

USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Preliminary Final Performance Report
Due date: July 31, 2018

Cover Page

Principle Investigator (PI):	Jim Pestka
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Fiscal Year:	2017
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:	5/3/17 - 5/2/18
Reporting Period End Date:	5/2/2018

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



8-6-2018

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?

Several research studies suggest these effects are 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. Current 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

We successfully generated a mouse organoid culture system as an *in vitro* model to investigate how DON and its congeners influence cholecystinin (CCK) secretion by enteroendocrine cells. We found that the positive control L-tryptophan significantly increased CCK secretion from organoids, but no effect was found for DON. It is possible that DON may not act directly on calcium sensing receptor (CaSR), as we originally hypothesized based on previous studies using STC-1 cells.

As an alternative, we 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. Firstly, compared to cultured cell lines, intestinal slices do not take up calcium sensing dye well within the recommended concentrations and incubation lengths, however going above those ranges stresses the cells. Secondly, 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.

We investigated a third model, enteric glial cells, to uncover 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 findings suggest 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, predoctoral students, and postdoctoral fellows.

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

We have presented our research at local, national and international meetings. We have published our findings in international public journals with high impact factors.

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Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17 award 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 award 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 award period?**

yes

If yes, how many?

1

3. **Have any post docs who worked for you during the FY17 award 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 award 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?

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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 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *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

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Publications, Conference Papers, and Presentations

Instructions: Refer to the FY17-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17 grant. Only include citations for publications submitted or presentations given during your award period (5/3/17 - 5/2/17). 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.

Journal publications.

Wu, W., Zhou, H.R., Bursian, S.J., Link, J.E., Pestka, J.J., 2017. Calcium-sensing receptor and transient receptor ankyrin-1 mediate emesis induction by deoxynivalenol (vomitoxin). *Toxicol Sci* 155, 32-42.

Status: Published

Acknowledgement of Federal Support: YES

Wu, W., Zhou, H.R., Pestka, J.J., 2017. Potential roles for calcium-sensing receptor (CaSR) and transient receptor potential ankyrin-1 (TRPA1) in murine anorectic response to deoxynivalenol (vomitoxin). *Arch Toxicol* 91, 495-507.

Status: Published

Acknowledgement of Federal Support: YES

Pestka, J.J., Clark, E.S., Schwartz-Zimmermann, H.E., Berthiller, F., 2017. Sex is a determinant for deoxynivalenol metabolism and elimination in the mouse. *Toxins (Basel)* 9.

Status: Published

Acknowledgement of Federal Support: YES

Schwartz-Zimmermann, H.E., Hametner, C., Nagl, V., Fiby, I., Macheiner, L., Winkler, J., Danicke, S., Clark, E., Pestka, J.J., Berthiller, F., 2017. Glucuronidation of deoxynivalenol (DON) by different animal species: identification of iso-DON glucuronides and iso-deepoxy-DON glucuronides as novel DON metabolites in pigs, rats, mice, and cows. *Arch Toxicol* 91, 3857-3872.

Status: Published

Acknowledgement of Federal Support: YES

Pestka, J.J., Clark, E.S., Schwartz-Zimmermann, H.E., Berthiller, F., 2017. Age is a factor in deoxynivalenol metabolism and elimination in the mouse. (Manuscript in preparation).

Status: Published

Acknowledgement of Federal Support: YES

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Pestka, J.J. and Zhou, H.R. Deoxynivalenol activates calcium sensing receptor (Manuscript in preparation).

Status: Published

Acknowledgement of Federal Support: YES

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

Other publications, conference papers and presentations.

Pestka, J.J. Control of gastroenteric hormones by CaSR. 3RD International Symposium On: The Calcium Sensing Receptor (CASR). Florence, Italy, March 2018

Status: Presented

Acknowledgement of Federal Support: YES