UNIVERSITY of GUELPH

ONTARIO AGRICULTURAL COLLEGE

DEPARTMENT OF ANIMAL BIOSCIENCES

PhD. Defence

In Vitro Toxicity Assessment of Mycotoxins Using Bovine Mammary Epithelial Cells and Their Remediation Using Yeast Cell Wall-Based Adsorbents

Ran Xu

Date: February 22nd 2023 at 1:30pm

The PhD Defence for Ran Xu has been scheduled for February 22nd, 2023 at 1:30pm. The defence will be held online via Teams and in 141: https://teams.microsoft.com/l/meetup-join/19%3ameeting_ZTg4NWVjYzQtNmM5NS00NThmLWI5OTAtOWY3MTFiNjgzMWY4%40thread.v2/0?context=%7b%22Tid%22%3a%22be62a12b-2cad-49a1-a5fa-85f4f3156a7d%22%2c%22Oid%22%3a%22fbd28915-dda5-478f-8ecb-a3682dcf0c3a%22%7d

The exam committee will consist of:

Examining Chair: Dr. Vern Osborne

Adv. Committee Member: Dr. Niel Karrow

Adv. Committee Member: Dr. Craig Bailey

Additional Graduate Member: Dr. John Cant

External Examiner: Dr. Tai Guo

Abstract:

Frequent occurrence of the mycotoxins deoxynivalenol (DON), enniatin B (ENB), beauvericin (BEA), ochratoxin A (OTA) and citrinin (CIT) in ruminant feed and feedstuff can be a potential threat to feed safety and ruminant health. Inadequate biodegradation of mycotoxins by rumen microflora following ingestion of mycotoxin-contaminated feeds can lead to their circulatory transport to tissues such as mammary gland. In this thesis, individual effects of these mycotoxins on barrier and innate immune functions of the bovine mammary epithelium was first investigated using a bovine mammary epithelial cell line (MAC-T). Cytotoxicity assay was performed to investigate individual effects of these mycotoxins on cell viability, and potency?. Measurement of transepithelial electrical resistance (TEER) and paracellular flux of FITC-40 kDa dextran, expression of genes coding the tight junction (TJ) proteins *zonula occludens-1*, *occludin*? and *claudin 3* were performed using qPCR to assess mycotoxic effects on paracellular permeability. Expression of *toll-like receptor 4* (*TLR4*) and *interleukin-6* (*IL-6*), *tumor necrosis factor*-α (*TNF-a*) and *transforming growth factor-β* (*TGF-β*) were assessed to evaluate mycotoxic effects on innate immune function genes. Assessment of three yeast cell wall-based mycotoxin adsorbents (yeast cell wall (YCW), yeast cell wall extract (YCWE) and post-biotic yeast cell wall -based blend (PYCW)) to mitigate toxicity was carried out using an *in vitro* approach combining chemical assays and cell culture bioassays following under conditions that simulated the ruminant gastrointestinal environment. Mycotoxicity assessment results indicated that exposure of all mycotoxins significantly decreased cell viability in a concentration-dependent manner. Tested mycotoxins differentially modulated TEER and FITC-dextran flux as well as mRNA expression of selected TJ proteins, pro- and anti-inflammatory cytokines and *TLR4*. Adsorbent efficacy assessment indicated that Hill and Freundlich adsorption isotherm models were well-fitted into the LC-MS data; all tested adsorbents contributed to sequestering the tested mycotoxins to varying degrees. Also, the tested adsorbents helped to mitigate adverse effects of mycotoxins on MAC-T cells. These findings support the hypothesis that the tested mycotoxins can disrupt barrier and innate immune homeostasis of bovine mammary epithelial cells, and that yeast cell wall-based adsorbents possess the capacity to adsorb selected mycotoxins.