USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report – NCE for FY18
Due date: July 12, 2019

Cover Page

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Fiscal Year:	2017 (NCE for FY18)
USDA-ARS Agreement ID:	59-0206-4-008
USDA-ARS Agreement Title:	Application of Hormonal Biomarkers for DON-3-Glucoside Risk Assessment.
FY17 USDA-ARS Award Amount:	$ 63,882
Recipient Organization:	Michigan State University Contract & Grant Administration Hannah Administration Building, Room 2 East Lansing, MI 48824-1046
DUNS Number:	193247145
EIN:	38-6005984
Recipient Identifying Number or Account Number:	RC103734
Project/Grant Reporting Period:	5/3/18 - 5/2/19
Reporting Period End Date:	05/02/19

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
FST	Deoxynivalenol Plant Metabolite and Congener Toxicity in Mini-Gut Organoid Cultures.	$ 63,882
	FY17 Total ARS Award Amount	$ 63,882

7/15/2019

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Soft Winter Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: *Deoxynivalenol Plant Metabolite and Congener Toxicity in Mini-Gut Organoid Cultures.*

1. What are the major goals and objectives of the project?

Effects of DON and its congeners are potentially mediated by neuroendocrine hormones produced by enteroendocrine cells (EECs), one of the four primary intestinal cell subtypes that populate the epithelial layer of the GI tract. DON-induced anorexia (mouse) corresponds to the CCK and PYY secretion by "I" cell EEC lineage in the duodenum and "L" cell EEC lineage of the ileum and colon, respectively. Emesis (mink) which corresponds to increased plasma PYY and 5-HT which is produced by the "EC" cell EEC lineage found throughout the GI tract. The available cell culture models have limitations for assessing the toxicity of DON metabolites and congeners. There has been much recent progress on the propagation of adult intestinal stem cells from animals making it now feasible to generate ever-expanding, three-dimensional epithelial organoid structures in mini-gut cell culture that replicate the in vivo epithelium of the intestine. We proposed to test the guiding hypothesis that DON, DON plant metabolites and DON congeners differentially regulate hormone secretion in mini-gut organoid cultures.

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.*

1) Major activities
Test the guiding hypothesis that DON, DON plant metabolites and DON congeners differentially regulate hormone secretion in mini-gut organoid cultures.

2) Specific objectives
Determine the effects of DON and its congeners on calcium activation and enteroendocrine hormone release in ex vivo gut models

3) Significant results
Mouse organoid cultures were employees as an *in vitro* model to investigate how DON and its congeners influence cholecystokinin (CCK) secretion by enteroendocrine cells. While the positive control L-tryptophan significantly increased CCK secretion from organoids, no effect was found for DON. Thus DON may not act directly on calcium sensing receptor (CaSR), as we originally hypothesized based on previous studies using STC-1 cells.

We also used *ex vivo* model using organotypic slices of mouse small intestine. We attempted to label freshly isolated 300 μm tissue slices with a calcium sensing Fluo-4AM dye in combination with confocal fluorescence microscopy to investigate whether DON treatment increases intracellular calcium concentration. Compared to cultured cell lines, intestinal slices did not take up calcium sensing dye well within the recommended concentrations and incubation lengths, however going above those ranges stresses the cells. Furthermore, because of the presence of muscle layers underneath the mucosal

layer, there is constant twitching of the specimen despite they are pinned to the bottom of chamber slides, making quantification of signals from rare enteroendocrine cells (less than 1% cells in the mucosal layer) impossible.

Finally, we investigated a third model, enteric glial cells, to discern how DON acts in the intestine. Enteric glia are a collection of glial cells residing within the walls of the intestinal tract. These regulate intestinal motility, a well-characterized reflex controlled by enteric neurons that is affected by DON in in vivo. Enteric glia also network with many non-neuronal cells of the including enterocytes, enteroendocrine and immune cells and are therefore emerging as important local regulators of diverse gut functions. Thus DON and its congeners might affect vomiting and anorexia by acting on the enteric glia. We teamed up with MSU colleague Dr. Brian Gulbransen in the Department of Physiology who uses confocal microscope system to test activation of enteric glial cells in externalized mouse intestine. While positive controls showed activation, DON, even at high concentrations failed to do so.

4) Key outcomes or other achievements

Our results indicate that while DON and its congeners induce release of enteroendocrine hormones in vivo, reconstitution of DON response in three different ex vivo intestinal models could not be demonstrated. Thus it will not be possible to use test structure activity effects of DON congeners using the latter approaches.

3. What opportunities for training and professional development has the project provided?

We have provided training for undergraduate student assistants and graduate students.

4. How have the results been disseminated to communities of interest?

Throughout the course of the grant we have presented our research at local, national and international meetings and published in high impact journals.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17-NCE period. The term "support" below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student's stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY17-NCE period?**
   No

   If yes, how many?

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY17-NCE period?**
   No

   If yes, how many?

3. **Have any post docs who worked for you during the FY17-NCE period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   No

   If yes, how many?

4. **Have any post docs who worked for you during the FY17-NCE period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   No

   If yes, how many?

Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY17-NCE period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

NOTE: Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.

Name of Germplasm/Cultivar	**Grain Class**	**FHB Resistance** (S, MS, MR, R, where R represents your most resistant check)	**FHB Rating** (0-9)	**Year Released**

Add rows if needed.

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Abbreviations for Grain Classes

Barley - BAR
Durum - DUR
Hard Red Winter - HRW
Hard White Winter - HWW
Hard Red Spring - HRS
Soft Red Winter - SRW
Soft White Winter - SWW

Publications, Conference Papers, and Presentations

Instructions: Refer to the FY17-NCE_FPR-Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17-NCE grant period. Only include citations for publications submitted or presentations given during your award period (5/3/18 - 5/2/19). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each reference/citation, you must indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in publication/ presentation. See example below for a poster presented at the FHB Forum:

> Conley, E.J., and J.A. Anderson. 2018. Accuracy of Genome-Wide Prediction for Fusarium Head Blight Associated Traits in a Spring Wheat Breeding Program. In: Proceedings of the XXIV International Plant & Animal Genome Conference, San Diego, CA.
> Status: Abstract Published and Poster Presented
> Acknowledgement of Federal Support: YES (poster), NO (abstract)

Journal publications.

Zhou, HR and Pestka, JJ. DON influences release of gastroenteric hormones via CASR. (manuscript in preparation)

Books or other non-periodical, one-time publications.

Other publications, conference papers and presentations.