SESSION 4:

FOOD SAFETY, TOXICOLOGY AND UTILIZATION OF MYCOTOXIN-CONTAMINATED GRAIN

Chairperson: David Kendra

INVESTIGATING THE IMPACT OF HISTOLOGICAL AND / OR CHEMICAL DIFFERENCES IN THE BRAN OF WHITE AND RED WHEAT NEAR-ISOGENIC LINES ON FUSARIUM MYCOTOXIN ACCUMULATION

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ABSTRACT

Hard white wheat (*Triticum aestivum* L.) is the newest U.S. class of wheat, and demand for this class has increased since milling it can provide higher flour yields and end-product appearance and taste are improved. However, many white wheat varieties are highly susceptible to Fusarium head blight (FHB), caused by *Fusarium graminearum* (teleomorph *Gibberella zeae*). Furthermore, the FHB mycotoxin, deoxynivalenol (DON) accumulates primarily in the bran layer of kernels. Since significant levels of DON can accumulate in infected wheat kernels, the bran may be a significant source of DON, particularly in whole white wheat products. Moreover, differences in bran thickness between white and red wheat kernels may impact the DON in such products, presenting a potential food contamination risk. Near-isogenic lines (NILs) of red and white winter wheat were developed for use in this study to examine potential bran differences and identify the impact of any genetic differences on the accumulation of DON in bran. Parents, NILs, and control varieties were artificially infected with FHB, and samples were collected from both infected and uninfected plants. For DON content analyses, samples from infected plants were visually sorted into kernels without damage and into *Fusarium* damaged kernels (FDK). To compare accumulation of DON in the bran layer, the same samples were pearl-milled to produce bran and non-bran fractions. DON accumulation in bran fractions was significantly higher than in non-bran fractions. The mean DON content in the bran fraction from the white parent was higher than for the red parent, but the mean DON content in the bran fraction from the red NILs was higher than for the white NILs. Continuing research is designed to determine if differences in DON accumulation are due to differences in the morphological or chemical properties of the white compared with the red bran layer.

NOVEL FEEDING BIOASSAY FOR CHARACTERIZATION OF DEOXYNIVALENOL-INDUCED FEED REFUSAL IN THE MOUSE

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ABSTRACT

Deoxynivalenol (DON) is a trichothecene mycotoxin that is resistant to heat processing, and as a result, contaminates grain products worldwide. DON causes feed refusal and growth retardation, the latter of which has been used to establish a Tolerable Daily Intake (TDI) in Europe. Despite being a primary adverse effect, the mechanisms of DON-induced feed refusal are not well understood. In order to relate feed refusal to other DON-induced physiological changes, the dose and timing at which feed refusal occurs needs to be elucidated. Using a novel mouse feeding bioassay, we determined DON's short-term effects on feed refusal following intraperitoneal injection. DON caused a significant, dose-dependent reduction in food intake that could be measured with-in two hours of exposure. In naïve mice, the lowest dose that caused feed refusal was 1 mg/kg bw. At 1 mg/kg bw DON, the duration of feed refusal was 3 hr; while at 5 mg/kg bw feed refusal lasted 6 hr. Mice appeared to become resistant to DON-induced feed refusal upon subsequent 24 hr exposures; yet, mice re- exposed to DON (5 mg/kg bw) 14 days after the last exposure exhibited a feed refusal response comparable to that of naïve mice. Taken together, these data suggest DON induced transient feed refusal. Furthermore, these data suggest that feed refusal and possibly other DON-induced physiological changes can be affected by the number of DON exposures and time since the last exposure. This information is important because risk assessments of DON have been based on chronic feeding studies.

CONVERSION OF DEOXYNIVALENOL TO 3-ACETYLDEOXY-NIVALENOL IN BARLEY-DERIVED FUEL ETHANOL CO-PRODUCTS WITH YEAST EXPRESSING TRICHO-THECENE 3-*O*-ACETYLTRANSFERASES

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ABSTRACT

The trichothecene mycotoxin deoxynivalenol (DON) is a common contaminant of distillers dried grains with solubles (DDGS; a co-product of fuel ethanol fermentation). Even low levels of DON (≤ 5 ppm) in DDGS pose a significant threat to the health of monogastric animals. Enzymes known as trichothecene 3-O-acetyltransferases convert DON to 3-acetyldeoxynivalenol (3ADON), and may reduce its toxicity. Two *Fusarium* trichothecene 3-*O*-acetyltransferases (FgTRI101 and FfTRI201) were cloned and expressed in yeast during a series of small-scale ethanol fermentations using barley. During the fermentation process, FgTRI101 converted 9.2% to 55.3% of the DON to 3ADON, resulting in DDGS with reductions in DON and increases in 3ADON in the Virginia winter barley cultivars Eve, Thoroughbred and Price, and experimental line VA06H-25. Analysis of barley mashes prepared from the barley line VA04B-125 showed that yeast expressing FfTRI201 were more effective at acetylating DON than those expressing FgTRI101; DON conversion for FfTRI201 ranged from 26.1% to 28.3%, whereas DON conversion for FgTRI101 ranged from 18.3% to 21.8% in VA04B-125 mashes. Ethanol yields were highest with the industrial yeast strain Ethanol Red, which also consumed galactose when present in the mash. This work demonstrates the potential of using transgenic yeast to modify DON during commercial fuel ethanol fermentation and has been published in the journal *Biotechnology for Biofuels*.

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COMPARISON OF DON ACCUMULATION IN BRAN AND FLOUR FRACTIONS OF FHB INFECTED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) in wheat is caused by the fungus *Fusarium graminearum*, which infects wheat heads at anthesis. One of the biggest causes of loss due to infection by *F. graminearum* is the production of mycotoxins in the infected grains, which are mainly type B trichothecenes. Studies have reported that a higher fraction of the total DON in the kernel accumulates in the bran. The aim of this study is to determine if genetic variation for resistance to toxin accumulation in the bran exists in locally adapted soft wheat varieties. Thirty-nine locally adapted cultivars and breeding lines (20 soft red, 19 soft white) having varying levels of visual FHB resistance and whole grain DON ppm were selected. The genotypes were planted in a multi-year (2009, 2010 and 2011) and multi-location study (2 locations per year) with artificial inoculation using two different methods. The two inoculation methods were 1) grain spawn followed by misting and 2) spray inoculation followed by bagging to maintain humidity. Visual symptoms were measured at 21dpi as % Incidence and % Severity. *Fusarium* damaged kernel scoring was carried out on harvested and cleaned subsamples by comparing to a set of standards. Accumulation of toxin was quantified by the GC-MS at the University of Minnesota, on a whole grain sample, and milled bran and flour fractions. Toxin (DON) data is presented for samples from 2009 and 2010.

A NEW UNDERSTANDING OF DON'S MECHANISMS OF ACTION

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ABSTRACT

Deoxynivalenol (DON, vomitoxin), a trichothecene mycotoxin produced by the field fungus *Fusarium*, is a very frequent contaminant of cereal-based foods throughout the world. A joint committee of the WHO-FAO designated DON as a global public health concern based on its capacity to cause growth suppression and emesis in experimental animals as well as its etiologic association with non-infectious human gastroenteritis. Biomarker studies have recently confirmed that human exposure to this toxin is relatively common and closely correlated with grain consumption. This problem has been exacerbated by recent changes in global climate and agricultural practices that have greatly increased fusarial blight. The inherent challenge of balancing risks (human growth stunting and acute illness) and benefits (availability of essential dietary staples) associated with consuming DON-containing grains has created a public health dilemma. DON's anorexic and emetic effects are highly consistent with aberrant hormonal and neuronal signaling within the "gut-brain axis" that is responsible for appetite control, however, the underlying mechanisms for such dysregulation remain undetermined. We propose that DON induces anorexia and emesis by aberrantly inducing secretion of gut satiety hormones by enteroendocrine cells. This hypothesis is based on our studies demonstrating that: 1) in the mouse, a emesis-resistant species, DON-induced anorexia corresponds with rapid, robust elevation in plasma levels of the gut satiety hormones cholecystokinin (CCK) and peptide YY (PYY), 2) in the mink, an emesis-susceptible species, DON causes both anorexia and emesis, and 3) DON induces CCK secretion in the murine STC-1 enteroendocrine cell, an established model for bitter taste receptor activation. Our hypothesis is further supported by studies of other researchers demonstrating that CCK and PYY dose-dependently induce anorexia and emesis in animals as well as humans. The rationale for conducting this research is that once it is known how DON disrupts regulation of the gut-brain axis, it will enable the rational design of targeted cell, animal and epidemiological studies to better understand the potential for adverse chronic and acute effects in individuals who consume this and related trichothecene mycotoxins.The expected outcomes of these studies will be an increased understanding of trichothecene-induced anorexia and emesis relative to critical initiating events, hormonal mediators, neuronal targets, and longevity of these effects. These findings will have a positive impact, because it will be an initial step in the path to predicting the specific thresholds of DON and other foodborne toxins for eliciting adverse human effects as well as the persistence and reversibility of these effects. Such knowledge can facilitate more precise science-based safety assessment and result in improved management strategies that reduce the risk of foodborne illness from DON and other trichothecenes while at the same time assuring food security through the availability of low cost, nutritionally important wheat- and barley-based foods.

THE ECONOMICS OF MYCOTOXIN CONTAMINATION TO THE MILLING INDUSTRY AND CONSUMERS

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ABSTRACT

Since there appears to be limited natural genetic disease resistance in our grains, Mother Nature holds the upper-hand in the manifestation of the diseases and the toxins many of them produce. It is imperative to fund and provide research for identification and implementation of disease resistance into our grain supply. Mycotoxin outbreaks occur from year to year and we as processors, ingredient manufacturers and food companies have to be extremely careful not to permit these compounds into our food products and ultimately reaching the consumer. The European Union has specific regulations for deoxynivalenol (DON) and Ochratoxin A in food systems. Canada only has standards for deoxynivalenol in Soft Wheat with no standards for Hard Red Winter or Spring wheats. The United States has standards for Aflatoxin in Corn, Guidelines for DON in wheat products only (bran, germ, flour) and none for Ochratoxin in Food Products. With the recent food recalls, the Food Safety Modernization Act and consumer demands for safer foods, the pressure is on us – the Processing Industry – to remove all concerns regarding potential contaminants and food safety for the consumer. To the processor, this responsibility becomes a very expensive exercise of determining initially whether to Accept or Reject in-bound commodities solely based on mycotoxin levels. The Canadian Food Inspection Agency (CFIA) and the United States Food Inspection Agency (FDA) have stepped up and/or staffed up to increase surveillance by on-site inspections and collecting of samples for mycotoxin analysis. In-house testing is necessary to assure the grain is acceptable for food use and this directly impacts food prices. The cost of mycotoxin test kits, time and labor to sample and conduct the analysis could cost the processor from .5M to $1M annually. If the local crop is unacceptable and one must reach out beyond the local grain draw area for origination, freight costs could be an additional $9M for a given geographic location. Therefore, as a processor, we would encourage even greater research efforts to develop resistant varieties that provide the producer a better quality crop, the processor a raw material that is more consistent year-to-year for quality and the consumer a quality retail product mitigating rising food prices.

RELATION OF 8-KETOTRICHOTHECENE STRUCTURE TO ANOREXIGENIC RESPONSE IN THE MOUSE

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ABSTRACT

The trichothecene mycotoxin deoxynivalenol (DON) is well known to cause food refusal in experimental animals. However, the relative anorexic potencies of structurally related 8-keto-trichothecene are not known. A simple food refusal bioassay employing the mouse was used to compare the effects of 8-keto-trichothecene following by oral and intraperitoneal (ip) exposure. The results suggested that, similar to DON, the anorexic effects of 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) were transient (lasting only a few hours) and food intake recovered within 16 h. In contrast, the food refusal responses to nivalenol (NIV) and fusarenon X (FX) were markedly different, persisting from 36 to 96 h depending on administration route. For both ADONs, the no observed adverse effect levels (NOAEL) and lowest observed adverse effect levels (LOAEL) were 0.5 and 1 mg/kg bw for ip, respectively, and 1 and 2.5 mg/kg bw for oral, respectively. The NOAEL and LOAEL for FX were 0.025 and 0.25 mg/kg bw, respectively, for both ip and oral exposure. The NOAEL and LOAEL for NIV were 0.01 and 0.1 mg/kg bw, respectively, for ip and 0.1 and 1, respectively, for oral exposure. To summarize, the anorexic effects of 8-ketotrichothecene followed the rank order NIV>FX>DON=15-ADON=3-ADON, based on the NOAEL and LOAEL, with effects being greater when administered ip as compared to oral exposure. 3-ADON and 15-ADON caused acute anorexia, similar to DON, whereas, the anorexic effects of NIV and FX were more persistent.