**USDA-ARS/**
**U.S. Wheat and Barley Scab Initiative**
**FY05 Final Performance Report (approx. May 05 – April 06)**
**July 14, 2006**

## Cover Page

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| **Fiscal Year:** | **2005** |
| **FY05 ARS Agreement ID:** | **NA** |
| **Agreement Title:** | **Dose-Response Thresholds in DON Feeding Trials: Metabonomic and Proteomic Markers.** |
| **FY05 ARS Award Amount:** | **$ 45,126** |

**USWBSI Individual Project(s)**

| USWBSI Research Area* | Project Title | ARS Adjusted Award Amount |
|---|---|---|
| FSTU | Dose-Response Thresholds in DON Feeding Trials: Metabonomic and Proteomic Markers. | $ 45,126 |
| | **Total Award Amount** | **$ 45,126** |

_____ 07/10/06
Principal Investigator Date

* BIO – Biotechnology
CBC – Chemical & Biological Control
EDM – Epidemiology & Disease Management
FSTU – Food Safety, Toxicology, & Utilization
GIE – Germplasm Introduction & Enhancement
VDUN – Variety Development & Uniform Nurseries

**Project 1:** *Dose-Response Thresholds in DON Feeding Trials: Metabonomic and Proteomic Markers.*

**1. What major problem or issue is being resolved and how are you resolving it?**

The toxicology database used in the DON risk analysis conducted by regulatory agencies and authoritative bodies such as the Joint Food and Agricultural Organization/ World Health Organization Expert Committee on Food Additives is inadequate. Weaknesses in the hazard characterization increase uncertainty resulting in recommended tolerable daily intakes that are lower than necessary to ensure safety of consumers. The critical effect used for setting the current tolerable daily intake for DON was slightly reduced weight gain in mice during a 2-year study. This occurred in the absence of any unequivocal toxic effects and its mechanistic basis is unknown. We are currently in the process of conducting metabonomic studies for the purpose of developing a better understanding of the mechanistic basis for the reduced weight gain and identifying possible mechanism-based biomarkers. This information will reduce the uncertainty in both the DON hazard characterization and the exposure assessment. At the present time we have completed a dose-response gavage study using pure DON and B6C3F1 mice and have collected tissues and serum for on-going metabonomic studies and future proteomic studies. The serum and tissues are currently being used in developing analytical approaches using data-dependent LC/MS-MS to assess the dose-dependent changes in the metabolite profiles. Future studies will assess changes in relative protein abundance. We believe that the information gained from these on-going studies will reveal changes in specific metabolites and proteins that will provide critical information needed to reveal the underlying mechanistic basis for the reduced weight gain. In addition, the metabolite profiles will be useful for conducting short-term feeding studies in mice to assess the effectiveness of processing methods to reduce the effects of DON and thus refine the exposure assessment.

**2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):**

**Accomplishment:** We have conducted a dose-response gavage experiment using pure DON. The dose selection produces reduced weight gain and feed intake at the highest level and no effects at the two lower levels compared to saline gavaged and unhandled control groups. The tissues have been collected and stored and are being used for methods development using a newly acquired linear ion trap mass spectrometer (Finnigan LTQ with Ion Max ion source and Dynamic Nanospray Probe). A great deal of time, energy and money has been expended in developing the expertise to use this new instrument for both the metabonomic and proteomic analysis. Additional training has been scheduled (July 23-27, 2006) at the Thermo Electron Corporation Training Institute to learn Advanced Operations ("Biotech") which provides in depth training in nanospray ionization techniques and the use of the proteomics software. Currently we are using data dependent scanning to generate ion maps and spectrum lists for over 800 compounds in each tissue/serum extract. The intensities for each nominal mass is plotted so as to produce a metabolite profile for controls and treated tissues/serum. Replicate analyses are produced for each sample and averaged. The averaged values for each sample are used to produce a metabolite profile for each treatment group

with the mean and SD for each nominal mass. In addition to visual assessment of the segmented plots, we are developing an analytical method which will allow us to calculate the fold change for each metabolite relative to the other groups. The goal is to create a heat map for the fold change that can be analyzed statistically to determine responses that are statistically significant among treatments.

This is a long-term research project and thus we included a specific objective (Objective 3) in our recently approved 5 year USDA-ARS NP108 Project Plan – "Deoxynivalenol mechanism based bioassay". The milestones and expected outcomes for the objectives extend over the entire 60 months of the approved project. In summary, in this first year we have conducted a preliminary experiment, acquired a sophisticated instrument, developed expertise and training in the use of the instrument, and are in the process of developing approaches for analysis of the metabonomic data.

**Impact:** This is the first time that a metabonomic/proteomic approach has been implemented for the purpose of developing a better understanding of the mechanistic basis for the critical toxicological effect utilized in the DON risk assessment. This is a first step towards reducing the uncertainty in the risk assessment and could potentially result in revision of the current recommended tolerable daily intakes. Increase in the tolerable daily intake for DON could ease concern by importers of US agricultural products that are often contaminated with low levels of DON.

**As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:** There is now a focused effort by a well funded, equipped and trained ARS research team to fill existing gaps in the DON risk assessment. This is the beginning of a long-term commitment that could result in the transfer of information resulting in increased value of US exports and improved consumer confidence in the safety of the food supply.

**Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.**

**NONE**