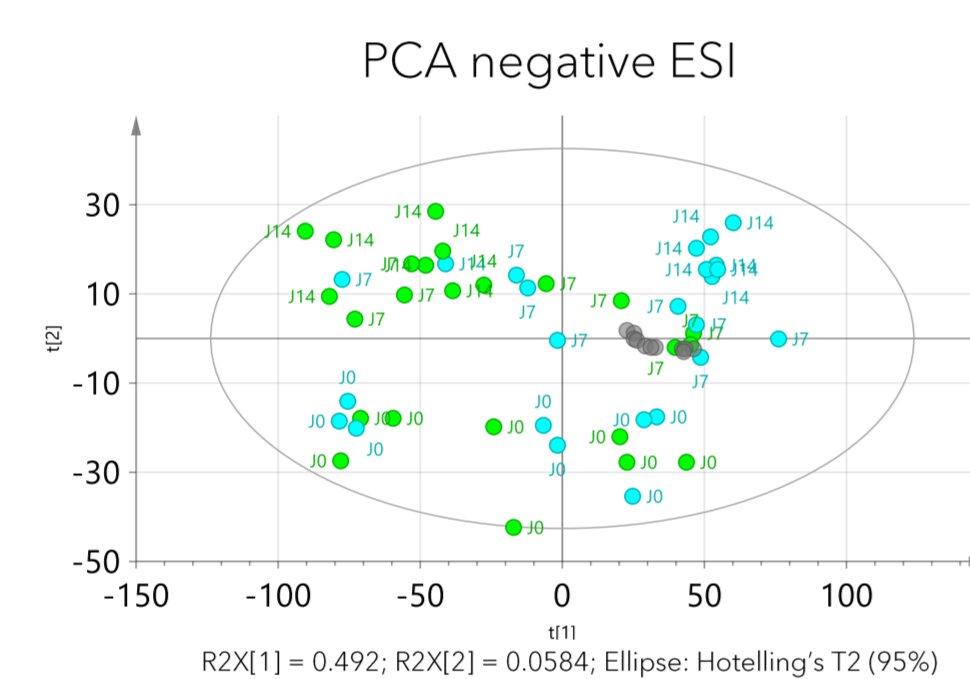


D7 and D14

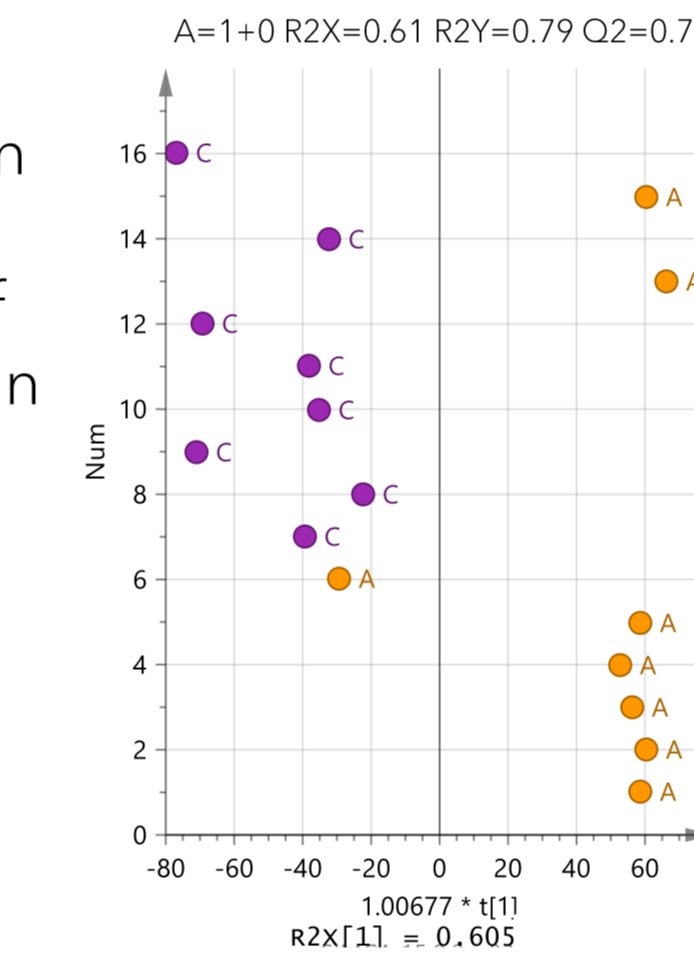


Untargeted analysis

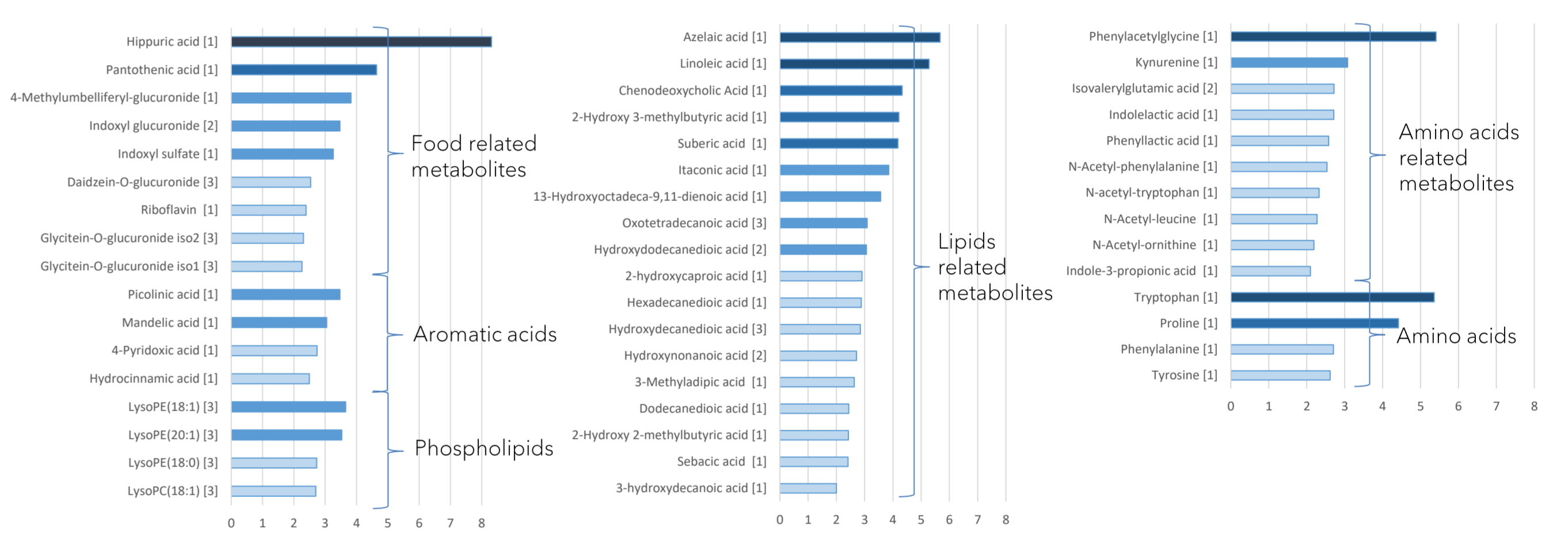
Negative ionization mode : 11744 filtered features
Positive ionization mode : 10075 filtered features



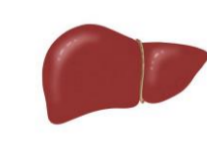
PLS-DA:
No discrimination at D7
Discrimination of diet at D14 only in negative ESI



Fold Change:
Log2 of annotated metabolites (EMs/Ctrl)



Liver collection

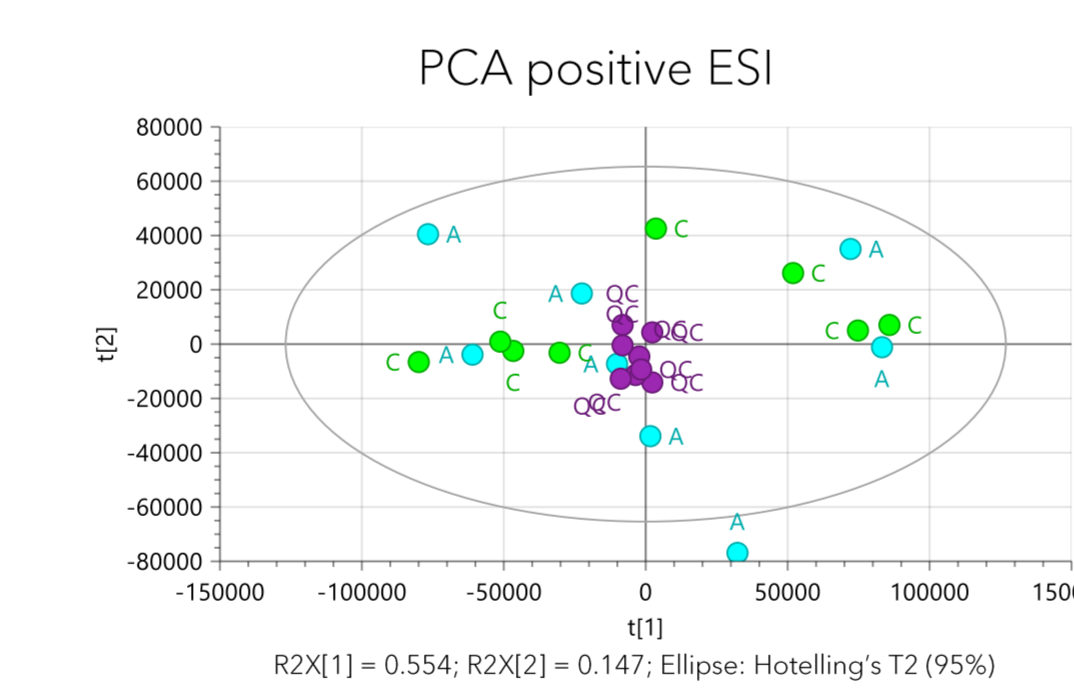
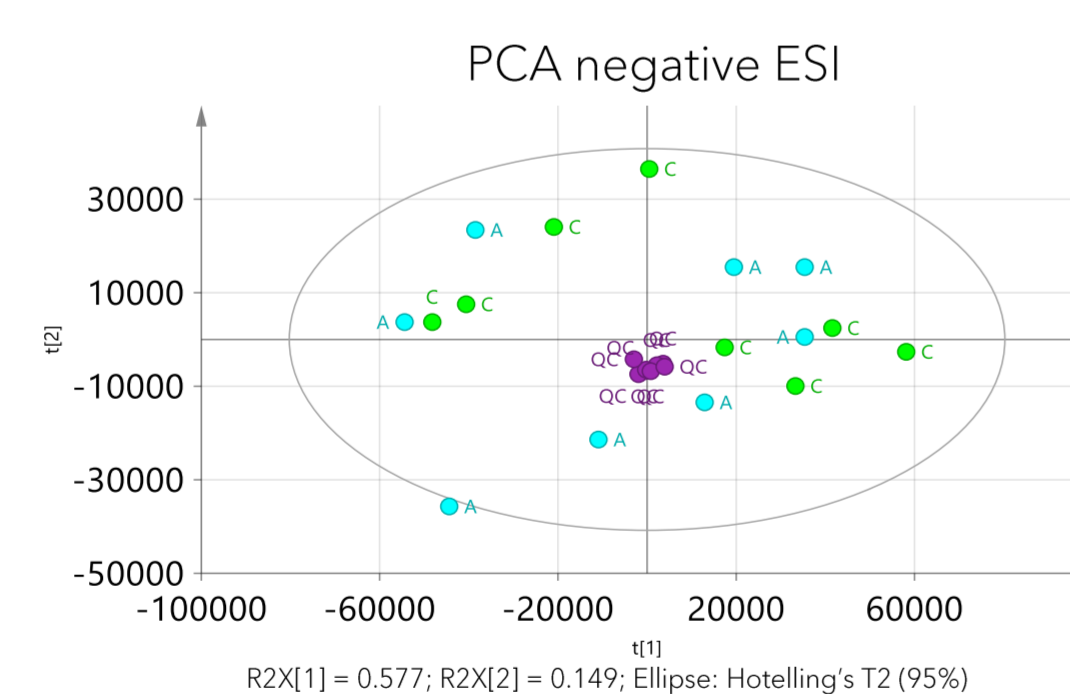


D14

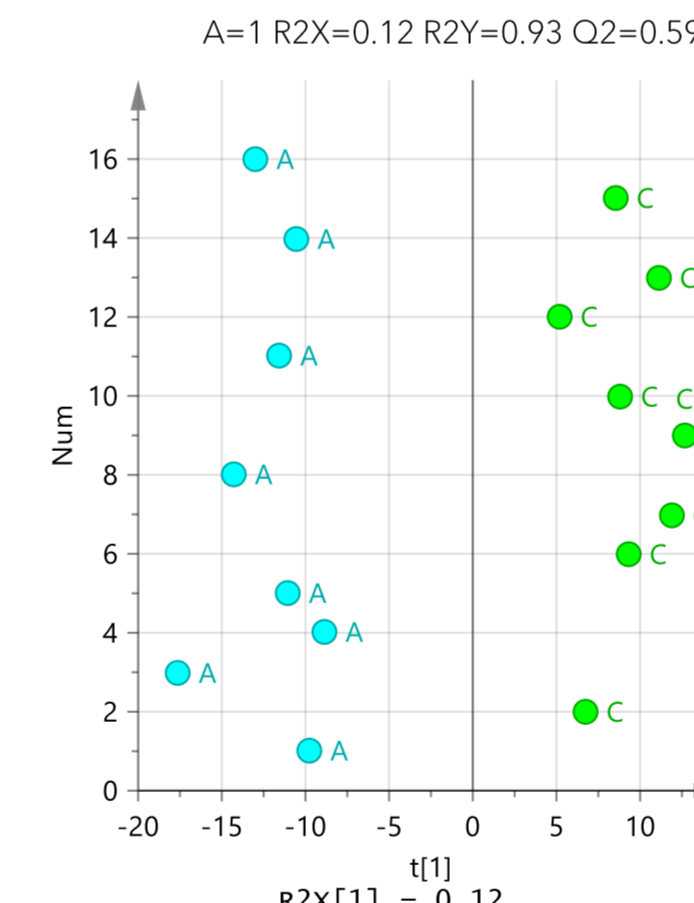


Untargeted analysis

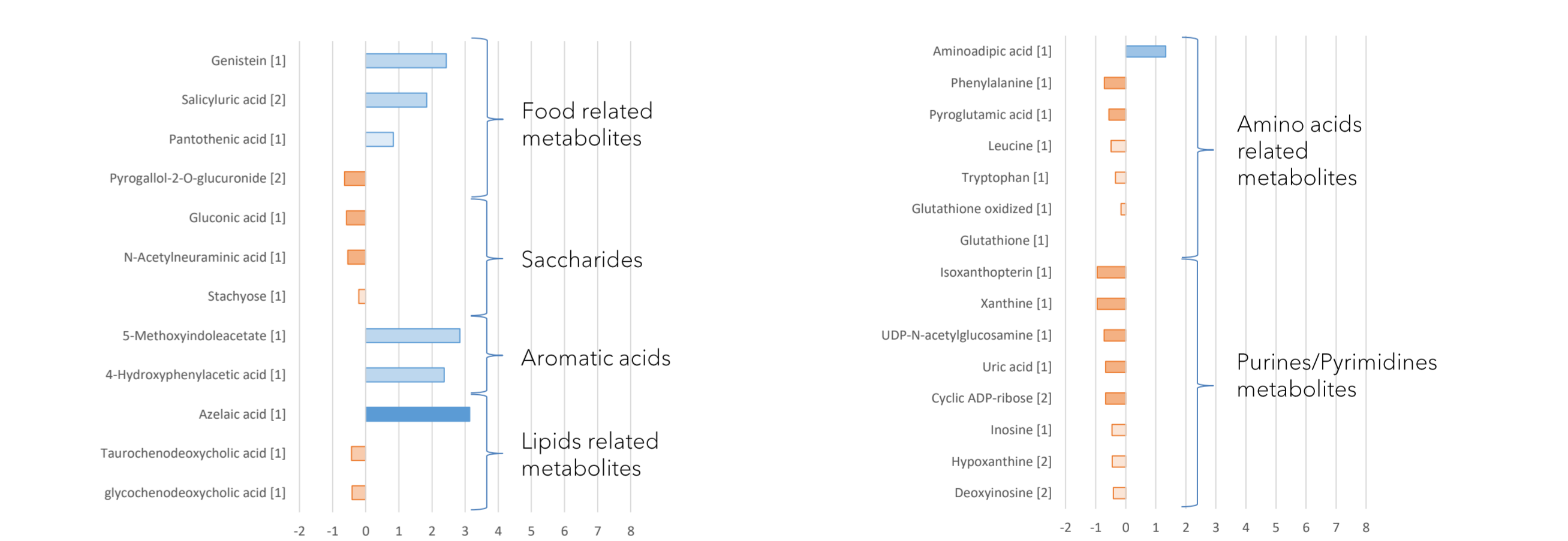
Negative ionization mode : 4652 filtered features
Positive ionization mode : 7801 filtered features



OSC-PLS-DA:
Discrimination of diet only in negative ESI



Fold Change:
Log2 of annotated metabolites (EMs/Ctrl)



Urine collection

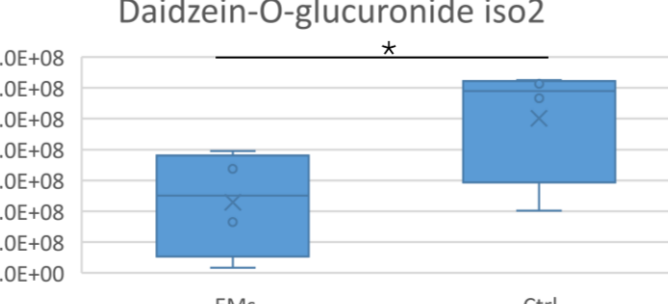
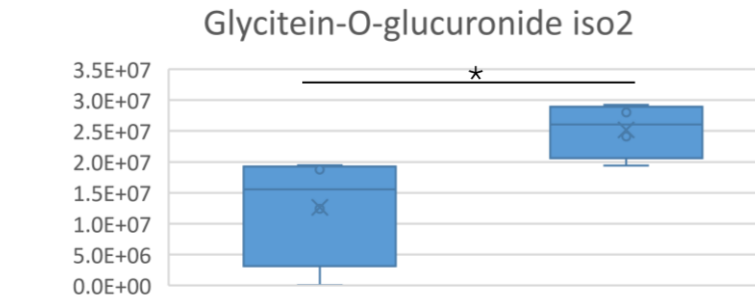
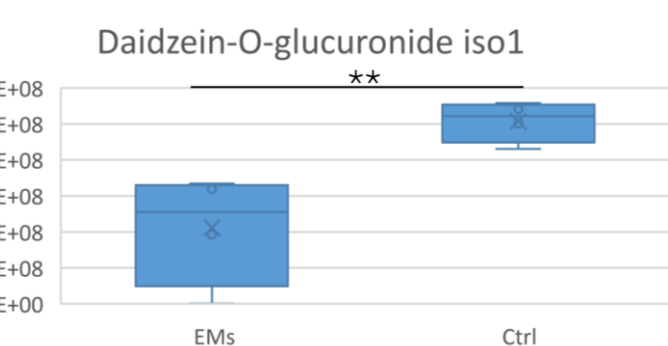
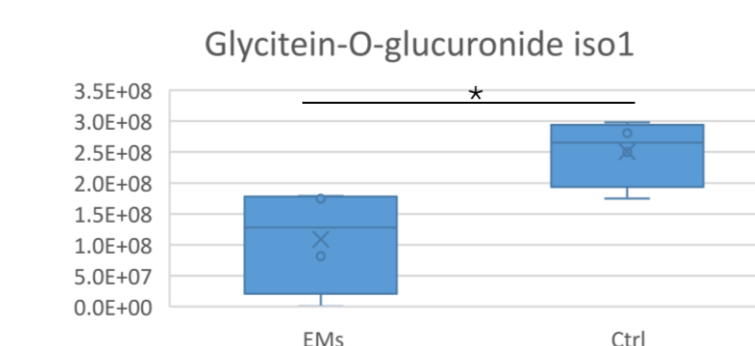
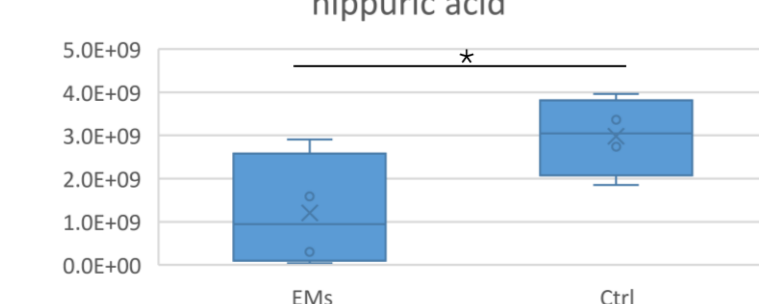


D14



Targeted analysis

Targeted analysis by screening of HRMS [M-H]⁻ ions of discriminant food related metabolites: 5 of them are significant in urines



Conclusions

- In plasma or liver: only 1 ionization mode significant, only with PLS-DA or OSC filtration, only after 14 days of diet
- ⇒ Weak effect of the mixture of the emerging mycotoxins beauvericin and enniatins according to metabolomics data
- Discrimination according to food metabolites and to uric acid derivatives (no weight gain observed [2])
- ⇒ Potential renal effect of EMs
- Opposite modulation of food metabolites in plasma (untargeted data) versus in urine (targeted data)
- ⇒ Confirmation of the renal effect of EMs
- From pathways enrichment analysis (MetExplore) "glutathione metabolism" and "phenylalanine metabolism" significantly affected
- ⇒ Increase of flavonoids concentration in plasma due to renal effect ⇒ decrease of inflammation (glutathione metabolism) through modulation of phenylalanine and lipid metabolism, similarly as Shorde *et al.* [5]

References

- [1] Streit E. *et al.* J. Sci. Food. Agric., (2013) 93, 2892
- [2] Novak B. *et al.* Food Chem. Toxicol., (2021) 158, 112719
- [3] Jacques C. *et al.* Arch. Tox., (2021) 95, 3303
- [4] Giacomoni F. *et al.* Bioinformatics, (2015) 31, 1493
- [5] Shorde R.L. *et al.* Metabolomics (2022) 18, 84

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