Genetic response of red yeast supplementation in feed to mycotoxin contamination in laying hens

Shahrbanou Hosseini[1,2], Bertram Brenig[1,2], Wanaporn Tapingkae[3,4], Kesinee Gatphayak[3,4]*

[1]Department of Animal Sciences, University of Goettingen, Göttingen, Germany

[2]Institute of Veterinary Medicine, University of Goettingen, Göttingen, Germany

[3]Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand

[4]Functional Feed Innovation Center, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand

Introduction

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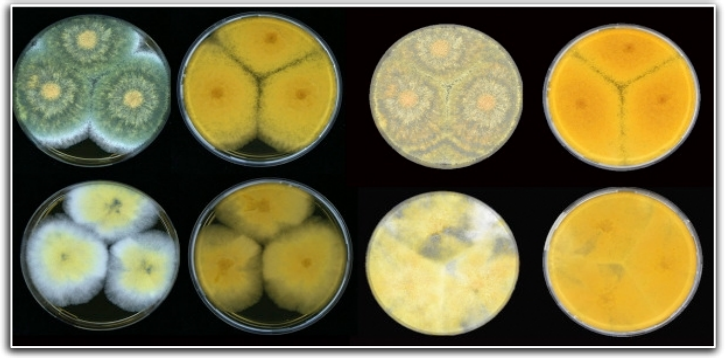
Mycotoxins are toxic secondary metabolites produced by filamentous fungi that contaminate agricultural products

- **Feed and food contaminated with toxic fungi can cause disease and death in animals and humans, raising global concerns for food safety and health**
- **Agricultural products can be contaminated simultaneously with multiple mycotoxins that can increase the risk of adverse health effects in animals and humans**

Mycotoxin



Chen et al., 2017

Groundnut

Sorghum

Nleya et al., 2018

Wheat

Maiz

Ladeira et al., 2017

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Feeding animals with mycotoxin contaminated diets leads to:

Decrease

- Feed intake
- Feed efficiency
- Weight gain
- Reproductive performance

Increase

- Susceptibility to infectious diseases
- Impaired immunity
- Higher rate of mortality

- Harmful fungi are the major cause of feed contamination in poultry, which can affect different organs such as liver, kidneys, spleen, gastrointestinal tract

- Consumption of contaminated feed results in birds health damage, significant economic losses in the poultry industry, and a safety risk to human consumers

Image: Nleya et al., 2018

Introduction

- **So far, different strategies have been developed to control mycotoxin contamination, which classified into:**

➢ **preharvest strategies** in the field to prevent mycotoxin production range from good agricultural practices to the use of biological control agents

➢ **postharvest strategies** to control mycotoxin contamination in the process of harvesting, storage, and processing

Harvest

- Time control
- Crop rotations
- Quick drying
- Optimum moisture

Storage

- Physical methods (e.g. temperature)
- Chemical methods (e.g. preservatives)
- Biological methods (e.g. yeast)

Processing

- Physical methods (e.g. washing)
- Chemical methods (e.g. alkaline)
- Biological methods (e.g. yeast)

Images: Sun et al., 2022

Introduction

- Biological agents have proven to be more effective, specific, and environmentally friendly to control mycotoxin contamination
- Yeast is a promising detoxification strategy for the poultry feed industry, which being able to degrade mycotoxin in diets

❖ **Red yeast** is a novel yeast used as a biological binder of mycotoxins in poultry feed that:

- ➢ Absorb mycotoxin through the cell wall
- ➢ Acts as a prebiotic with antioxidant properties
- ➢ Possess high nutritional value to improve production traits in poultry species

Tapingkae et al., 2022; Husakova et al., 2021

Objectives

- Since poultry are fed with a mixture of various grains and oilseed meals, they are at higher risk of co-exposure to a combination of different mycotoxins
- However, the genetic mechanism underlying detoxification processes in poultry in response to multiple mycotoxins and its interaction with organic binder remains to be elucidated

- The main goal of this study was to investigate the genetic mechanism underlying feeding with red yeast supplementation in interaction with multiple mycotoxins in laying hens using RNA sequencing
- To gain more insights for the development of counter strategies to eliminate the adverse effects of mycotoxins in poultry species and increase food safety to avoid health concerns for human consumers

Image: https://www.foodsafetyafrica.net/wageningen-university

Materials and Methods

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Experimental groups: Hy-Line brown layers (288 Animals)

Control	Red yeast	Mycotoxins	Red yeast + Mycotoxins
72 Laying hens	72 Laying hens	AFB1, T-2, OTA, ZEA, DON 72 Laying hens	72 Laying hens

- Birds were fed with experimental diets at 23 weeks of age for a duration of 9 weeks
- Collecting liver tissue samples from four animals in each experimental group at 32 weeks of age
- Isolation of RNA from liver tissue for RNA_Seq analysis
- Sequenced on an Illumina NovaSeq6000 Platform, aiming PE150 reads per sample
- Analysis of differentially expressed genes in RY1.0 vs. CON | MT100 vs. CON | RY1.0+MT100 vs. CON
- Functional enrichment analysis in RY1.0 vs. CON | MT100 vs. CON | RY1.0+MT100 vs. CON

Control: Con | *Red yeast: RY1.0 (1.0 g/kg feed)* | *Mycotoxins: MT100 (100 µg/kg feed)* | *Red yeast + Mycotoxins: RY1.0+MT100*

Aflatoxin B1: AFB1; T-2 toxin: T-2; Ochratoxin A: OTA; Zearalenone: ZEA; Deoxynivalenol: DON

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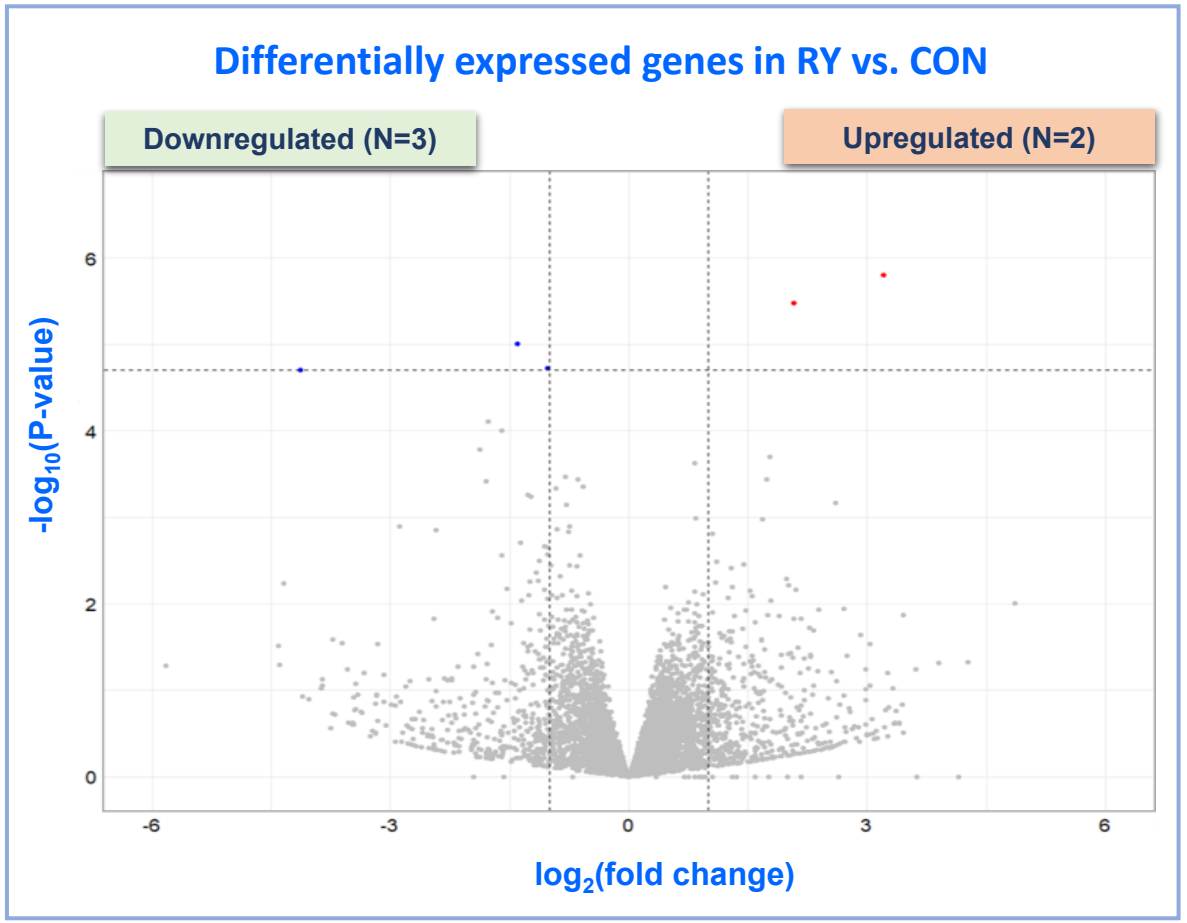
Differentially expressed genes in red yeast versus control (RY vs. CON)



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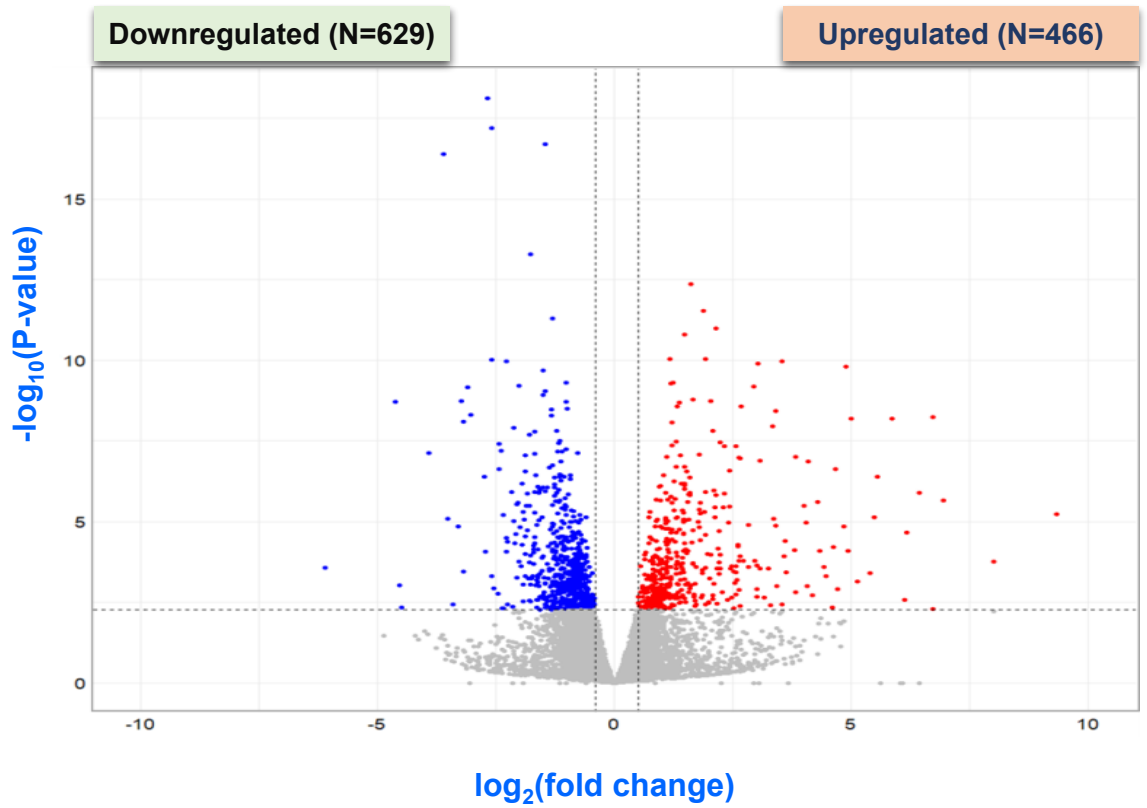
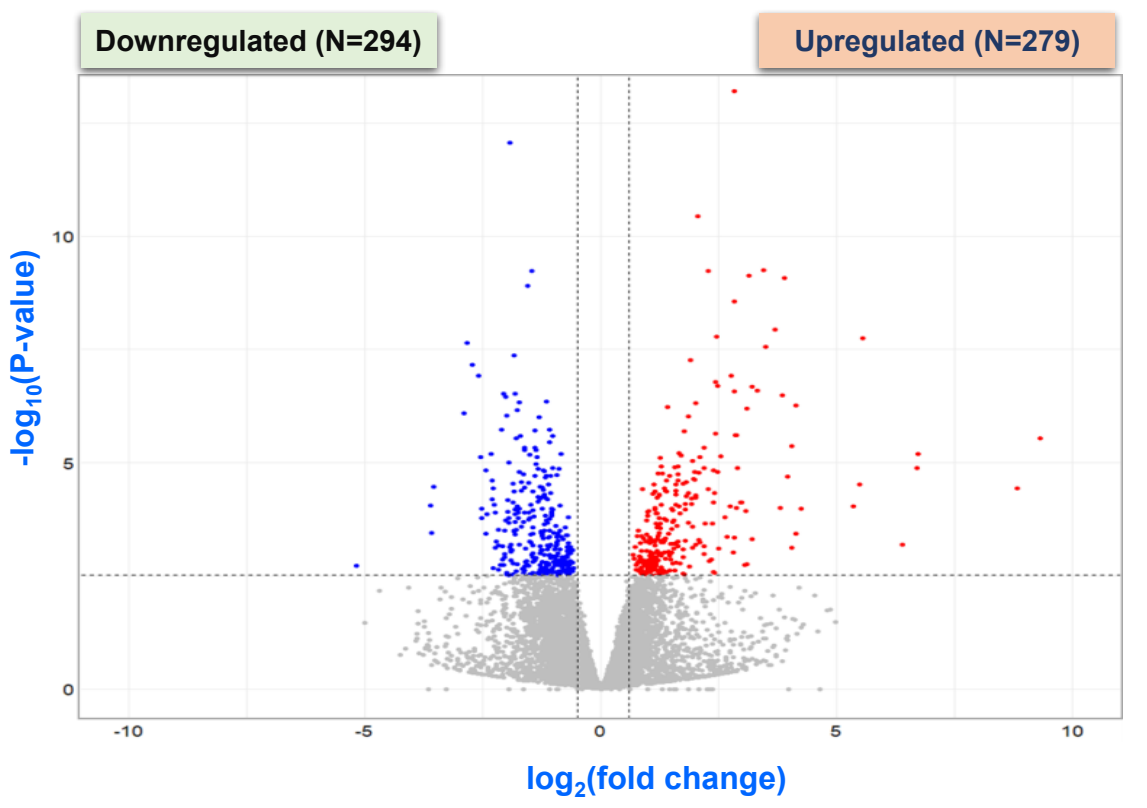
Differentially expressed genes in mycotoxins versus control (MT vs. CON) and red yeast + mycotoxins versus control (RY+MT vs. CON)

Differentially expressed genes in MT vs. CON



Differentially expressed genes RY+MT vs. CON



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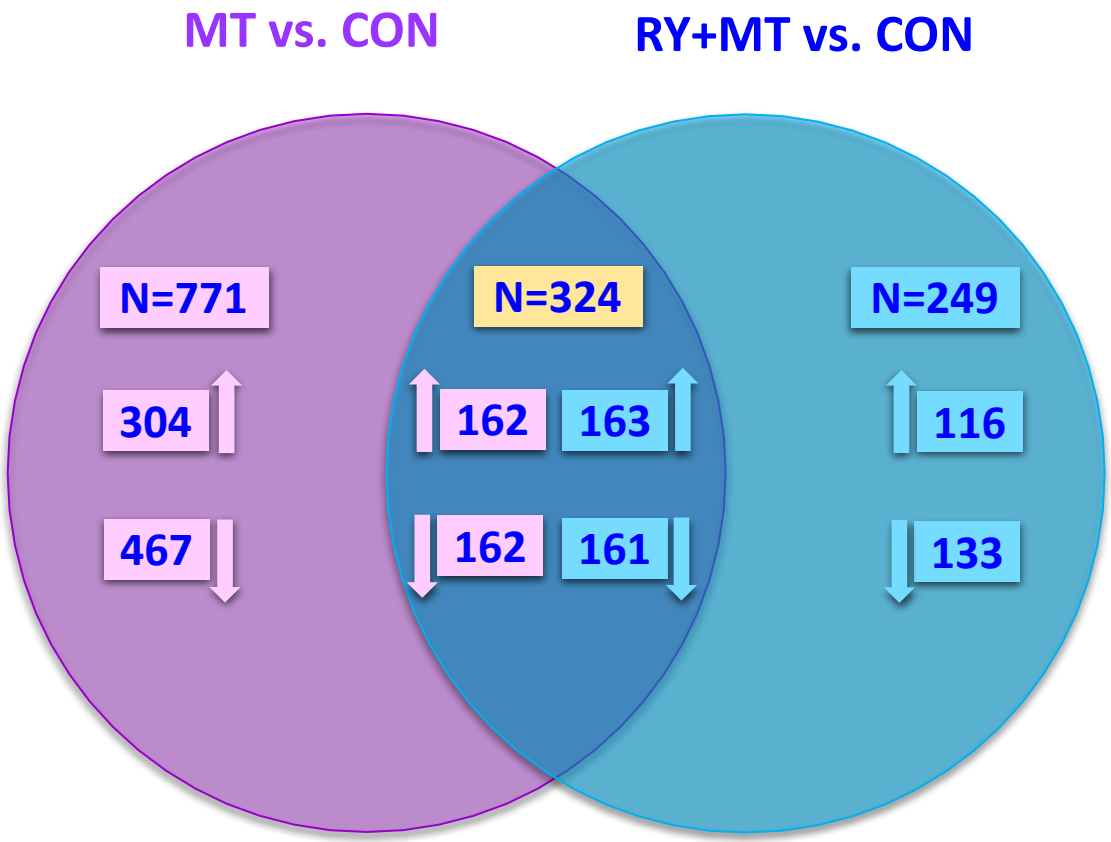
Significantly differentially upregulated↑ and downregulated↓ genes comparing MT vs. CON and RY+MT vs. CON



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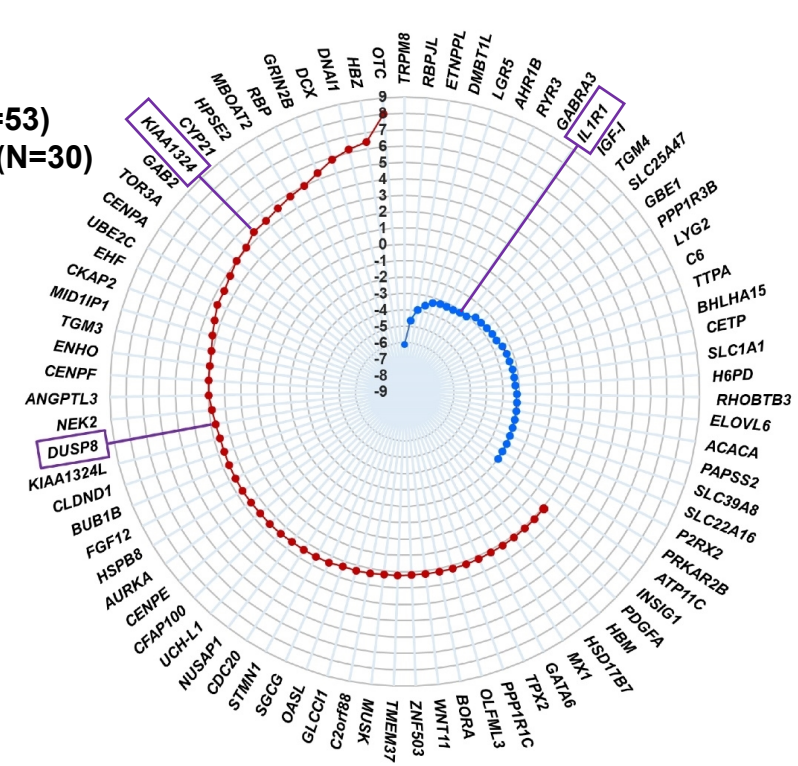
Top significant differentially expressed unique genes in MT vs. CON and RY+MT vs. CON

MT vs. CON

- Upregulated (N=53)
- Downregulated (N=30)



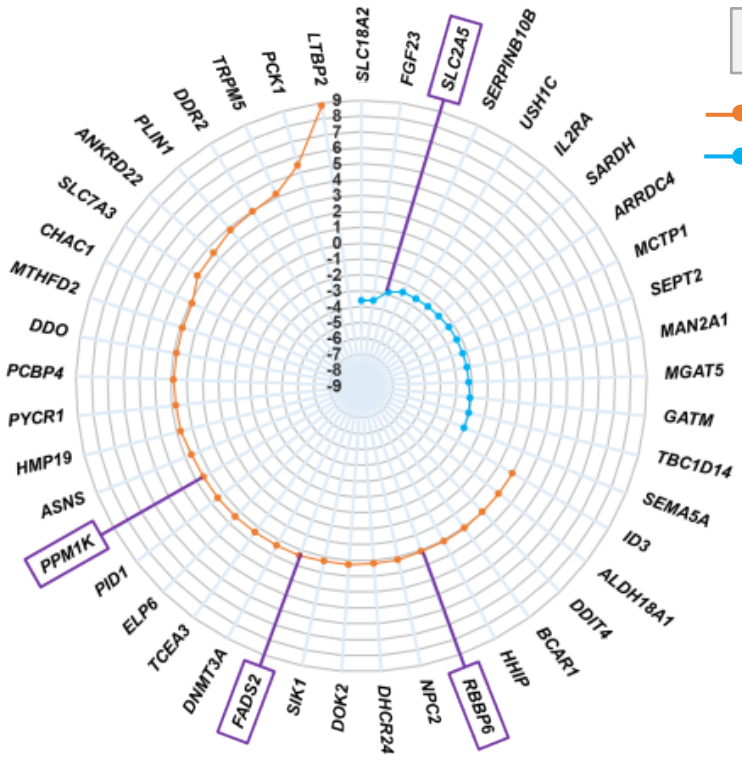
DUSP8: Apoptosis gene

KIAA1324: Tumor suppressor gene

IL1R1: Immune and inflammatory response gene

RY+MT vs. CON

- Upregulated (N=30)
- Downregulated (N=25)



RBBP6: Essential for preventing DNA damage

PPM1K: Essential for cell survival and development

FADS2: Functional gene in fatty acid metabolism

SLC2A5: Glucose and fructose transporter in cell

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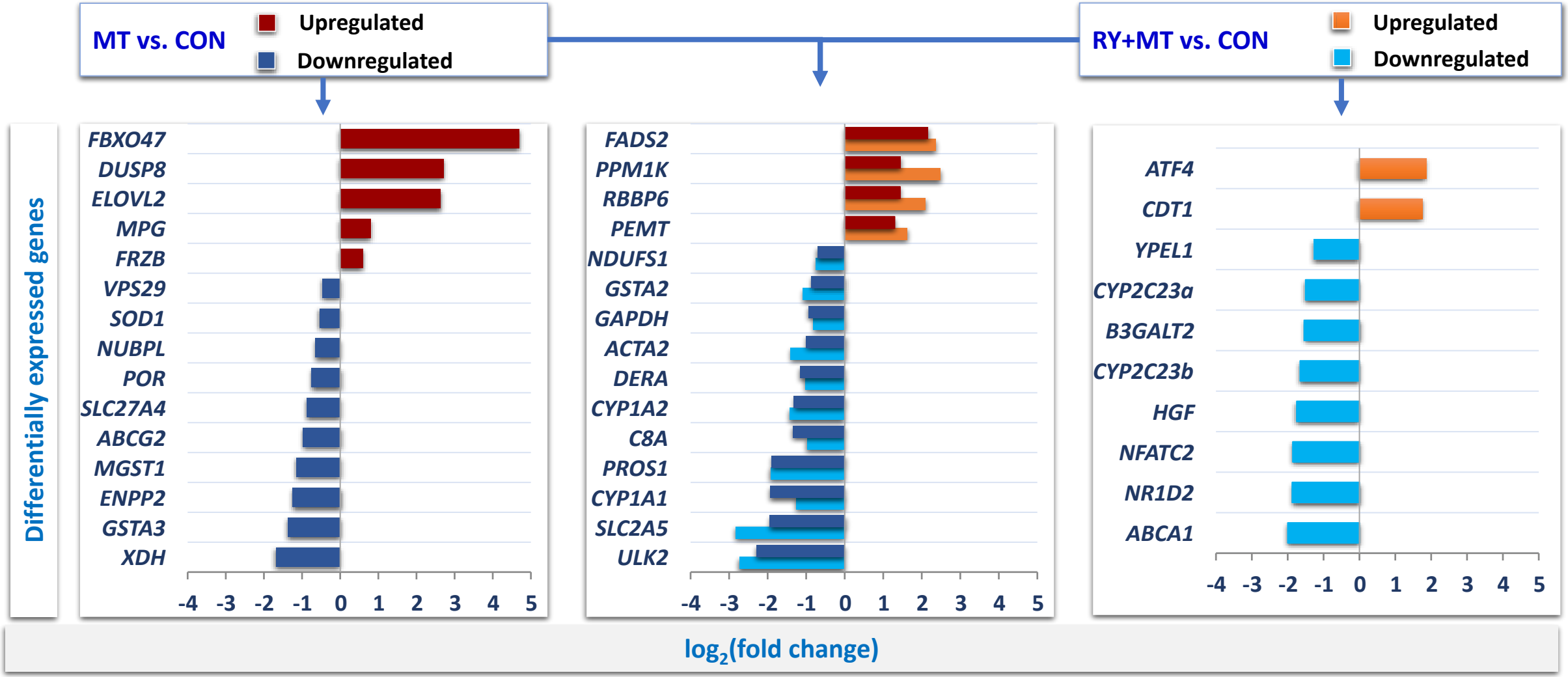
Candidate genes involved in mycotoxicity in MT vs. CON and RY+MT vs. CON



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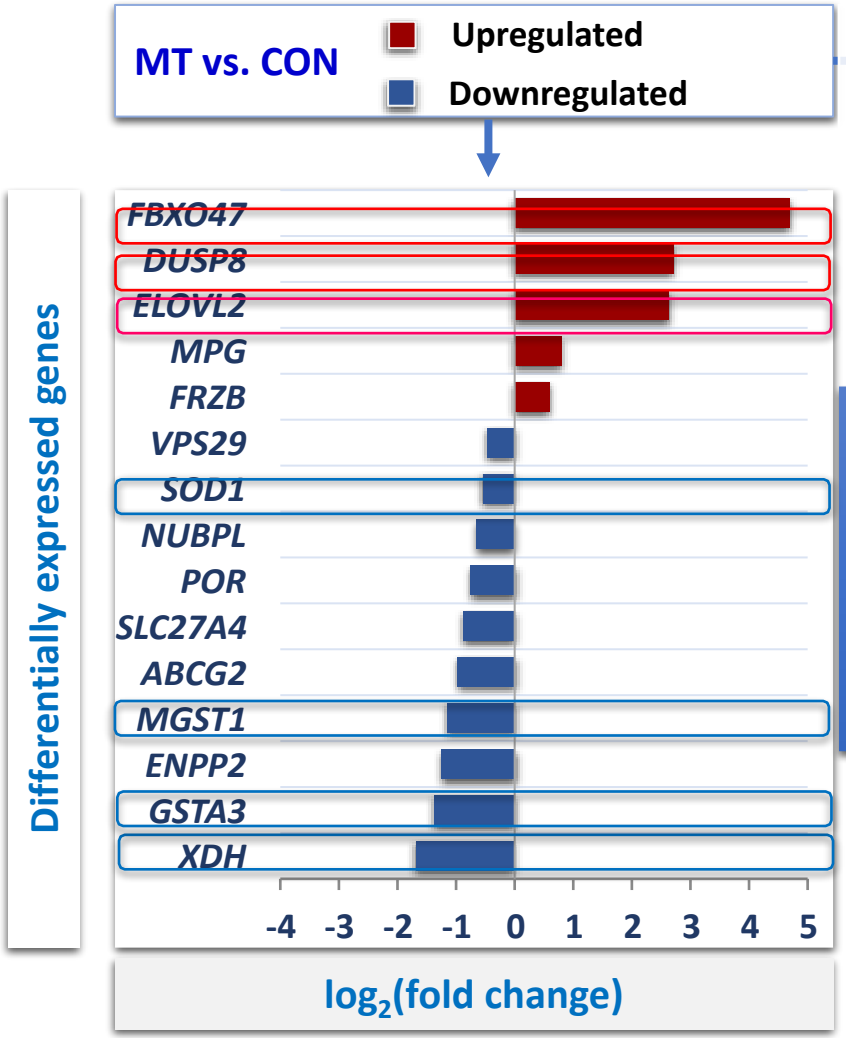
Candidate unique genes involved in mycotoxicity in MT vs. CON



- ***FBXO47: Tumor suppressor gene***
- ***DUSP8: Apoptosis gene***
- ***ELOVL2: Fatty acid biosynthesis gene***

- ***XDH: Gene involved in the oxidative metabolism***
- ***GSTA3 and MGST1: Genes involved in phase II detoxification process***
- ***SOD1: An antioxidant gene***

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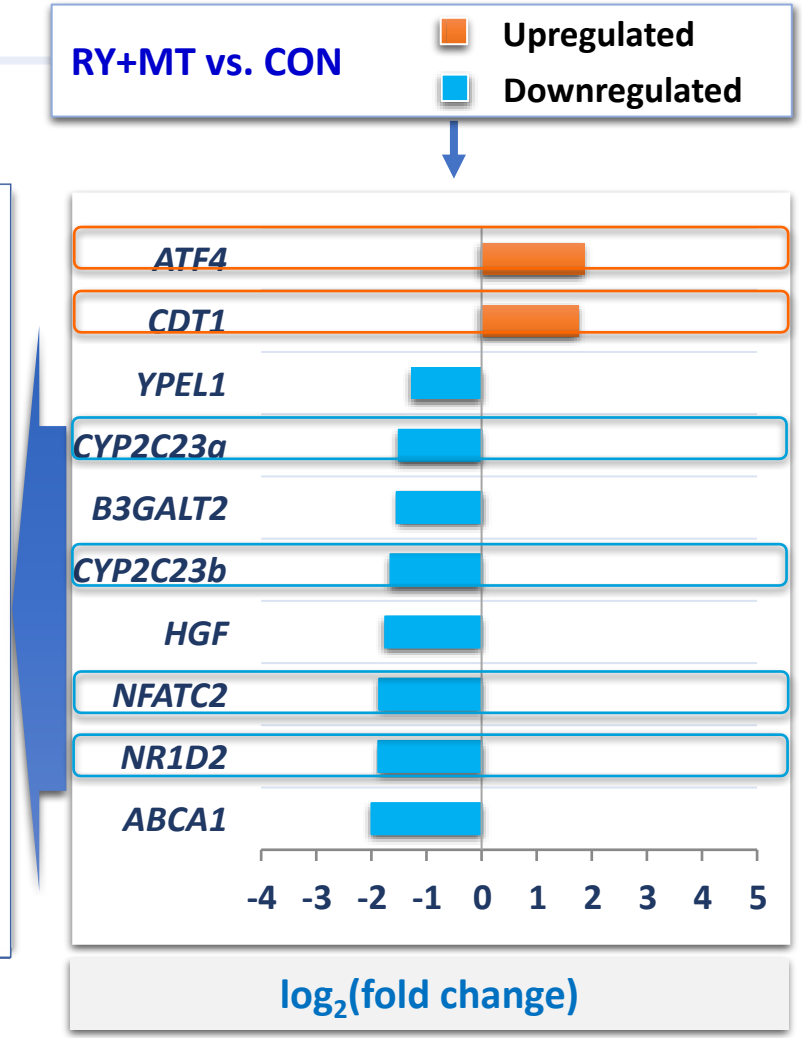
Candidate unique genes involved in mycotoxicity in RY+MT vs. CON

Differentially expressed genes

- *ATF4: Stress response gene*
- *CDT1: Regulates DNA replication initiation*
- *NFATC2: A tumorigenesis gene*
- *NR1D2: Gene involved in lipid metabolism*
- *CYP2C23a and CYP2C23b: Genes involved in xenobiotic metabolism*



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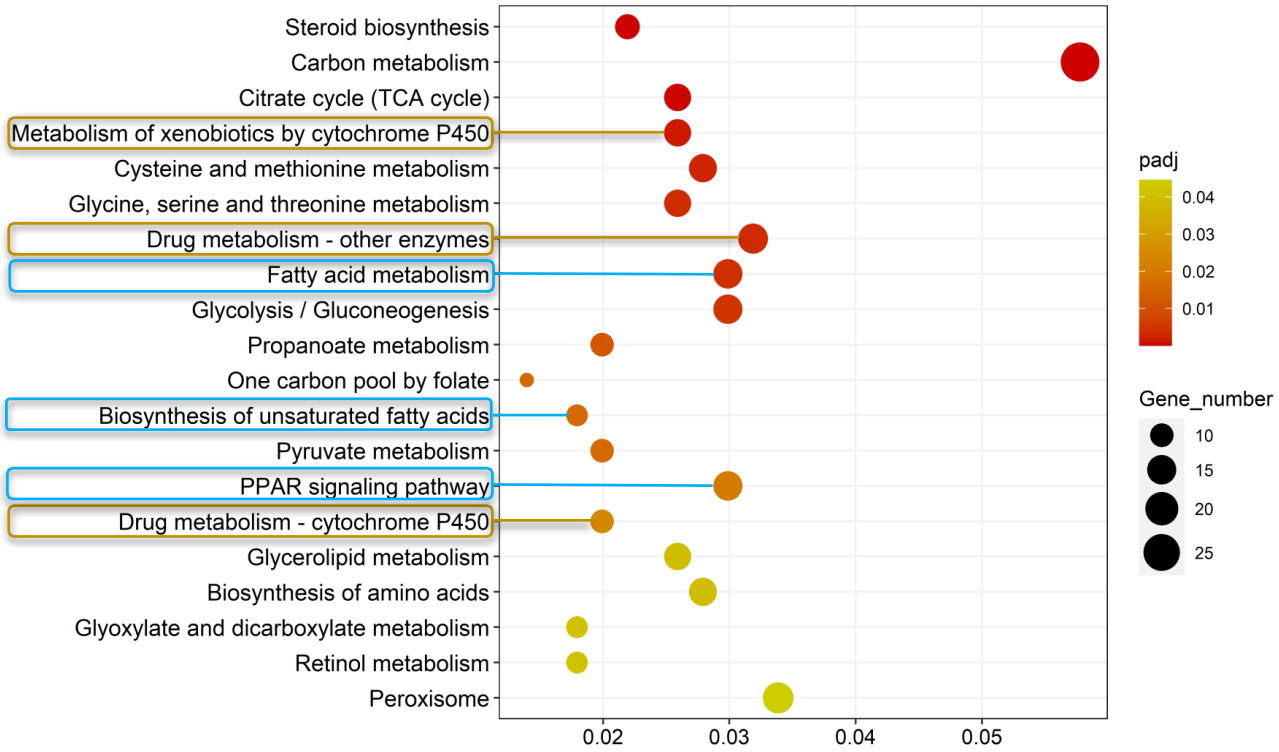
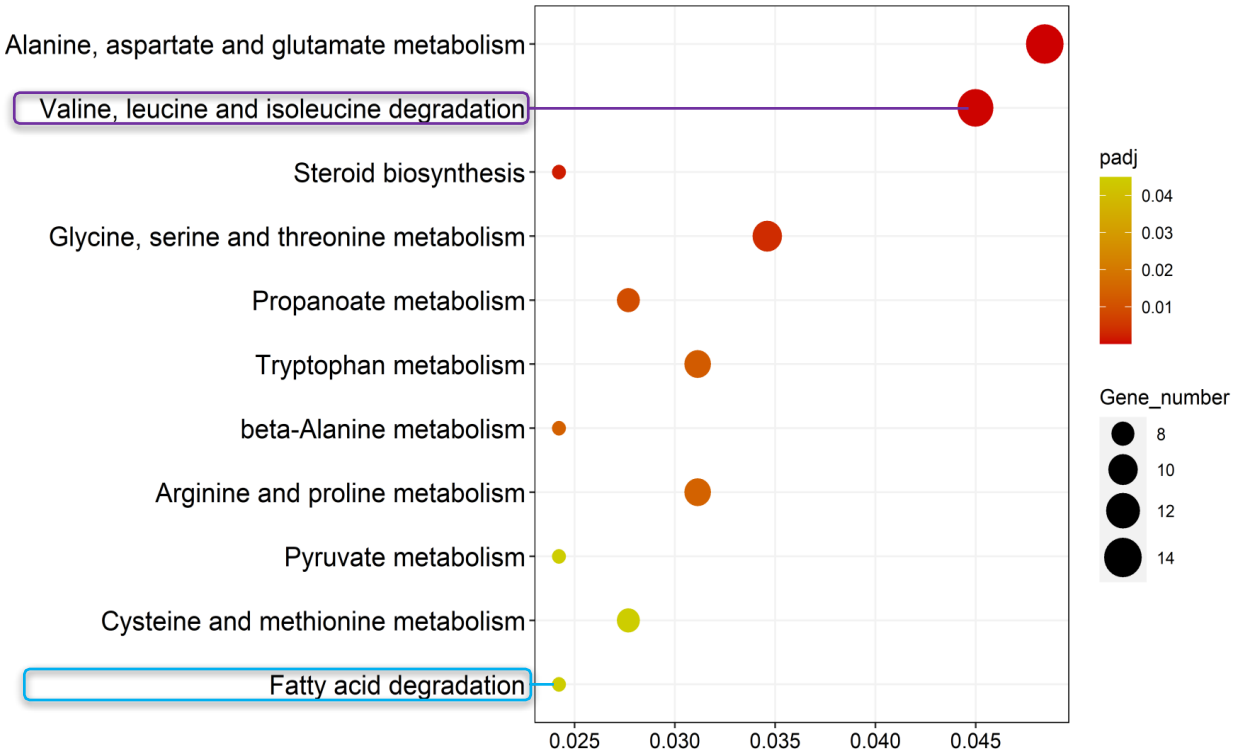
Significantly enriched KEGG pathways in MT vs. CON and RY+MT vs. CON

Enriched pathways MT vs. CON



Enriched pathways RY+MT vs. CON



Detoxification related pathways

Lipid metabolism related pathways

Amino acids related pathway

Lipid metabolism related pathway

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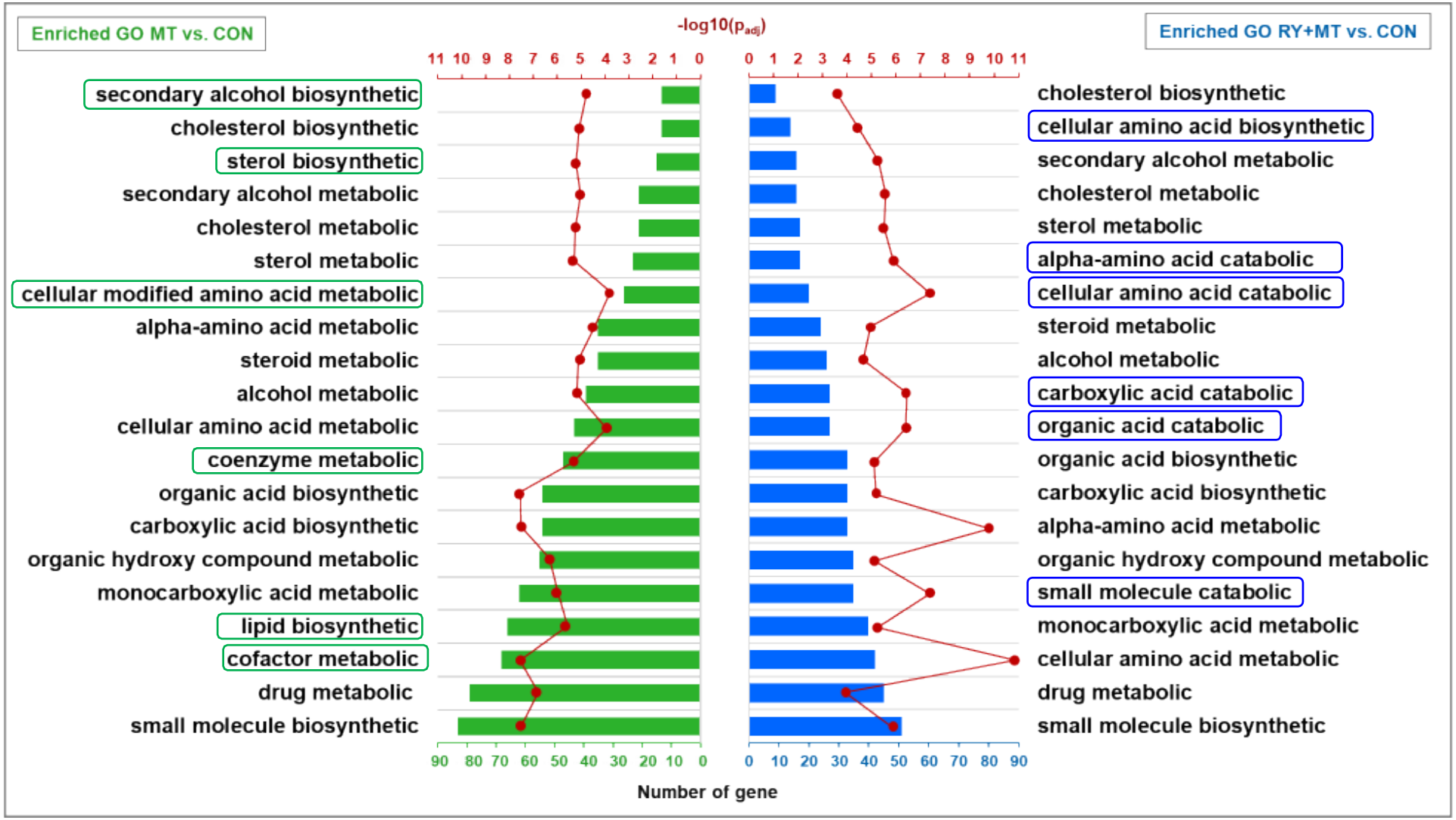
Significantly enriched gene ontology (GO) terms in MT vs. CON and RY+MT vs. CON



Conclusions

- This study indicated expression changes of genes involved in detoxification mechanism in response to feeding with multiple mycotoxins.
- Red yeast as a feed additive significantly reduced the adsorption of biologically active mycotoxins in feed.

- Red yeast can act as a mycotoxin binder and can be used as an effective strategy in the poultry feed industry.
- This feeding strategy can eliminate the adverse effects of mycotoxins to animals and increase food safety for human consumers.

Dr. Shahrbanou Hosseini

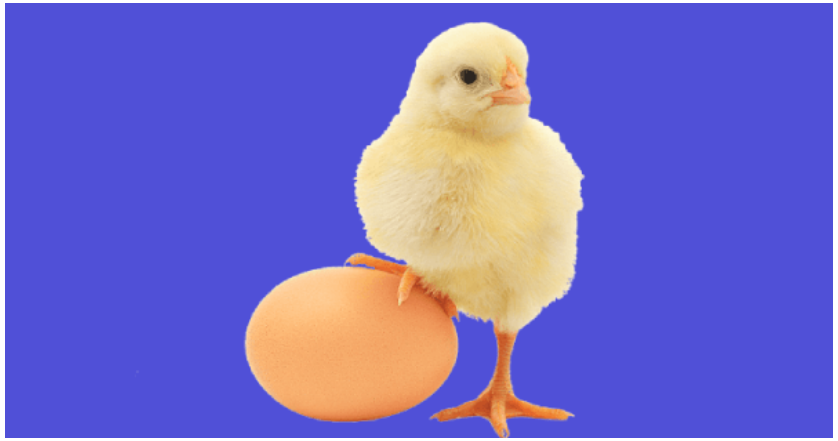
Molecular Biology of Livestock and Molecular Diagnostics

Department of Animal Sciences

University of Goettingen

Göttingen, Germany

shahrbanou.hosseini@uni-goettingen.de



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Genetic assessment of the effect of red yeast (Sporidiobolus pararoseus) as a feed additive on mycotoxin toxicity in laying hens

Shahrbanou Hosseini[1, 2*] Bertram Brenig[1, 2] Sunattinee Winitchakorn[3]
Chanidapha Kanmanee[3] Orranee Srinual[3, 4] Wanaporn Tapingkae[3, 4]
Kesinee Gatphayak[3, 4]

[1] Molecular Biology of Livestock and Molecular Diagnostics, Department of Animal Sciences, University of Goettingen, Germany
[2] Institute of Veterinary Medicine, Faculty of Agricultural Sciences, University of Göttingen, Germany
[3] Department of Animal and Aquatic Sciences, Chiang Mai University, Thailand
[4] Functional Feed Innovation Center, Faculty of Agriculture, Chiang Mai University, Thailand

Image: https://thepoultrypunch.com/2020/05/mycotoxins-and-biomarkers-of-exposure-win-the-battle-against-mycotoxins/

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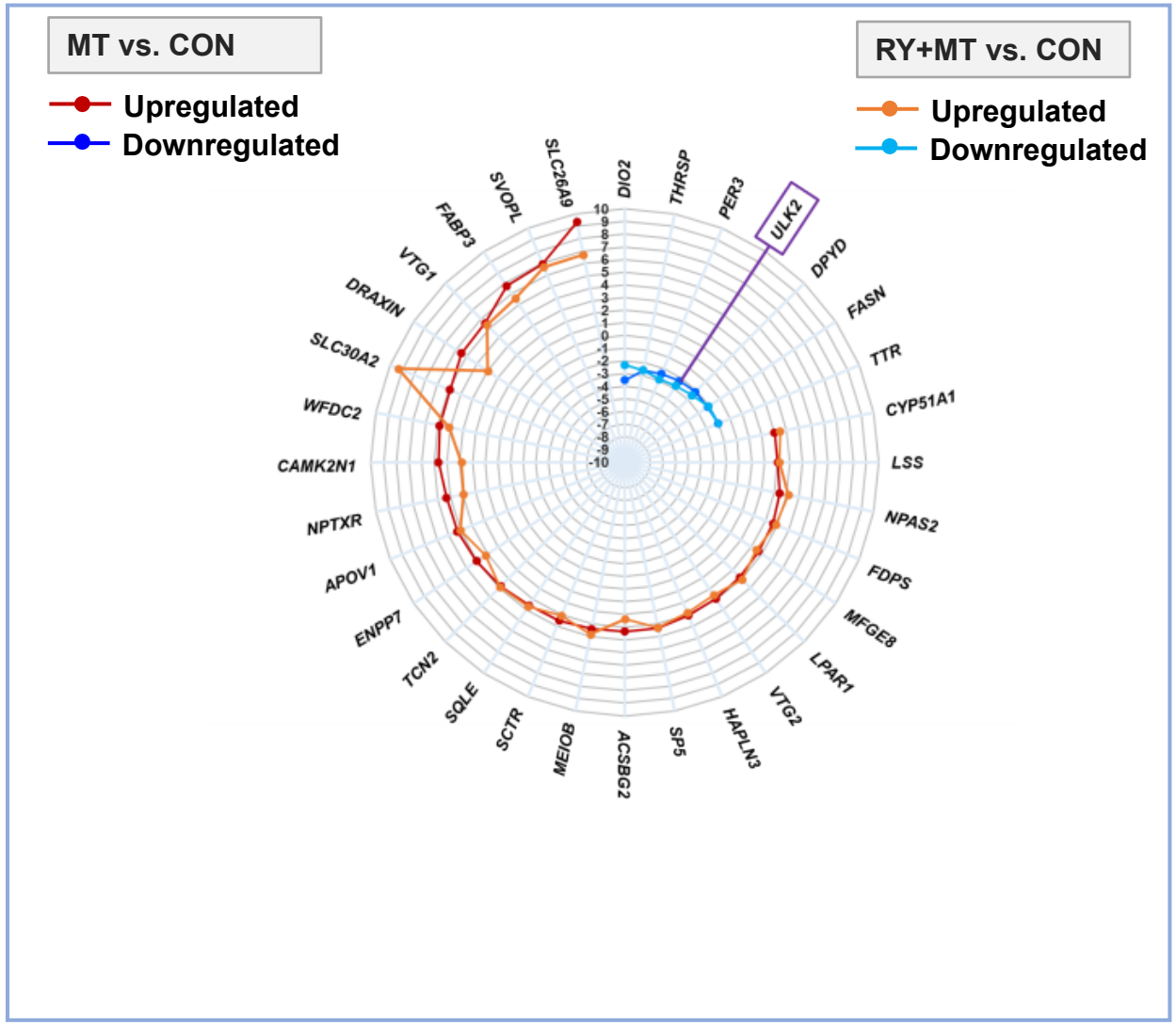
Top significant differentially expressed common genes in MT vs. CON and RY+MT vs. CON



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Candidate common genes involved in mycotoxicity in MT vs. CON and RY+MT vs. CON

MT vs. CON
- Upregulated
- Downregulated

RY+MT vs. CON
- Upregulated
- Downregulated

Differentially expressed genes

- *CYP1A1 and CYP1A2:Genes involved in phase I detoxification process*
- *GSTA2: Involved in phase II detoxification process*