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POST HARVEST DON REDUCTION STRATEGIES FOR CANADIAN WESTERN SPRING WHEAT, DURUM AND BARLEY

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1. **Project title, ADF file number and reporting period.**
   Post Harvest DON reduction Strategies for Canadian Western Spring Wheat, Durum and Barley – Proj #: **20170292**.

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4. **Abstract (Not more than 250 words).**
   Deoxynivalenol (DON) is the major secondary metabolite produced by *Fusarium graminearum*. *F. graminearum* can infect cereals such as: wheat, durum, barley, rye, triticale, and corn. This toxin can make the grain unmarketable for producers. This study examined methods to recover high quality (low DON) wheat and barley from infected seed lots. Seed sorting by BoMill (Near Infrared Transmittance single seed sorting), air fractionating grain cleaning, the combination of the two and oxidation of the mycotoxin through introduction of Ozone gas produced by an ozone generator into the air stream of an aeration bin during grain drying immediately following harvest. The BoMill technology effectively sorted both wheat and barley into high and low DON fractions resulting in recovery of significant quantities of low DON wheat especially when using the Fusarium calibration combined with the appropriate vitreous kernel setting and precleaning. Air fractionation effectively separated infected wheat and barley based on relative grain density but the air speed settings had a significant effect on effectiveness. The combination of air fractionation followed by sorting of mid DON fractions on the BoMill was the most effective method of recovering high quality wheat and barley from material that otherwise had few marketing opportunities. Ozone treatment during drying by aeration significantly reduced DON concentration (up to a 50% reduction) and would be effective when grain is only marginally too high to market but in more extreme cases the combination of air fractionation and BoMill sorting are recommended.

5. **Introduction:**

Deoxynivalenol (DON) is the major secondary metabolite produced by *Fusarium graminearum*. *F. graminearum* can infect cereals such as: wheat, durum, barley, rye, triticale, and corn (Wegulo 2012). Infection of wheat is sporadic and occurs when spores are carried by wind or splashed by water during rainfall during the flowering stage of plant growth, (Beyer et al. 2010; Wegulo 2012). The infection spreads from one spikelet to others if ambient conditions remain humid with moderate heat (Beyer et al. 2010; Wegulo 2012). Wheat kernels infected by *F. graminearum* appear shrunken and chalky, with a white to pinkish color, these kernels are termed fusarium damaged kernels (FDK) (Delwiche et al. 2005).

Wheat is one of the highest produced cereals in Canada with a total production of 30.8 million tonnes, and 14.4 million tonnes from Saskatchewan alone in 2018 (Statistics Canada 2018). In 2016, FDK was found in up to 97% of Saskatchewan Canada Western red spring wheat samples, while in 2017 only up to 13% were observed (Canadian Grain Commission 2018a). If wheat contains greater than 4% FDK visually, it is downgraded to feed, but >10% it is downgraded further to salvage (Canadian Grain Commission 2018b). Downgraded grain represents a significant economic loss to the producer.

Barley is a key feed grain in Canada and like wheat has been susceptible to fusarium infection. In 2020, Canada produced 10.7 million tonnes of which 5.8 million tonnes is used domestically as feed, an addition 2 million tonnes are exported of which a signficant protion is also used in feeds internationally (Stats Canada, 2020).

There are currently few technologies commonly in use by the feed industry to reduce DON levels. The most common method used historically and currently to reduce DON level is by blending contaminated grain with uncontaminated grain (Trenholm et al. 1989). Blending may present a significant risk to feed producers, as DON can vary significantly within truckloads, the resultant feed may also have variable to high DON content (Trenholm et al. 1989). Acquisition of sorting technologies to reduce DON can represent a significant investment with variable returns. One such sorting machine is the BoMill, which uses near-infrared transmittance to sort grain based on Fusarium, protein, or vitreousness. Also available are colour sorters with near infrared, which sort grain by detecting defects in colour and shape. The least expensive sorting technology available may be the fractionating aspirator which sorts grain based on density.

Near-infrared transmittance (NIT) grain sorting technology measures the transmittance of near-infrared light which is emitted from individual grain kernels and predicts their relative chemical composition. A previous technology developed by Perten Instruments (Stockholm, Sweden) used near-infrared reflectance and had the capability of sorting 60 kernels per second with accuracy of sorting FDK from sound kernels of 99%, and further sorted kernels according to DON level (Peiris et al. 2010). Peiris et al. (2010) and Beyer et al. (2010) observed that sound kernels had a

different NIR absorbance than FDK. BoMill AB (Sweden) developed higher capacity single kernel sorting technology using NIT in their IQ and TriQ seed sorters. Using proprietary calibrations, these machines can sort wheat, barley, and durum, based on Fusarium damage, protein, vitreousness, and falling number. The BoMill IQ seed sorter collects individual kernels in a rotating singulator designed for the specified grain variety. Kernels pass under the NIT detector where transmittance information about that kernel is related to the ejector system to eject the kernel into one of six fractions. The Fusarium specific calibration is used to sort FDK by extent of fungal damage, and thereby by DON level.

Air fractionation is loosely based on technology of air classification used in flour milling. Air classification uses an air stream and centrifugal force to grind and carry fine flour particles to a container while coarse particles fall to have further particle size reduction (Voorhees 2013). Whereas air classification is a destructive process, air fractionation is a preservative sorting process. The air fractionation process uses an impeller fan to create an air stream that carries grain when released from the hopper above and in front of the fan. The horizontally travelling grain then falls into one of seven containers based on grain density or is carried up an incline into a cyclone chamber to deposit fine material in an eighth container. Air is recirculated through the machine, and ultra fine particles are caught by a secondary cyclone chamber.

Chemical degradation of mycotoxins is possible using the oxidizing agent ozone (McKenzie et al 1997; Wang et al. 2017; Piemontese et al. 2018). Effectiveness of DON degradation by gaseous ozone is dependent on initial DON level, exposure time, ozone concentration, and grain moisture (Awad et al. 2010; Wang et al. 2017). Where grain moisture wasn't a main factor, ozone exposure time was increased, while concentration was decreased with a resultant DON reduction of 30 to 50% (Wang et al. 2017; Piemontese et al. 2018). When grain moisture content was increased, ozone exposure time was reduced while concentration was increased, with the same DON reduction of 30 to 50% (Li et al. 2015; Wang et al. 2016). To date, the effect of temperature on the efficacy of gaseous ozone on DON reduction has not been determined. Consumption of DON contaminated feed by livestock results in inhibition of protein synthesis, reduced feed intake, organ dysfunction, and immunosuppression (Yunus et al. 2012; Gallo et al. 2015; Piemontese et al. 2018). Of the livestock species, pigs are the most sensitive to DON,

whereas chickens and beef cattle are the least sensitive (Maresca 2013). Dairy cattle are considered as sensitive as pigs, in addition to reduced feed intake they have reduced milk fat, and DON present in milk (Gallo et al. 2015). There is evidence of maternal transfer of DON to piglets in utero (Sayyari et al. 2018). Mice have reduced feed intake, body weight gain, increased liver and kidney enzymes, and decreased white and red blood cells (Piemontese et al. 2018). DON exposure to sow oocytes impairs oocyte maturation and increases intracellular aberrations, altering sow reproductive function (Malekinejad et al. 2007). Tissues with high protein synthesis rates such as the spleen, kidney, and ileum are affected more significantly by a large single dose of DON, before tissues with lower rates, such as skeletal and cardiac tissue (Tiemann and Dänicke 2007).

Broiler chickens and turkey poults have a similar low oral bioavailability and rapid biotransformation of DON (Devreese et al. 2015). A review by Awad et al. (2010) noted that broilers displayed no adverse effects to reduced feed intake, weight gain, and efficiency at 10 to 15 mg/kg of diet, whereas Wang and Hogan (2018) observed reduced feed intake and efficiency at 6 to 8 mg/kg of feed. DON alters gastrointestinal morphology by having shorter and narrower villi, and shallower crypts, which may in turn alter nutrient absorption (Wang and Hogan 2018). Aflatoxicosis was prevented in turkey poults by ozone treatment of the feed (McKenzie et al. 1998).

6. Objectives and the *progress towards meeting each objective*

Objectives (*Please list the original objectives and/or revised objectives if Ministry-approved revisions have been made to original objective. A justification is needed for any deviation from original objectives)*	**Progress (e.g. completed/in progress)**
To develop and examine strategies to reduce the Mycotoxin deoxynivalenol (DON) in Barley and Wheat post harvest on farm	**Completed**
To examine strategies to reduce the Mycotoxin deoxynivalenol (DON) in Barley Post Harvest during seed cleaning	**Completed**

Please add additional lines as required.

7. Methodology:

A series of studies were conducted to determine the most practical and effective on farm methods to detoxify fusarium infected wheat and barley. These strategies included: Sorting seed using a near infrared transmittance system (BoMill) and specifically testing a new fusarium calibration as well as new settings for vitreous kernal settings. The impact of seed cleaning prior to sorting with a BoMill was also investigated to determine if the level of unsorted/rejected seeds can be reduced and overall efficiency of sorting can be improved. Studies were also conducted using 2 makes of air fractionation systems to determine if they can effectively remove fusarium infected kernels based on separation due to changes in relative grain density and thereby reduce the level of toxin in the wheat and barley. A study was also conducted to determine if a combination of air fractionation followed by BoMill sorting would be a more effective method of reducing DON concentrations and increasing the rate of recovery. Studies were also conducted to determine if abrading the grain prior to air fractionation would effectively reduce DON concentration of the final grain fractions. A series of studies was also conducted to determine if DON can be reduced through oxidation by treatment of infected grain with ozone treatment during aeration post harvest. A final study was conducted to determine if reducing DON through sorting technology will improve broiler performance and improve intestinal integrity.

Each study is described, including the detailed description of experimental design, in the Results and discussion section below.

8. Results and discussion:

8.1 Determination of sorting capability and grain recovery of deoxynivalenol contaminated wheat using different calibrations and settings from the near-infrared transmittance technology of BoMill AB

Abstract

Fusarium infection of wheat causes the production of the secondary metabolite deoxynivalenol (DON), which affects animal performance. Limited post-harvest sorting technologies are available to remove moderately infected kernels and improve overall grain recovery below 5ppm DON. Proprietary near-infrared seed sorting calibrations for *fusarium* damage have recently been developed by BoMill AB (Sweden) to achieve this objective. Two main experiments were conducted to test the sorting efficacy of the BoMill IQ using the new calibrations. Wheat with 8-10 ppm DON was pre-cleaned to remove debris, small, and shriveled kernels prior to sorting. The impact of HVK setting and calibration (Fusarium and

Protein) was tested. Recovery of low *fusarium* grain using the Fusarium calibration settings was 43.4, 50.3, and 44.9%, with rejection rates of 38.7, 29.0, and 22.7% and average DON in recoverable fractions was 2.0, 1.7, and 2.4 ppm, using the HVK, HHVK, and HHHVK settings respectively. Using the Protein calibration settings, grain recoveries were 46.2 and 51.1%, with rejection rates of 35.6, and 17.5% and average DON in recoverable fractions was 1.6, and 3.0 ppm, using the HVK, and HHVK settings, respectively. Therefore, it was determined that the Fusarium calibration using the HHVK setting was most effective, as it resulted in lower average DON with lower rejection rates. The second experiment examined sorting efficiency using the optimal setting on a pooled 6 ppm wheat, and a 15 ppm wheat. Grain recovery from the pooled and 15 ppm wheat was 69.0 and 46.1%, with an average DON of 1.3 and 2.2 ppm, respectively. Sorting using a BoMill equipped with the *Fusarium* calibration and HHVK setting will effectively sort a range of wheat samples, including pooled samples, into low DON fractions, increasing the grain value and minimizing any negative effects on the animal.

Introduction

Proprietary near-infrared transmittance (NIT) calibrations have been developed to sort wheat based on chemical composition changes within grain, including relative protein content, grain vitreousness, and the changes caused by *Fusarium* damage (BoMill AB, Sweden). BoMill AB manufactured both large and small scale single seed sorters, the IQ which sorts 1 kg/hr, and the TriQ, which sorts up to 3 Ton/hr. Kautzman et al. (2015) found that sorting based on protein content also sorted the grain into low and high DON fractions using the larger BoMill TriQ. However, the challenges related to the unsorted seed and other material that was not sorted were faced by the researchers, reducing the overall effectiveness of the process (personal communication Rex Newkirk 2019).

The objective of the study was to determine if increased grain recovery of below 5 ppm DON is possible with grain pre-cleaning and the NIT sorter made by BoMill AB using calibrations specifically designed for *Fusarium* infected kernels. Hypothesis one was that pre-cleaning grain will allow more efficient seed sorting by removing unsortable material regardless of BoMill IQ calibration or setting. The second hypothesis was that using a combination of a Fusarium calibration and one of the three HVK (hard vitreous kernel) settings will improve grain recovery of grain below 5 ppm DON with fewer rejects with the BoMill IQ than using the Protein calibration. The third hypothesis was that the optimal calibration and setting determined in experiment 2 could with similar results, sort other sources of wheat with different initial DON concentrations, and growing years.

Materials and Methods

Experiment 1

Canadian Hard Red wheat naturally contaminated with DON (W1) was grown in 2014 Saskatchewan (N 52˚50'17.87", W 107˚34'12.42"). The experiment was designed as a 2 x 2 factorial arrangement for both the Fusarium and Protein calibrations based on grain pre-cleaning (cleaned wheat and uncleaned wheat) and BoMill IQ setting (Fusarium: HVK and HHHVK, Protein: HVK and HHVK). Each calibration setting was replicated four times in the experiment. Wheat was cleaned by sieving with a 5.25 size slotted sieve, and further hand cleaned as sieving did not completely clean the grain. The singulator disc used was a #14 with 90 slots. A setting spectral curve was run for 600 seconds on a BoMill IQ (model 1002, version 2.0.79, BoMill AB, Sweden) to create a spectral histogram for each treatment to allow grain segregation. Fraction borders were created as part of the spectral histogram and were set equal (16.66 %) for fractions 1 through 6 for each treatment. The wheat sample size was 1.0 kg for each replication. The BoMill IQ creates eight fractions; fraction one (F1) is the highest DON wheat fraction, fractions

2 to 6 (F2 to F6) are low DON wheat fractions, rejects are kernels that were not sat in the singulator disc slots properly or were outside of the calibration, and the unsorted fraction that was not sorted when the machine determined that it was emptied. The machine was set to automatically stop when sensing that it was emptied. Each fraction was weighed to determine the fraction proportion (%) after sorting.

The Protein calibration has five separate settings ranging from LVK to HHVK, and sorting occurs based on relative crude protein content where LVK would sort a low crude protein content, and HHVK would sort a high crude protein content. The *Fusarium* calibration was built and specialized from samples sorted by the Protein calibration; settings range from LVK to HHHVK. The settings that are not used for both calibrations were outside of the setting spectral limits for the wheat used in the current study (LVK, and MVK).

Experiment 2

Cleaned wheat samples from Experiment 1 (Fusarium: HVK, HHHVK; Protein: HVK, HHVK) were used for Experiment 2. In addition, one more setting was used (*Fusarium*: HHVK) to test the full range of settings available from BoMill AB. The same procedure was adhered to as in Experiment 1. Fraction proportion, grain density, and DON analysis was performed for each calibration setting. The average grain density was obtained by using a 100 ml graduated cylinder and observing the weight (g) and volume (i.e.: (g/ml)*500; g/0.5L then converted to kg/hL).

Experiment 3

Two additional wheat sources of Canadian Hard Red wheat were acquired to test the ability to sort other sources of wheat; one from Kenaston, SK (W2) with an approximate DON concentration of 13-15 ppm. A pooled wheat sample (W3) was created by combining equal

portions of cleaned W1, W2, and bulk wheat sourced from the Canadian Feed Research Centre (tested negative for DON), to make 1 kg for each replication with a total of four replications.

Using the Fusarium HHVK setting, which was determined to be optimal in Experiment 2 with a moderate rejection rate and grain recovery, and low average DON, W2 and W3 were processed through the BoMill. Grain proportion, density, and DON concentration were measured using the same procedures as Experiment 1, and 2. The grain from W3 was further separated by the kernel size using the #12 singulator to allow sorting, since the large kernels were not fitting into the singulator disc slots.

DON Analysis

DON testing was performed using the Vicam DON-V ELISA test (room temperature method) and Vertu Lateral Flow Reader (Milford, MA, USA). The manufacturer procedures with the standard dilution techniques were used to obtain DON concentration. A sub-sample from every treatment replication was ground on the Fine setting using a BUNN G1 HD grinder (Springfield, IL, USA). Five grams of ground wheat was weighed into an extraction tube, and then 20 ml of distilled water was added to the tube. The sample was vortex mixed for attachment specified maximum for 2 min. Contents of the extraction tube were filtered using the supplied filter paper into another extraction tube, and then 100 µL of the filtrate was placed into 1ml distilled water and vortex mixed. A hundred microlitres of the supplied diluent was placed into a sample vial with 100 µL of diluted filtrate and vortex mixed. Then 100µL of the mixture was transferred to the sample well of DON-V cassette (1 drop/s) and developed for 3 minutes. After cassette development, the cassette was placed in the Vertu Lateral Flow Reader to obtain DON results.

Grain recovery and rejection rate

Total grain recovery was determined by combining the weights of all sortable fractions (fractions 1 to 6) with DON concentrations under 5 ppm to obtain the proportion recovered. Rejection rate was determined by observing the weight of material that was sorted into the rejects fraction.

Statistical Analysis

Analysis of variance was performed using JMP (version 12; SAS Institute, Inc., Cary, NC, USA). The experimental model for Experiment 1 was a Complete Randomized Design, $Y_{ijk}=\mu+C_i+S_j+C*S_{ij}+e_{ijk}$, where: Y_{ijk} = observation, μ = mean, C_i = cleaning effect (I = 1 to 2), S_j = setting effect (S = 1 to 2), $C*S_{ij}$ = cleaning and setting interaction, and e_{ijk} is the error term. The model of Experiment 2 was $Y_{ij}=\mu+T_i+e_{ij}$, where: Y_{ij} = observation, μ = mean, T_i = fraction effect (I = 1 to 8), and e_{ij} is the error term. The second model for Experiment 2 was $Y_{ij}=\mu+S_i+e_{ij}$, where: Y_{ij} = observation, μ = mean, S_i = setting effect (Fusarium I = 1 to 3; Protein 1 to 2), and e_{ij} is the error term. The model for the Experiment 3 was $Y_i=\mu+W_i+e_{ij}$, where: Y_{ij} = observation, μ = mean, W_i = wheat effect (I = 1 to 3), and e_{ij} is the error term. Significant differences were indicated at $P < 0.05$, and trends discussed at $0.05 < P < 0.1$.

Results

Cleaning

There was no interaction between grain pre-cleaning and setting for both the Fusarium and Protein calibrations. Pre-cleaning grain significantly reduced the amount of unsorted grain for the Fusarium and Protein calibration settings, and the Protein HVK setting was lower in F3 than the HHVK setting, but there was no difference between cleaned and uncleaned grain sorted into all other fractions (Table 1 and 2). The Fusarium HVK F3 tended to be lower than the HHHVK setting, and the Protein HVK F4 also tended to be lower than the HHVK setting. The setting had a significant effect on sorting in the Fusarium calibration for all fractions except F5

and the unsorted fraction. The setting had a significant effect on sorting into all fractions except the unsorted fraction for the Protein calibration.

Calibration and BoMill IQ setting

The effects of calibration and BoMill IQ setting on the distribution and DON concentrations of DON contaminated wheat fractions are shown in Table 3 and 4. The Fusarium calibration settings F1, F4, and F6 had significantly different DON concentrations between settings. The HVK Fusarium setting F1 had higher DON concentration than the HHVK and HHHVK settings. The HHHVK Fusarium setting had higher DON concentrations than the HVK and HHVK settings for F4 and F6. As the Fusarium calibration settings changed from HVK to HHHVK, there was more grain sorted into the HHHVK setting versus the HVK setting for F1, F2, and F3, with a tendency for F4 (0.052) and F6. The rejects decreased from HVK to HHHVK. For the Protein calibration settings, the DON concentrations were higher for the HHVK setting than the HVK setting for F4, F5, and F6, and a tendency for F3. The HHVK Protein calibration setting had higher grain sorted into each fraction except for F1 and the unsorted fraction.

Grain recovery of Experiment 2

The grain recovery, rejection rate, and DON concentrations for experiment 2 are shown in Table 5. The recovery of wheat under 5 ppm DON for the Fusarium HHVK setting was higher than the HVK setting, but not different from the HHHVK setting which was the intermediate. A recoverable fraction was specified as any fraction between F1 and F6 that was under 5 ppm DON. The rejection rate decreased from the HVK to HHHVK settings. The average DON

concentration tended to be higher for the Fusarium HHHVK setting. The grain recovery for the Protein calibration HHVK setting was higher than the HVK setting. The Protein HHVK setting rejection rate was lower than the HVK setting. The average DON concentration in recoverable fractions for the Protein calibration HHVK setting was higher compared to the HVK setting.

Comparison of wheat source

The effect of HHVK Fusarium setting on wheat source on distribution, fraction DON concentration, grain recovery, rejection rate, and DON results are shown in Table 6, 7, and 8. The wheat from W3 required further kernel separation by size to fit the singulator while sorting properly. For fractions 1 and 2, W3b (large kernels) had the greatest amount of grain sorted into each fraction, W2 and W3a the least, and W1 was the intermediate. Fractions 3 and 4 demonstrated similar behaviours, except W2 was also an intermediate. In fractions 5 and 6, W3b was greater than the other wheat sources, but W1, W2, and W3a were not different from each other. W3b in the rejections fraction had least material, whereas W2 and W3a the greatest; and W1 was the intermediate. The unsorted material in W1 tended to be higher than the other wheats, and no material remained in the machine for W3b after stopping.

The DON concentration in W3b were lower than the other wheat sources, which were not different from each other in fractions 1, 4, and the rejects fraction. In fraction 2, W3b had the lowest DON concentration, W2 the highest, W1 the intermediate, and W3a was not different from W1 and W2. Fraction 3 demonstrated similar results to fraction 2, except W1 and W3a were both intermediates. Fraction 4 and the rejects fraction demonstrated different results than the other fractions, where W3b had the lowest DON concentration, but the other wheat sources DON concentrations were not different from each other. W3b in fraction 5 had the lowest DON concentration, and W1 and W2 the highest, but W3a was not different from the other wheat

sources. In fraction 6, W2, W3a, and W3b had the lowest DON concentration, and W1 the highest. The unsorted fraction was not significant.

The source W3 had the highest grain recovery, whereas W2 had the lowest recovery; W1 was the intermediate. W2 had a higher rejection rate than both W1 and W3. The DON concentration for W3 was lower than either W1 or W2.

Discussion

Grain pre-cleaning

Grain cleaning is an important part of grain milling to remove impurities such as straw, dust, and small or broken kernels (Hazel and Patel 2004). Pre-cleaning grain reduces the bulk material such as straw and chaff, which blocks the singulator channels, preventing kernels from falling into the slots properly. Kautzman et al. (2015) postulated that cleaning grain prior to sorting would reduce outliers such as rejects, and grain sorted into the high DON fraction. As Kautzman et al. (2015) did not pre-clean their samples, afterwards, they discussed that pre-cleaning grain prior to sorting may reduce the rejects and material sorted into the high DON fraction. The post study discussion of Kautzman et al. (2015) is not supported by the current experiment, as only the unsorted material remaining in the machine was reduced by grain cleaning, in the current study only one wheat class was used, whereas three were used in theirs. The differences in chemical composition between the wheat classes would be adjusted for by calibration setting and singulator disc slot shape and size. A major difference between the current and previous study is that they used the larger BoMill TriQ, whereas we used the BoMill IQ, but the two machines were designed to behave in a similar manner. Pre-cleaning allows more

efficient sorting, as kernels can fall into singulator slots more easily, and less material would remain in the machine to clog it during large capacity sorting.

Calibration and BoMill IQ setting

BoMill AB created multiple calibrations and settings to meet the needs of the user. The BoMill IQ segregates grain into six sortable fractions, and two unsortable fractions and sorting borders are set based on the initial spectral histogram. There is no one setting within a calibration that can sort all grain, as there exist broad variations in protein content, growing conditions, and genetic differences between wheat varieties. It was important to achieve DON segregation within the spectral calibration and have as low a rejection rate as possible with a high grain recovery, when taking the results in context. A study performed by Peiris et al. (2010) observed NIR absorption band differences at 1160-1220nm and the 1395-1440nm regions and had a high accuracy for the removal of Fusarium damaged kernels. It could be speculated that BoMill combined with the previously observed absorption bands and further elucidated other absorption bands to create their calibrations and settings. As NIR spectral calibrations are proprietary to BoMill AB, the absorption patterns were not known to the researchers of this study. Kautzman et al. (2015) only had the Protein calibration available to them, so it was important to build on and compare the calibration they used, as well, and report grain recovery. Although there were significant differences in grain density both between fractions and between settings, these changes are not commercially relevant (data not shown).

HVK setting within calibration affects DON segregation into fractions. In the Fusarium calibration HHHVK setting we observed a reduction of DON in fraction 1, this appears a redistribution of high DON grain into other fractions, and dilution of low DON grain sorted into fraction 1. We observed the opposite effect in the Fusarium HVK setting where fraction 1 had a

high DON concentration but a smaller amount sorted into the fraction, and the HHVK setting was the intermediate of the two settings. This indicates that the Fusarium HVK setting had a higher specificity for the wheat sample to segregate and create low DON fractions but had a high rejection rate. The Fusarium HHHVK results are an example of sorting at the edge of the spectral capability, and the user will also observe higher DON concentrations in fractions 2 and 6. Alternatively, the results observed in the Protein calibration settings demonstrated the expected behaviour with increasing HVK setting the DON is redistributed from the rejects fraction into the sortable fractions.

Different wheat sources

Using multiple sources of wheat to test the applicability and range of this technology, the current experiment indicates that the Fusarium calibration using the HHVK setting can effectively sort and recover grain from a wide range of initial DON concentrations. Using a bichromatic optical sorter Delwiche et al. (2005) was able to reduce the average DON concentration by 51%. They estimated for the optical sorter to reduce the DON in wheat to under 2 ppm would require two successive sorts and a maximum DON concentration of 5.7 ppm. In comparison, we achieved a reduction of 76-81%, with an average DON under 2 ppm, which would be appropriate for further milling or livestock feed. The differences observed in grain recovery, DON concentration, and rejection rate between the three sources of wheat appears to be primarily attributable to the initial DON concentration. Wheat 2 had the highest initial DON concentration of 13-15 ppm, indicating that a greater proportion within the sample would be damaged by *fusarium*, which would reduce the possible recovery of low DON grain and increase the rejection rate. It is important to note that the DON concentration between W1 and W2 were not different, which demonstrates that the damage caused by *fusarium* creates consistent

chemical changes across cultivation years and growing conditions. If the pooled wheat sample W3 was not separated by kernel size, it may be speculated that those results would be similar to that of W1 for recovery and DON. The sorting process using the BoMill could be further optimized by sorting and removing the highest DON fraction (fraction 1), then recombining and re-sorting, which may reduce the DON concentration in each fraction and the overall rejection rate.

Conclusions

In summary, the NIT technology developed by BoMill AB (Sweden) was studied to evaluate the effects of grain pre-cleaning on sorting efficiency, to compare the grain recoveries under 5 ppm DON from the BoMill IQ HVK settings of the Fusarium and Protein calibrations, and the applicability to a wide range of initial DON concentrations. Pre-cleaning the grain significantly reduced the unsorted material, thereby improving sorting efficiency. The Fusarium HHVK setting is capable of segregating a wide range of DON concentrations, recovering 46 to 68% of grain with DON concentrations under 2 ppm. Near-infrared transmittance seed singulation technology is a non-destructive and effective method to reduce DON concentration and improve wheat quality. Further improvements to the sorter would improve sorting capability and efficiency.

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Table 1. Effects of grain pre-cleaning and BoMill IQ setting on grain distribution using the BoMill AB Fusarium calibration

	Fusarium Calibration							
	HVK[2]		HHHVK[2]					
	Distribution (%)					P		
Fraction	Cleaned	Uncleaned	Cleaned	Uncleaned	SEM	C[1]	S[1]	C*S[1]
1	10.3	9.4	16.0	13.4	1.13	0.154	0.001	0.471
2	10.4	9.7	14.0	13.4	0.68	0.344	<0.001	0.889
3	9.6	8.7	12.1	11.9	0.26	0.075	<0.001	0.248
4	8.7	8.1	10.4	10.3	0.42	0.408	<0.001	0.559
5	7.9	7.5	8.5	8.6	0.76	0.761	0.291	0.870
6	6.7	6.3	8.5	8.4	0.46	0.587	0.001	0.821
Rejects	38.7	39.4	22.7	24.1	0.95	0.307	<0.001	0.749
Unsorted	7.3[a]	10.6[b]	7.4[a]	9.4[b]	0.52	<0.001	0.294	0.237

[a-b]Means with different letter superscript in rows are significantly different at $P < 0.05$.
[1]Cleaning; setting; interaction of cleaning and setting
[2]Hard vitreous kernel, where HVK is a lower relative protein content, and HHHVK high relative protein content

Table 2. Effects of grain pre-cleaning and BoMill IQ setting on grain distribution using the BoMill AB Protein calibration

	Protein Calibration							
	HVK[2]		HHVK[2]					
	Distribution (%)						P	
Fraction	Cleaned	Uncleaned	Cleaned	Uncleaned	SEM	C[1]	S[1]	C*S[1]
1	12.4	10.0	15.0	15.6	0.96	0.377	0.001	0.136
2	12.1	10.8	14.4	14.9	0.56	0.453	<0.001	0.146
3	10.5	9.5	13.6	12.8	0.35	0.035	<0.001	0.812
4	9.0	8.9	12.2	11.0	0.30	0.061	<0.001	0.082
5	8.2	7.9	10.9	9.6	0.47	0.137	<0.001	0.279
6	6.5	6.7	10.4	9.5	0.41	0.461	<0.001	0.197
Rejects	35.6	35.8	17.5	18.5	0.82	0.488	<0.001	0.626
Unsorted	5.5[a]	9.8[b]	5.9[a]	10.0[b]	1.69	0.029	0.848	0.943

Means with different letter superscript in rows are significantly different at $P < 0.05$.

[1] Cleaning; setting; interaction of cleaning and setting

[2]Hard vitreous kernel, where HVK is a lower relative protein content, and HHVK high relative protein content

Table 3. Effect of BoMill IQ setting within calibration on distribution of deoxynivalenol contaminated wheat into fractions.

Fusarium Calibration						Protein Calibration				
	Distribution (%)						Distribution (%)			
Fraction	HVK[1]	HHVK[1]	HHHVK[1]	SEM	P	Fraction	HVK[1]	HHVK[1]	SEM	P
1	10.3[b]	12.2[ab]	16.0[a]	1.21	0.026	1	12.4	15.0	1.04	0.136
2	10.5[b]	12.1[ab]	13.9[a]	0.55	0.005	2	12.1	14.4	0.66	0.046
3	9.6[c]	11.2[b]	12.1[a]	0.21	<0.001	3	10.5	13.6	0.33	<0.001
4	8.7	10.3	10.4	0.47	0.052	4	8.9	12.2	0.28	<0.001
5	7.9	9.1	8.5	0.87	0.636	5	8.2	10.9	0.55	0.013
6	6.7	7.6	8.5	0.48	0.069	6	6.5	10.4	0.4	<0.001
Rejects	38.7[a]	29.0[b]	22.7[c]	0.81	<0.001	Rejects	35.6	17.5	0.7	<0.001
Unsorted	7.3	8.2	7.4	0.67	0.600	Unsorted	5.5	5.9	1.43	0.83
SEM	0.64	0.4	0.99			SEM	0.69	0.56		
P	<0.001	<0.001	<0.001			P	<0.001	<0.001		

[a-c]Means with different letter superscript in rows are significantly different at $P < 0.05$.
[1]Hard vitreous kernel, where HVK is a lower relative protein content, and HHHVK high relative protein content

Table 4. Effect of BoMill IQ setting within calibration on deoxynivalenol segregation.

Fusarium calibration						**Protein calibration**				
	DON[1] (ppm)						DON[1] (ppm)			
Fraction	HVK	HHVK	HHHVK	SEM	P	Fraction	HVK	HHVK	SEM	P
1	54.2[a]	36.3[b]	33.4[b]	4.16	0.013	1	37.8	42.2	6.73	0.656
2	5.0	2.9	3.0	0.68	0.107	2	3.6	3.6	0.35	0.953
3	2.0	1.4	2.0	0.19	0.112	3	1.6	2.4	0.27	0.077
4	1.2[a]	1.5[a]	2.0[b]	0.14	0.009	4	1.2	2.2	0.19	0.013
5	1.0	1.3	2.6	0.58	0.169	5	0.9	3.6	0.22	<0.002
6	0.7[a]	1.6[a]	9.4[b]	1.21	0.001	6	0.8	8.7	0.4	<0.001
Rejects	7.4	6.8	9.5	0.95	0.155	Rejects	8.9	7.4	1.09	0.379
Unsorted	5.00	4.8	6.3	0.58	0.190	Unsorted	8.3	6.1	1.18	0.254
SEM	1.47	1.03	2.15			SEM	3.24	1.38		
P	<0.001	<0.001	<0.001			P	<0.001	<0.001		

[a-b]Means with different letter superscript in rows are significantly different at $P < 0.05$.

[1]Deoxynivalenol

[2]Hard vitreous kernel, where HVK is a lower relative protein content, and HHHVK high relative protein content

Table 5. Effect of BoMill IQ HVK setting on grain recovery (%), rejection rate (%), and average deoxynivalenol concentration (ppm), sorting deoxynivalenol contaminated wheat using a BoMill IQ and associated calibrations

	Fusarium calibration				
	Setting				
	HVK[1]	HHVK[1]	HHHVK[1]	SEM	P
Recovery (%)	40.8[b]	50.3[a]	45.1[ab]	1.82	0.015
Rejection rate (%)	38.7[a]	29.0[b]	22.7[c]	0.81	<0.001
Deoxynivalenol (ppm)	1.6	1.8	2.4	0.22	0.068
	Protein calibration				
	Setting				
	HVK[1]	HHVK[1]		SEM	P
Recovery (%)	46.2	51.0		0.80	0.005
Rejection rate (%)	35.6	17.5		0.70	<0.001
Deoxynivalenol (ppm)	1.6	2.9		0.13	<0.001

[a-c]Means with different letter superscripts in rows are significantly different $P < 0.05$.

[1]Hard vitreous kernel, where HVK is a lower relative protein content, and HHHVK high relative protein content

Table 6. Effect of sorting different sources of DON contaminated wheat using the BoMill IQ Fusarium HHVK setting on grain distribution.

	Distribution (%)					
Fraction	W1[1]	W2[2]	W3a[3]	W3b[3]	SEM	P
1	12.3[b]	10.8[c]	9.5[c]	16.5[a]	0.34	<0.001
2	12.1[b]	9.0[c]	9.6[c]	14.9[a]	0.22	<0.001
3	11.3[b]	10.6[b]	9.7[c]	14.7[a]	0.19	<0.001
4	10.3[b]	10.1[b]	9.1[c]	13.5[a]	0.13	<0.001
5	9.1[b]	9.1[b]	9.1[b]	12.3[a]	0.26	<0.001
6	7.7[b]	7.3[b]	8.4[b]	12.0[a]	0.35	<0.001
Rejects	29.0[b]	37.3[a]	38.1[a]	16[c]	0.27	<0.001
Unsorted	8.2	5.8	6.5	-	0.66	0.066
SEM	0.40	0.22	0.36	0.3		
P	<0.001	<0.001	<0.001	<0.001		

[a-c]Means with different letter superscripts in rows are significantly different $P < 0.05$.

[1] initial average deoxynivalenol concentration of 8 to 10 ppm

[2] initial average deoxynivalenol concentration of 13 to 15 ppm

[3] Initial average deoxynivalenol concentration of 6 to 8 ppm

[3]W3 was separated by seed size to enable sorting where W3a was the smaller seeds and W3b the larger seeds

Table 7. Effect of sorting different sources of DON contaminated wheat on DON separation using the BoMill IQ Fusarium HHVK setting.

	DON[1] (ppm)					
Fraction	W1[2]	W2[3]	W3a[4]	W3b[4]	SEM	P
1	36.3[a]	34.2[a]	46.0[a]	1.8[b]	4.24	<0.001
2	2.9[b]	5.1[a]	4.3[ab]	0.4[c]	0.34	<0.001
3	1.4[b]	3.2[a]	2.2[b]	0.4[c]	0.17	<0.001
4	1.5[a]	1.4[a]	1.6[a]	0.2[b]	0.15	<0.001
5	1.3[a]	0.8[ab]	1.7[a]	0.2[b]	0.22	0.002
6	1.6[a]	0.5[b]	0.8[b]	0.2[b]	0.14	<0.001
Rejects	6.8[a]	7.5[a]	8.4[a]	1.0[b]	0.49	<0.001
Unsorted	4.8	5.8	4.7	-	0.56	0.343
SEM	1.03	0.77	2.77	0.11		
P	<0.001	<0.001	<0.001	<0.001		

[a-c]Means with different letter superscripts in rows are significantly different $P < 0.05$.

[1]Deoxynivalenol

[2] initial average DON concentration of 8 to 10 ppm

[3] initial average DON concentration of 13 to 15 ppm

[4] Initial average DON concentration of 6 to 8 ppm

[4]W3 was separated by seed size to enable sorting where W3a was the smaller seeds and W3b the larger seeds

Table 8. Effect of Fusarium HHVK setting on different sources of wheat with different initial deoxynivalenol concentrations on grain recovery (%), rejection rate (%), and deoxynivalenol (ppm)

	W1	W2	W3	SEM	P
Recovery (%)	50.3^{b}	41.6^{c}	69.2^{a}	1.46	<0.001
Rejection rate (%)	29.0^{b}	37.3^{a}	28.3^{b}	0.25	<0.001
Deoxynivalenol (ppm)	1.8^{a}	1.8^{a}	1.1^{b}	0.14	0.019

Means with different letter superscripts in rows are significantly different $P < 0.05$

8.3 Effect of density sorting DON contaminated wheat using fractional pneumatic separation technology to separate grain into high and low contaminated fractions to improve grain recovery

Abstract

Deoxynivalenol (DON) contamination of wheat occurs when the grain is infected by *Fusarium* spp. Fusarium infection causes a reduction of grain density and alters kernel size. There are few technologies that are cost effective and capable of reducing DON contamination on farm. Fractional pneumatic separation uses an air stream on falling grain to segregate into fractions based on aerodynamic properties and grain density, thereby segregating wheat by relative DON concentration. Three wheat samples were used with DON concentrations of W1 = 8-10 ppm, W2 = 13-15 ppm, and W3 = 6-8 ppm. Treatments were processed through a GCS-200, dial set to 33.5 Hz, with 60 kg wheat used per treatment (15 kg/rep). Each of the five fractions (F1 to 4 + cyclone) for the three wheat samples were weighed to determine grain distribution, grain density observed, and DON concentration measured. Usable recovered grain represented 52.5, 42.5, and 59.2 % of total material from W1, W2, and W3 respectively from fractions 1 and 2. The DON concentration of the recovered material was 1.8, 2.0, and 1.1 ppm for W1, W2, and W3 respectively. The grain density for each wheat sample was highest in fraction 1 and significantly decreased with each fraction, with the least dense fraction being the cyclone. This study demonstrates that fractional pneumatic separation is effective for segregating DON contaminated wheat by grain density, thereby segregating by DON concentration and recovering significant quantities of what was low quality wheat. Initial DON concentration affects the recoverable

grain, where a lower DON concentration recovers larger quantities of grain, and a higher initial concentration recovers a lower quantity of grain.

Introduction

Fractional pneumatic separation (FPS) is the process of separating whole grains and impurities by dropping grain into an air stream to be separated into fractions by relative density. FPS is a flexible technology that can be used anywhere with an appropriate power source and a means to store fractionated grain. Separation is dependent on grain velocity dropping into the airflow, airflow velocity, angle, and aerodynamic properties of the grain (Piven 2018). A vertical air stream is not effective for FPS as the distribution band is too narrow for grain separation (Gorial and O'Callaghan 1990).

Due to a reduction in grain density caused by *Fusarium* spp. infection, we hypothesize that it is possible to partition wheat from low to high DON concentration. FPS will be able to separate low from high-density grains, thereby recovering significant quantities of wheat below 5 ppm, rendering significant portions of grain suitable for livestock production.

Different sources of wheat infected with *Fusarium* spp. will segregate into fractions by FPS according to DON level and grain density, but contamination levels between wheats will alter segregation behaviour.

Materials and Methods

Wheat

Three samples of Canadian Hard Red wheat sourced from Saskatchewan was used. Two of the sources were naturally contaminated with DON, and the third had no detectable levels of DON. Wheat 1 (W1) was sourced, in 2014, from around North Battleford, SK (N 52˚50'17.87", W 107˚34'12.42"). Wheat 2 (W2) was sourced, in 2016, from the Kenaston, SK area. The third

wheat sample was sourced from the bulk bin at the Canadian Feed Research Centre. DON concentrations of W1 was on average 8-10 ppm, W2 was 13-15 ppm, and a pooled wheat source (W3) was created by mixing 5 kg of the three wheat sources together to create a replication (15kg) measured 6-8 ppm.

Grain cleaner

A GCS-200 Grain Cleaner (Apple Valley, MN) equipped with a ¾ horsepower variable frequency motor, electronic dial set to 33.5 Hz was used. The adjustable fraction control levers remained in the upright position, and the hopper bin control opened approximately 1/3 open to allow grain flow. The grain fell from a hopper in front of an upward angled air stream. As the grain fell, it was hit by the air stream and pushed along the machine to one of four fractions where the first fraction is the lowest DON and the fourth fraction is higher DON, or to the end of the machine called the cyclone, which has the highest DON. Airstream was controlled by the variable frequency drive motor and electronic dial. To collect the cyclone material, an aluminum air duct transition (rectangular to circular) was attached to the cyclone outlet, and a large garbage bag taped over the circular outlet. Buckets were placed at each of the four fraction outlets.

Experimental procedure

Each treatment (W1, W2, W3) had 15 kg wheat per replication, and was replicated four times (60 kg total per treatment). Grain was placed in the hopper, the machine started, and air stream was allowed to equilibrate for 30 s. During this process, the hopper control was opened to allow grain flow. After processing through the fractionator, the machine was turned off, and grain from each fraction was emptied into labeled bags for testing. Each fraction from a replication was weighed, grain density observed, and tested for DON level.

Grain density

Grain density for each fraction was measured twice and averaged using the Cox Funnel method, or a 100ml graduated cylinder, and observing the weight and volume.

DON Testing

DON testing was performed using the Vicam DON-V ELISA test (room temperature method) and Vertu Lateral Flow Reader. Manufacturer procedures with standard dilution techniques were used to get DON levels. A sub-sample of grain was ground using a BUNN HD G1. Five grams of ground wheat were weighed into an extraction tube with 20 ml distilled water was added to the tube. The sample was then vortex mixed on accessory maximum for 2 minutes. The contents of the extraction tube were filtered using the supplied filter paper into another extraction tube, then 100µL of the filtrate was placed into 1ml distilled water and vortex mixed. Using the supplied diluent, the 100µL of diluent was placed into a sample vial with 100µL of diluted filtrate and vortex mixed, then 100µL of mixture was transferred to the sample well of DON-V cassette (1 drop/s) and developed for 3 minutes. After cassette development, the cassette was placed in the Vertu Lateral Flow Reader to obtain DON results.

Statistical analysis

Statistical analysis was performed using JMP (version 12; SAS Institute, Inc., Cary, NC, USA). The statistical model used for individual wheats was a CRD design $Y_{ij}=\mu+\alpha_{ij}+e_{ij}$, where Y_{ij} = observation, μ = mean, α_{ij} = fraction (I = 1 to 5), e_{ij} = error term. The model used to examine the differences between the wheats was CRD design $Y_{ij}=\mu+W_{ij}+e_{ij}$, where Y_{ij} = observation, μ = mean, W_{ij} = wheat (I = 1 to 3), e_{ij} = error term. Mean separation was performed using the Tukey-Kramer method with significance determined at $P<0.05$.

Results

Grain distribution

The results for grain distribution are shown in table 1. In response to FPS and the setting used, the three fractionated wheat sources demonstrated a bell curve distribution, where the majority of materials were located in fractions 2 and 3. Within W1 and W3, fractions 2, 3, 4 were significantly different from each other, but fraction 1 and the cyclone were not significantly different. Within W2, fraction 1 had a very small sample size, fractions 2 and 3 were not significantly different from each other, but fraction 4 and cyclone fraction were significantly different from the other fractions. In fraction 1 between wheats, W1 and W3 were significantly different from each other. In fraction 2, W2 had a significantly lower yield than W1 or W3. In fraction 3 and 4, W2 had a significantly higher yield than W1 or W3. W2 had a significantly higher amount of material in the cyclone fraction than W1 or W3.

Grain density

The results for grain density are shown in table 2. Within W1, the grain density was higher in fraction 1 and significantly decreases with every fraction. Within W2, the results reflect the results from W1 with the exception of the small sample size in fraction 1. Within W3, fractions 1, and 2 were not significantly different, but had a significantly higher grain density than the other fractions. Between W1, W2, and W3, fractions 1, 3, and 4 were not significantly different from each other. For fraction 2, W2 had a significantly lower grain density than W1 or W3. In the cyclone fraction, W2 had a significantly higher grain density than W1, and W3 was not different from W1 or W2.

DON results and grain recovery

The DON concentration results are shown in table 3. In W1, fractions 1 and 2 are significantly lower than the other fractions. For W2 and W3, fractions 1 to 3 were significantly lower than fraction 4 and the cyclone fraction. Between wheats in fraction 1, W3 had

significantly lower DON concentrations than W1. In fraction 2, W3 had significantly lower DON concentrations than W1 and W2. W1 in fraction 4 and the cyclone fraction had significantly higher DON concentrations than W2 and W3. The grain recovery for W1, W2, and W3 were 52.8, 42.7, and 58.8 % respectively. The DON concentration of the grain recovered was 1.7, 1.5, and 1.0 ppm.

Discussion

Using DON contaminated wheat presents challenges due to the inconsistencies between wheat sources, cultivars, and varieties. Currently the industry practice to reduce DON contamination is through grain blending. The CFIA regulatory guidelines for complete livestock feeds are as low as 1 ppm (CFIA 2017). Uncleaned soft wheat intended for human consumption, in Canada, must be less than 2 ppm (CFIA 2017). Achieving the DON concentrations outlined in the CFIA guidelines requires grain blending or a technological approach to reduce DON concentrations. Expense is a dominating concern for technological acceptance since optical sorters and NIT sorters are very expensive. In contrast, FPS is a relatively inexpensive method and provides ease of use not found to other relative sorting technologies.

Fusarium damage causes a reduction in grain density. The damage and alteration in density is not consistent between kernels, creating a gradation. The changes in grain density allow segregation of wheat by DON concentration using FPS, recovering up to 58.8 % of material with a DON concentration under 2 ppm. DON was concentrated in the least dense fractions. The initial DON concentration affects the overall grain recovery, with higher initial DON concentrations having a lower grain recovery. A central bell distribution, where the majority of material is sorted into the central fractions is ideal for maximal recovery but must be adjusted based on individual wheat sources, DON concentration, and recovery objectives. In

comparison, a one sort pass using an optical sorter having a set rejection rate of 10%, achieved approximately 50 % DON reduction (Delwiche et al. 2005; Delwiche 2008). In this study, similar grain recoveries were observed as Tkachuk et al. (1991) when a specific gravity separator was used having similar initial DON concentration.

It may be possible to increase recovery by reprocessing the moderately high fraction 3, although the DON concentration would incrementally increase in the recovered fraction. The concentrated DON material represents a small proportion of the total material. The intermediate fraction with DON concentrations under 10 ppm has an increased grain density compared to the cyclone and fraction 4. By adjusting the electronic dial, decreasing the air speed, and shifting the bell distribution grain would fall into earlier fractions.

Conclusions

FPS is an effective technology to segregate *fusarium* infected wheat into high- and low-density fractions and recover significant quantities of low DON grain. The recovered grain had a DON concentration under 2 ppm, a maximum recovery of 58.2 %. Passing the moderately high fraction back through the machine may increase grain recovery. Improvement and refinement of the FPS technology could improve grain recovery results. It would also be valuable to standardize the initial DON concentration, grain density, and other factors to reduce time and resources in the optimization of results.

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Table 1. Effect of fractional pneumatic separation[4] on grain distribution into fractions of different sources of DON contaminated wheat

	Distribution (%)				
Fraction	W1[1]	W2[2]	W3[3]	SEM	*P*
1	0.3^{bz}	-	1.0^{az}	0.14	0.002
2	52.2^{aw}	42.5^{bw}	58.2^{aw}	1.83	<0.001
3	38.5^{ax}	41.3^{aw}	32.2^{bx}	1.26	0.001
4	7.5^{by}	12.2^{ax}	7.2^{by}	0.72	0.001
Cyclone	1.0^{bz}	3.3^{ay}	2.0^{abz}	0.49	0.031
SEM	1.32	1.23	0.45		
P	<0.001	<0.001	<0.001		

Significance of P<0.05 in rows indicated by letters a, b, c

Significance of P<0.05 in columns indicated by letters w, x, y, z

[1] initial average DON level of 8 to 10 ppm

[2] initial average DON level of 13 to 15 ppm

[3] Initial average DON level of 6 to 8 ppm

[4]Air fractionator was GCS-200 Grain Cleaner, dial set to 33.5

Table 2. Effect of air fractionation[1] on grain density of wheat segregated into fractions from different sources of DON contaminated wheat.

	Density (kg/hL)				
Fraction	W1[2]	W2[3]	W3[4]	SEM	*P*
1	83.7^{v}	-	81.4^{v}	0.55	0.065
2	78.7^{abw}	78.3^{bv}	79.0^{av}	0.09	0.001
3	74.7^{x}	74.2^{w}	74.2^{w}	0.16	0.108
4	65.5^{y}	66.0^{x}	65.0^{x}	0.40	0.267
Cyclone	51.7^{bz}	56.5^{ay}	54.3^{aby}	0.65	0.002
SEM	0.34	0.48	0.49		
P	<0.001	<0.001	<0.001		

Significance of P<0.05 in rows indicated by letters a, b, c

Significance of P<0.05 in columns indicated by letters v, w, x, y, z

[1]Air fractionator was GCS-200 Grain Cleaner, dial set to 33.5 Hz

[2]Initial grain density 74.5 kg/hL

3

[4]Initial grain density 75.7 kg/hL

Table 3. Effect of air fractionation[1] on DON separation into fractions using different sources of DON contaminated wheat.

	DON (ppm)				
Fraction	W1[2]	W2[3]	W3[4]	SEM	*P*
1	1.4^{az}	0.9^{aby}	0.1^{by}	0.22	0.009
2	1.8^{az}	2.0^{ay}	1.1^{by}	0.15	0.004
3	9.8^{y}	10.0^{y}	7.0^{y}	0.89	0.066
4	39.8^{ax}	29.0^{bx}	28.1^{bx}	3.09	0.046
Cyclone	108.9^{aw}	63.3^{bw}	75.8^{bw}	3.72	<0.001
SEM	1.63	2.81	3.3		
P	<0.001	<0.001	<0.001		

Significance of P<0.05 in rows indicated by letters a, b, c

Significance of P<0.05 in columns indicated by letters w, x, y, z

[1]Air fractionator was GCS-200 Grain Cleaner, dial set to 33.5

[2] initial average DON level of 8 to 10 ppm

[3] initial average DON level of 13 to 15 ppm

[4] Initial average DON level of 6 to 8 ppm

8.4 Improvement of grain recovery using air fractionation and near-infrared transmittance (BoMill) sequentially.

Introduction

In sections 8.2 and 8.3 above we examined the potential to sort infected wheat using a BoMill IQ, near infrared sorting system and air fractionation. Both methods were effective at separating seeds with high levels of DON from those seeds containing little to no toxin. However each method has shortfalls. The BoMill requires a large capital investment and has a nominal sorting capacity of 3 MT per hour per unit. In addition, since the seeds must fit within a small pocket in the drum, the seed needs to be similar size and be relatively free of chaff and foreign materials. Air fractionation is an effective method of cleaning and sorting seeds and is able to remove fusarium damaged kernels from sound kernels based on a reduction in grain density. The air fractionation systems are simple and inexpensive and are able to separate out the highest and lowest quality kernels, however, it produces an intermediary fraction(s) that contain both high and low DON seeds. Based on the work in section 8.1 it would appear these intermediate fractions may be able to be sorted into high and low DON using a BoMill. The combination of the two systems could be a practical approach to sorting fusarium infected grain. The grain would first be sorted using an air fractionator effectively producing 3 fractions, the first being the most dense grain with the lowest levels of DON, an intermediate fraction that contains both high and low DON kernels which have similar grain densities and therefore cannot be effectively sorted using this technology, and a high DON fraction which also contains the chaff and foreign materials. The intermediate fraction would then be sorted on a BoMill effectively creating high and Low DON fractions. This would result in a more efficient use of the BoMill by cleaning the grain while reducing the overall volume requiring to be sorted base on the more complex system.

Hypothesis
Our hypothesis is that using the air fractionation and BoMill IQ segregation methods in combination can result in more efficient and effective segregation than either technology alone.

Materials and methods

Air fractionator

Using a GCS-200 Grain Cleaner (Apple Valley, MN), with a ¾ horsepower variable frequency drive motor. The fraction control levers remained in the upright position. The machine creates five fractions where the first two are low DON fractions, the last three fractions have progressively higher DON levels. The cyclone is the last fraction and was modified with an aluminum air duct transition (rectangular to circular) to enable material collection with a garbage bag. The third fraction contains grain with moderately high DON levels which still contains low DON grain.

BoMill

Using a BoMill IQ (model 1002, version 2.0.79, BoMill AB, Sweden), with the previously determined optimal setting (unpublished) *Fusarium* HHVK, the third fraction from air fractionation was processed through. A spectral histogram was created over 600 seconds for each wheat to allow grain segregation

and set sorting borders of 16.7% for each fraction. The BoMill IQ creates eight fractions, where the first contains the highest DON material, the 2nd to 6th fractions are generally the low DON fractions, but the 2nd may have higher levels than the low DON fractions. The 7th fraction is any material that does not fit within the calibration, or if grain does not fit in the singulator pockets properly, or if two grains sit in one pocket. The 8th fraction is the unsorted fraction which remains unsorted in the machine after it has stopped, in this case it was combined with the rejects, as further processing would not be practical. Previous grain recovery data using the BoMill IQ (unpublished) will be used to compare grain recovery.

Wheat

Three sources of wheat were acquired for this experiment, Wheat 1 (W1) from Saskatchewan (N 52˚50'17.87", W 107˚34'12.42") in 2014, Wheat 2 (W2) from the Kenaston area (SK) in 2016, and bulk wheat from the Canadian Feed Research Centre. A pooled wheat sample (W3) was created by combining 5 kg of each wheat and mixing together to make 15 kg or one replication. W1 was naturally contaminated with DON with an average DON level of 8 to 10 ppm. W2 was also naturally contaminated with DON, with an average level of 13 to 15 ppm. The bulk wheat had no detectable level of DON. A total of 60kg (15kg per replication) of wheat per treatment was processed through the machine at the electronic dial set to 33.5 Hz, with the hopper control open 1/3 to allow grain flow. After processing through the air fractionator, each fraction was weighed to determine distribution, and a DON test run on a sub-sample to determine grain recovery. Each of the wheats third fraction with a moderately high DON level was then processed through the BoMill IQ. The seven new fractions from each wheat were weighed after processing, and a DON test run on a sub-sample.

DON testing

DON testing was performed using the Vicam DON-V ELISA test (room temperature method) and Vertu Lateral Flow Reader. Manufacturer procedures with standard dilution techniques were used to get DON levels. A sub-sample of grain was ground using a BUNNFive grams of ground wheat was weighed into an extraction tube, then 20 ml distilled water was added to the tube. The sample was then vortex mixed on accessory maximum for 2 minutes. Contents of the extraction tube were filtered using the supplied filter paper into another extraction tube, then 100µL of filtrate was placed into 1ml distilled water and vortex mixed. Using the supplied diluent, 100µL of diluent was placed into a sample vial with 100µL of diluted filtrate and vortex mixed, then 100µL of mixture was transferred to the sample well of DON-V cassette (1 drop/s) and developed for 3 minutes. After cassette development, the cassette was placed in the Vertu Lateral Flow Reader to obtain DON results.

Results and Discussion

Fraction 3 from air fractionation comprised 29.6 to 36.3% (Table 1) of the original mass sorted and that fraction was used in the current experiments. The proportions of each sorted sample collected in fractions 1-6 and the rejects after sorting fraction 3 (intermediate fraction from air fractionation) from 3 different wheat sources are shown in table 1. In theory based on the settings used, the grain should be equally sorted into each fraction at 16.6% and ideally have no rejects but that is not the case. A significant portion of the grain was still rejected 33.8 to 46.7%. It was hoped that precleaning the grain using the air fractionation would significantly reduce the proportion of the grain that was not sortable due to size. The material used in this experiment was relatively free of chaff and other foreign materials so the rejects are likely due to being either too large or too small to effectively fit in the pockets in the

chosen disc. Although air fractionation cleaning separates the grain based on density, it doesn't specifically sort based on size. Sorting the grain into similar sizes using sifting technology would likely increase the recovery of the grain but this would require multiple drums for the BoMill so small, medium and large seeds can be sorted after sieving. The remaining grain was sorted into relatively equal fractions from 1-6, however.

Table 1. Distribution of DON contaminated wheat after sequential sorting GCS-200[1] grain cleaner fraction 3 with a BoMill IQ[2]

	Distribution (%)				
Fraction	W1[4]	W2[4]	W3[4]	SEM	*P*-value
Initial[3]	36.3	39.9	29.6		
1	13.6[a]	8.5[b]	10.2[b]	0.56	<0.001
2	13.2[a]	9.2[b]	10.2[b]	0.47	<0.001
3	12.0[a]	9.2[b]	10.0[b]	0.26	<0.001
4	10.2[a]	9.0[b]	9.9[a]	0.22	0.006
5	9.2	9.2	10	0.27	0.123
6	7.6	8.1	9.5	0.56	0.101
Rejects	33.8[c]	46.7[a]	40.3[b]	0.92	<0.001

Significance indicated at P<0.05
Means not connected by same letter in rows are significantly different

[1] Motor set to 33.5 Hz

[2] Used the Fusarium calibration and HHVK setting
[3] Amount of material taken from third fraction of air fractionation
[4] W1=wheat 1, W2=wheat 2, W3=wheat 3

Table 2 shows the DON levels in fractions sorted by the BoMill from 3 sources of grain initially sorted on an airfractionator. Fraction 1 contained high levels of DON while fractions 4 to 6 had relatively low levels of DON. This supports the hypothesis that the BoMill can be used to effectively sort grain that was not effectively sorted by air fractionation.

Table 3 shows the overall recovery of wheat under 5 ppm using air fractionation, BoMill and a combination of the two on 3 sources of infected wheat. The data shows that a combination of the two is not only more practical from a capital investment and processing perspective but results in a greater overall recovery of wheat. It is also worthwhile noting that the pooled wheat sample (W3) was sorted equally efficiently as individual samples of wheat. Since both systems sort grain based on relative differences within the material sorted it was thought that a pooled samples would be sorted less effectively as the relative differences could be simply due to

differences in growing conditions and/or variety. However, in this study pooled samples were effectively sorted.

Conclusions

Based on the data to date it would suggest both the Bomill and Airfractiontion technology can be used to effectively sort fusarium infected grain and the combination of the two is the most effective approach.

Table 2. DON levels of wheat after sequential sorting the third fraction from GCS-200 grain cleaner[1] using a BoMill IQ[2]

	DON (ppm)				
Fraction	W1[3]	W2[3]	W3[3]	SEM	*P*-value
Initial	9.9	10	7	0.98	0.066
1	43.6	45.7	36.1	3.1	0.123
2	3.6[b]	8.0[a]	5.8[ab]	0.96	0.029
3	2.5[b]	4.0[a]	2.6[b]	0.25	0.004
4	1.8	2.2	1.8	0.17	0.18
5	2.0[a]	0.7[c]	1.3[b]	0.15	<0.001
6	2.5[a]	0.4[b]	0.8[b]	0.12	<0.001
Rejects	8.1	7.6	7.2	0.76	0.72

Significance indicated at P<0.05

Means not connected by same letter in rows are significantly different

[1] Motor set to 33.5 Hz

[2] Used the Fusarium calibration and HHVK setting

[3]W1=wheat 1, W2=wheat 2, W3=wheat 3

Table 3. Recovery of DON contaminated grain <5.0ppm after using different sorting methods.

	Grain recovery (%)		
Sorting method	W1[4]	W2[4]	W3[4]
Air fractionation[1]	52.5	42.5	59.2
BoMill IQ[2]	50.5	46.1	69
Sequential sorting[3]	71.5	56.6	72.3

[1] GCS-200 grain cleaner, motor set to 33.5 Hz

[2] Used the Fusarium calibration and HHVK setting

[3] Sorting the third fraction of [1] with [2]

[4]W1=wheat 1, W2=wheat 2, W3=wheat 3

8.4 Effects of grain moisture and temperature on ozone degradation of DON in contaminated wheat

Abstract

The mycotoxin deoxynivalenol (DON) has major impacts on wheat quality and safety worldwide. In Canada, wheat is harvested at upwards of 20 % moisture content, and environmental temperatures can have wide variation from freezing to above 30 °C. Currently, there are few treatments available to treat DON contaminated bulk grain post-harvest, that can reduce the overall DON concentration. Ozone is a strong oxidizing gas that has been found previously to degrade DON. In this study, gaseous ozone was used to treat DON contaminated wheat at different grain moisture contents, and temperatures. Using a custom PVC tube apparatus to simulate bulk grain aeration, 1 kg aliquots of wheat were moisture adjusted to 10.8, 15, or 20 % moisture, and treated with 7.5 mg l^{-1} ozone or air for 24 h. There was significant DON degradation of 25 % for the 20 % moisture grain compared to the air and ozone treated 10.8 % moisture grain. To simulate Canadian post-harvest temperatures, the PVC tubes were submerged in 2 °C, or 29 °C water, at 20 % grain moisture, and treated with 7.5 mg l^{-1} ozone for 24 h. Temperature did not affect ozone degradation of DON, as the DON concentrations for all temperatures were significantly lower than the initial DON. Ozone utilization decreases with treatment duration. Grain moisture content significantly affects the efficacy of DON degradation using ozone. Temperature had no effect on ozone efficacy. Treatment of bulk grain contaminated with DON post-harvest with low levels of ozone during aeration can reduce the overall DON concentration.

Introduction

There are currently only a few technologies available that are capable of reducing DON concentrations post-harvest. Chemical methods of DON reduction have had mixed success but may have chemical residues remaining on the grain after treatment, for example, sodium carbonate increases sodium concentration (Young 1986; Abramson et al. 2005). Chemical treatment of grain can pose significant health risks to the workers performing the treatments (Awad et al. 2010). Ozone is an inorganic molecule consisting of three oxygen molecules and has strong oxidizing properties (McKenzie et al. 1997; Wang et al. 2016; Piemontese et al. 2018). When produced, it is a colourless gas with a sharp distinctive odour and decomposes into O_2 (Health Canada 2010; Wang et al. 2016). It is "generally regarded as safe" by the U.S. Food and Drug Administration with the upper limits of human exposure being 0.1 ppm (FDA 2019). Concentrations above 0.1 ppm may cause irritation to mucus membranes (FDA 2019). Ozone has been previously found to reduce DON concentrations in grain, with no chemical residues, and minimal alterations to feed wheat grain quality (Young 1986; Li et al. 2015; Piemontese et al. 2018). Ozone is commonly used for purification of air and water, treatment of industrial waste, a bleaching agent, microbial control on produce, and pest control (Hansen et al. 2013; Margot et al. 2013; Kaur et al. 2019).

In Canada and many other growing regions, grain is stored after harvest prior to marketing. In many cases, air is blown through the grain for 24 or more hours immediately after harvest to cool and dry to render it stable during storage in a process called aeration (Jayas and White 2003). It may be possible to add ozone during grain drying and storage to oxidize the DON, thereby reducing the overall concentration and toxicity, provided that there is sufficient air flow to all areas of the bin, and sufficiently high ozone concentration to oxidize large quantities of DON. Previous studies used 20-100 mg ozone/L or higher, up to 20 % moisture contents, at

25 °C, and treated for 2-16 h observed DON reductions between 9 and 57 % (Li et al. 2015; Piemonetese et al. 2018). These studies illustrate that ozone concentration, exposure time, and grain moisture are major factors for DON reduction in wheat.

We hypothesize that DON can be reduced by adding practical levels of ozone (7.5 mg/L) to incoming air during simulated bin aeration. However, the duration of the treatment, the temperature and moisture content of the grain will affect the rate and extent of DON degradation.

Materials and Methods

Wheat

Canadian Hard Red wheat acquired in 2014 from (N 52˚50'17.87", W 107˚34'12.42") in Saskatchewan was naturally contaminated with DON (approximated DON concentration 8-10 ppm). The grain moisture was either left as is, or adjusted from 10.8 % moisture to 15 % moisture, and 20 % moisture by $(1000 - target\ moisture\left(\frac{g}{kg}\right))/(g\frac{DM}{kg})$. Using 4 kg of moisture adjusted wheat per treatment (1 kg per replication), the treatments were 10.8 % no ozone (10.8-), 10.8 % with ozone (10.8+), 15% with ozone (15+), and 20 % with ozone (20+), replicated four times.

Ozone

This experiment was conducted in a fumehood at 22˚C.

Four ozone generators were acquired (model OZX-300ST, Enaly, Shanghai, China) and connected to an air dryer. To regulate air flow through the machines, each machine was connected to a mixed gas flow regulator (model # HRF 1425-580, ProStar by Praxair). Each regulator was set to 1.6 l min^{-1}. The ozone generators were set to the maximum ozone generation setting and run continuously during the treatment time. Ozone concentrations were measured at 0 h, 12 h, and 24 h, from the machine outlet and the tube outlet with an Aeroqual 200 Series ozone

monitor with the 1 to 10 ppm sensor. To get accurate readings, the ozone sensor tip was isolated into a PVC tube with additional air flow to dilute the ozone, and at the end of the tube was a wind anemometer to measure air speed (m/s). Ozone measurements were observed when the ozone level and air flow stabilized between 3 and 5 minutes.

Four ozone vessels were made using 7.62 cm diameter PVC tubes cut to 91.5 cm length, and a 7.62 cm to 5.08 cm transition piece PVC glued on either end, with each having a detachable end cap with rubber O-ring. A hole was drilled into each end cap, and a brass 4.7 mm hose barb with male fitting placed on the tubes with the outside edges sealed with seal tape. Vinyl tubing was attached at each end of the tube to enable delivery and measurement of ozone.

Experiment 1

The grain and distilled water for each treatment were weighed and combined in sealable plastic bags, then refrigerated for 16 h prior to treatment start to allow moisture penetration into the grain. Grain was removed from the refrigerator 30 minutes prior to the experiment start time and placed in their respective PVC tubes. The ozone machines were started and run for 5 minutes, after which an ozone reading was taken, then the tubing from the ozone machines was attached to the bottom of their respective ozone vessel. After 10 minutes of ozone flowing through the vessels, an ozone reading was taken at the gas outlet. At 12 h of ozone treatment, an ozone reading was taken from the outlet of the vessel, detached and the tube inverted so the outlet became the inlet. An ozone reading was also taken from the ozone generators while the tubing was detached from the ozone vessel. At 24 h, an ozone reading was taken from the vessel and the machines. After treatment, the grain was dried if necessary, at 50˚C until reducing to grain starting weight. Then DON analysis was performed. This procedure was repeated four times.

Experiment 2

Grain preparation was the same as experiment 1, except that all grain moistures were adjusted to 20%. Ozone generators and flow regulators were set to the same as experiment 1. Ozone readings from the ozone generators were taken at 0 h, and 24 h from after the tubes. To adjust the temperature of the ozone vessel environment, the vessels were placed in several large plastic bags and placed in a large garbage bin filled with either ice water (2 °C) or warm water (29°C). Water was heated in the bin with a Polystat microprocessor (model 12003-series, Cole-Palmer Instrument Company, Niles, Illinois) to 29˚C. The ozone vessels were ¾ covered in water and tied down to prevent the vessels from bobbing. For the ice water, ice was placed into the bin surrounding the ozone vessels until ¾ covered, then cold water was added to just below the ice surface. Water temperature was observed at 0, 12, and 24 h. Ozone readings were taken from the vessels at 0, 12, and 24 h, and the vessels were not inverted at 12 h. Ozone vessel outlet lines were vented into the fumehood. DON levels were measured after grain was dried.

DON analysis

DON testing was performed using the Vicam DON-V ELISA test (room temperature method) and Vertu Lateral Flow Reader. Manufacturer procedures with standard dilution techniques were used to get DON levels. A sub-sample was ground on the Fine setting using a BUNN G1 HD grinder. Five grams of ground wheat was weighed into an extraction tube, then 20ml distilled water was added to the tube. The sample was then vortex mixed on maximum for 2 minutes. Contents of the extraction tube were filtered using the supplied filter paper into another extraction tube, then 100µL of filtrate was placed into 1ml distilled water and vortex mixed. Using the supplied diluent, 100µL of diluent was placed into a sample vial with 100µL of diluted filtrate and vortex mixed, then 100µL of mixture was transferred to the sample well of

DON-V cassette (1 drop/s) and developed for 3 minutes. After cassette development, the cassette was placed in the Vertu Lateral Flow Reader to obtain DON results.

2.6. Statistical analysis

Statistical analysis was performed using a one was ANOVA with JMP (version 12; SAS Institute, Inc., Cary, NC, USA). The experimental design was a CRD for both experiments. The statistical model for experiment 1 was $Y_{ij} = \mu+M_{ij}+e_{ij}$, where Y_{ij} = observation, μ = mean, M_{ij} = moisture effect (I = 1 to 4), e_{ij} = error term. The statistical model for the second experiment was $Y_{ij} = \mu+T_{ij}+e_{ij}$, where Y_{ij} = observation, μ = mean, T_{ij} = temperature (I = 1 to 3), e_{ij} = error term. Means separation was performed using the Tukey-Kramer method, and significance observed at $P<0.05$.

Results and discussion

Experiment 1

The results for the effect of grain moisture content on ozone degradation of DON are shown in Table 1. The 10.8+, 10.8-, and 15+ moisture ozone treatments DON concentrations were not significantly different from the initial DON concentration. Air treatment of grain was not significantly different from the initial DON concentration. The 20+ DON concentration is significantly lower that the initial DON concentration and the 10.8+ treatment. Numerically the DON concentration decreased as the grain moisture content increased, indicating that ozone efficacy increased with increased moisture content. Li et al. (2015) observed a 57.3 % DON reduction when wheat was treated for 12 h at 60 mg l^{-1} and 17 % grain moisture, when the initial DON concentration was 10 ppm. Wang et al. (2016) reported a 58.35 % DON reduction at 20 % grain moisture, using 100mg l^{-1} ozone. In contrast to previous studies, a concentration of 7.5 mg l^{-1} ozone for 24 h was used in this study and achieved a 38 % DON reduction at 20 % grain

moisture. These results are in agreement with previous studies that increased grain moisture improves the efficacy of ozone treatment (Li et al. 2015; Wang et al. 2016).

Experiment 2

The results for the effects of temperature on ozone treatment efficacy on DON contaminated wheat are shown in Table 2. All temperature treatments (2, 22, 29 °C) were significantly different from the initial DON concentration. The 2, 22, and 29 °C treatments were not significantly different from each other. Prior to this study, the effect of temperature on ozone treatment efficacy of DON contaminated wheat had not been explored. These results indicate that it may be possible to use ozone in the variable temperatures that occur in Canada post-harvest.

Ozone utilization over time

The results for the effect of ozone utilization over time in DON contaminated wheat are shown in Table 3. Ozone was almost completely utilized at 0 h after being attached to the ozone vessel for 10 minutes. Ozone utilization decreased over time with the highest concentration of ozone found at 24 h of treatment. This reduction in ozone utilization is most likely a function of the grain moisture gradually reducing due to gas flow. As moisture content decreases the ozone does not react as readily to create reactive ions, so more ozone will escape the vessel unchanged. Ozone production may also decrease over time as the machine heats up (Brodowska et al. 2018).

Conclusions

The present study demonstrated that grain moisture content significantly influenced the reduction of DON in wheat. At 20 % grain moisture, there was a 38 % reduction in DON compared to the initial DON concentration. It was expected that the low temperatures used in

this study would negatively affect the ozone reduction of DON, but temperature had no significant effect. Ozone utilization was greatest at the start of treatment time, but reduced as time progressed. Using 7.5 mg l^{-1} ozone for 24 h was effective at reducing the overall DON concentration when grain moisture was increased. The present study demonstrates that it may be possible to use low levels of ozone under Canadian post-harvest conditions to treat DON contaminated grain and reduce the overall DON concentration.

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Table 1. Effect of treating moisture adjusted DON contaminated wheat with ozone on DON concentration

Moisture (%)	DON (ppm)
Initial DON	8.9[a]
10.8-	8[ab]
10.8+	8.3[a]
15+	7.1[ab]
20+	5.5[b]
SEM	0.64
P	0.011

Means with different letter superscripts are significantly different $P < 0.05$
(+) indicates treatment with ozone
(-) indicates treatment with air

Table 2. Effect of treating moisture adjusted DON contaminated wheat at different temperatures with ozone on DON levels.

Temperature (˚C)	DON (ppm)
Initial	8.9[a]
2	6.5[b]
22	5.9[b]
29	6.3[b]
SEM	0.37
P	0.0004

Means with different letter superscripts are significantly different $P < 0.05$

Table 3. Effect of time on ozone[a] usage over 24 hours in 20 % grain moisture content DON contaminated wheat

Time (h)	Ozone mg l^{-1}
0	0.23
12	2.9
24	3.6
SEM	0.24
P	<0.0001

[a] initial ozone concentration = 7.5 mg l^{-1}

8.5 Effects of seed sorting on *Fusarium graminearum* contaminated wheat on the performance and gastrointestinal morphology of broiler chickens

Objective
The objective of this trial is to determine if detoxification of wheat through grain sorting can be used to render infected wheat suitable for broiler chicken production.

Hypothesis
Broiler chicken performance and gastrointestinal morphology of birds fed diets containing wheat that has had fusarium damaged kernels removed through a combination of air fractionation and sorting by Near Infrared Transmittence to less than (3 ppm) in the diet will be improved compared to the unsorted control.

Materials and methods

Animal use was approved for this experiment by the University of Saskatchewan Animal Care Committee (protocol # 20190068), with all procedures following the recommendations of the Canadian Council of Animal Care.

Diets

All the diets were formulated to meet or exceed the nutrient requirements during all stages of growth (Aviagen, 2014). A commercial starter diet was provided from 0 to 7 days. The treatment starter diets were provided from 7 to 21 days of age, and grower/finisher diets provided from 22 to 35 days of age. There were nine treatments: a control (60% wheat), low DON sorted wheat included at 15 (L15), 30 (L30), 45 (L45), and 60% (L60) of diet, and high DON unsorted wheat included at 15 (H15), 30 (H30), 45 (H45), and 60% (H60) of diet. Nutrient composition (dry matter, crude protein, crude fiber, ether extract, ash, calcium, and phosphorous) of the diets was measured at Central Testing Laboratories Ltd, MB, Canada. The treatment starter diets were fed in crumble form, and the grower/finisher diets were fed in pellet form.

Table 1. Composition of diets used in broiler study

	Starter Diets								
Ingredient (%)	Control	L15	L30	L45	L60	H15	H30	H45	H60
Control Wheat	61.32	45.79	30.25	14.72	0	45.96	30.6	15.24	0
Sorted Low Wheat	0	15	30	45	60	0	0	0	0
Unsorted contaminated wheat	0	0	0	0	0	15	30	45	60
Soybean meal	27.83	28.33	28.84	29.34	29.02	28.17	28.5	28.84	29.06
Canola oil	5.73	5.76	5.79	5.83	5.81	5.75	5.77	5.79	5.81
Dicalcium phosphate	1.61	1.63	1.64	1.66	1.68	1.63	1.64	1.66	1.67
Limestone	1.2	1.2	1.19	1.19	1.19	1.19	1.19	1.19	1.18
Vitamin/mineral premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Enzyme	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt (as NaCl)	0.37	0.35	0.34	0.33	0.32	0.35	0.34	0.33	0.32
L-lysine	0.35	0.34	0.32	0.31	0.32	0.34	0.33	0.32	0.31
DL-methionine	0.28	0.29	0.29	0	0.31	0.29	0.3	0.3	0.31
Choline chloride	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
L-threonine	0.15	0.15	0.15	0.14	0.15	0.15	0.15	0.15	0.15
Valine	0.004	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02
L-isoleucine	0	0	0	0	0.01	0	0	0	0.002
Total	100	100	100	100	100	100	100	100	100
Analyzed composition (%)									
Crude Protein	22.44	22.68	21.57	21.26	21.1	22.2	21.72	21.57	20.83
AME, kcal/kg	3,232	3,265	3,243	3,214	3,232	3,231	3,226	3,233	3,244
Calcium	0.87	0.78	0.98	0.85	0.95	0.92	0.98	0.88	1.11
Phosphorous	0.73	0.73	0.77	0.76	0.77	0.77	0.79	0.79	0.8
Crude Fibre	2.77	2.67	2.51	2.67	2.79	2.53	2.51	2.57	2.49
Fat	6.67	7.07	7.05	6.84	7.11	6.8	6.94	7.02	7.32
Ash	5.44	5.19	5.59	5.33	5.62	5.43	5.62	5.53	6.03
Calculated amino acid (%)									

Lysine	1.36	1.36	1.37	1.37	1.37	1.36	1.37	1.37	1.37
Methionine	0.59	0.6	0.61	0.61	0.62	0.6	0.61	0.61	0.62
Methionine + Cysteine	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99

Table 2. Composition of finisher diets fed in broiler study.

	Finisher Diets								
Ingredient (%)	Control	L15	L30	L45	L60	H15	H30	H45	H60
Control Wheat	60	45	30	15	0	45	30	15	0
Sorted Low Wheat	0	15	30	45	60	0	0	0	0
Unsorted contaminated wheat	0	0	0	0	0	15	30	45	60
Soybean meal	18.83	19.36	19.9	20.54	21.77	19.36	19.89	20.57	21.82
Corn - ground	10.92	10.32	9.71	8.98	7.59	10.33	9.74	8.98	7.59
Canola oil	5.54	5.63	5.72	5.82	6	5.62	5.71	5.82	6
Dicalcium phosphate	1.36	1.37	1.39	1.4	1.41	1.37	1.38	1.39	1.4
Limestone	1.06	1.06	1.06	1.06	1.05	1.06	1.05	1.05	1.04
Vitamin/mineral premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Enzyme	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt (as NaCl)	0.37	0.35	0.34	0.33	0.32	0.35	0.34	0.33	0.32
L-lysine	0.37	0.35	0.34	0.32	0.28	0.35	0.33	0.31	0.27
DL-methionine	0.23	0.24	0.24	0.25	0.25	0.24	0.24	0.25	0.25
Choline chloride	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
L-threonine	0.13	0.13	0.13	0.12	0.11	0.13	0.13	0.12	0.11
Valine	0	0	0	0	0.04	0	0	0	0.03
L-isoleucine	0.03	0.03	0.03	0.12	0.01	0.03	0.02	0.02	0.001
Total	100	100	100	100	100	100	100	100	100
Analyzed composition (%)									
Crude Protein	19.07	19.25	18.82	18.68	18.85	19.05	18.61	17.99	18.59
AME, kcal/kg	3,241	3,223	3,220	3,229	3,283	3,258	3,185	3,214	3,267
Calcium	0.79	0.83	0.84	0.85	0.87	0.89	0.95	0.87	0.89
Phosphorous	0.66	0.69	0.68	0.69	0.71	0.69	0.7	0.7	0.71
Crude Fibre	2.17	2.09	1.95	2.23	2.39	2.07	2.18	2.21	2.28
Fat	6.84	7.05	7.02	7.16	7.26	7.13	6.8	7.09	7.43
Ash	4.85	4.9	4.84	5	5.05	4.87	5.04	5	4.99

Calculated amino acid (%)									
Lysine	1.11	1.12	1.12	1.12	1.13	1.12	1.12	1.12	1.13
Methionine	0.5	0.51	0.51	0.52	0.52	0.51	0.51	0.52	0.52
Methionine + Cysteine	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85

Wheat

The control wheat used tested negative for DON. To create low DON wheat a high DON contaminated wheat was sorted using a GCS 200 Grain Cleaner using setting 33.5 Hz, and retaining the first two fractions. The third fraction was further sorted using the HHVK Fusarium setting on a BoMill IQ (model 1002, version 2.0.79, BoMill AB, Sweden) and retaining fractions two through six, which constituted the fractions below 5ppm DON. The low DON sorted wheat was recombined and mixed to create a homogenous product with a DON level of 1.3ppm. The three wheat sources were analyzed for chemical composition, mycotoxins (aflatoxin, DON, zearalenone, ochratoxin, fumonisin), and amino acid content by Cumberland Valley Analytical Services. The high DON diets were formulated to be 5.5ppm, but after production the DON level was measured as 3.5ppm. Further investigation revealed that during grinding while manufaturing the feed, part of the DON was associated with the fine particle that were stuck in the air filtration system and this may account for the reduced DON concentration in the final diets.

Table 3. Treatments and analyzed DON concentration in starter and finisher diets.

Treatment	Wheat Inclusion	Starter	Finisher
		DON ppm (analyzed by PDS)	
1	Control No DON(60%)	0.05	0.07
2	Sorted Low DON (15%)	0.24	0.23
3	Sorted Low DON (30%)	0.39	0.30
4	Sorted Low DON (45%)	0.53	0.59
5	Sorted Low DON (60%)	0.70	0.80
6	Unsorted High DON (15%)	0.68	0.66
7	Unsorted High DON (30%)	1.55	1.38
8	Unsorted High DON (45%)	2.27	2.30
9	Unsorted High DON (60%)	3.05	2.75

Experimental procedures

A total of 360 newly hatched mixed sex Ross 308 broiler chicks were acquired from Prairie Pride Hatchery (Grandora, SK). The birds were randomly placed in 90 bioassay cages, with 4 birds per cage at the University of Saskatchewan Poultry Research Centre in Saskatoon, SK.
There were 9 treatments, which were replicated 10 times. The birds were housed in a temperature-controlled room, transitioning from 34C to 25C. The lighting program was 23L:1D from 0 to 7 days, and 18L:6D for the rest of the experiment. Feed and water were provided ad libitum. Cage dimensions were 29 cm high x 48 cm wide x 83 cm long. Culled or mortality birds that died for unclear reasons were necropsied at Prairie Diagnostic Services Inc. (Saskatoon, SK) to determine cause of death.

Growth Performance

Body weight and feed consumption were measured on days 7, 14, 21, 28, and 35. Average daily feed intake (FI), average daily gain (ADG), and feed to gain ratio (F:G) were calculated from body weight and feed consumption.

Organ weight

Birds were euthanized by cervical dislocation on day 35. The empty weights of the duodenum, jejunum, and ileum, with the mesentery removed were recorded for all birds except on from each cage. The duodenal segment consisted of the duodenal loop. The jejunal segment was from the end of the duodenal loop to the Meckel's diverticulum, and the ileal segment from the Meckel's diverticulum to the ileocecal junction. The liver and spleen weights were recorded for all the birds. Bird sex was also noted.

Intestine histology

One bird was randomly selected from each cage to collect segments from the middle of the duodenum, jejunum, and ileum. Tissues were gently rinsed in isotonic saline then fixed in 10% buffered formalin. The tissues were embedded in paraffin and sectioned at a 5µm thickness and stained with hematoxylin and eosin by Prairie Diagnostic Services Inc. (Saskatoon, SK). One longitudinal section of each tissue was prepared. The microscope used to analyze the slides was an Axiostar light microscope, using a x 5 objective, pictures of the intestinal epithelial structures were acquired. Between 10 to 16 villi were measured per bird to evaluate villus height, crypt depth, and villus width. Due to issues with the sample preparation of the duodenal tissues, they were not evaluated.

Statistical analysis

Statistical analysis was performed using JMP (version 12; SAS Institute, Inc., Cary, NC, USA). Cage was used as the experimental unit for performance parameters, and bird was used for the other analysis. The experimental design was an RCBD 4 x 2 +1 factorial. Significance was determined at ($P<0.05$) and mean separation was analyzed using the Tukey-Kramer method.

Results

Body weights are shown in Table 4. At 14 and 21 days of age, body weight was reduced with increasing levels of the sorted wheat however there was no effect of increasing levels of high DON wheat nor was there any effects of with wheat type at 28 or 35 d of age. At the highest level of inclusion (60% added wheat), there the final body weights of the low DON sorted wheat, high DON wheat and the zero DON control.

Feed intake is shown in Table 5. Feed intake between 0 and 14 days of age was affected by inclusion level of the Low DON sorted wheat but not in a consistent manner. Feed intake was not affected at any other ages.

Feed conversion efficiency are shown in Table 6. Feed conversion efficiency was increased by increasing levels of both low and high DON wheat at 14, 21 days of age but there was no affect at 28, 35 or overall 0-35 day efficiency.

Table 7 shows the effects of treatments on liver and spleen weights at 35 D of age. There were no effects of treatment on organ weights. Table 8 shows the effects of treatment on Intestinal section weights. There were no statically significant effects on intestinal weights. Table 9 shows the effects of treatments on intestinally villi heigh, width and cript depth in the jejunum. There were no effects of treatment on these parameters except villus width was affected by inclusion level of low sorted wheat but not in a consistent manner. The low sorted wheat at 60% also had the higher villus width at 60% inclusion than the high DON wheat and the control.

Discussion

Surprisingly inclusion of high DON wheat did not affect growth rate, feed conversion, feed intake, growth rate, intestinal villi measurements or organ weights. This is likely due to the lower level of DON in these final diets than had been predicted when producing the diets. The lack of response further supports the understanding that broiler chickens are less sensitive to DON than Swine. Inclusion of low DON sorted wheat reduced growth rate, feed intake up to 21 days of age but increased feed efficiency. Based on the lack of response to the High DON wheat, the changes may be due to the inadvertent sorting of another nutrient or antinutrient in wheat using the Bomill. Unfortunately what this may be is not known. A full panel of mycotoxins was analysed and the low DON diet did not have appreciable levels of any of those compounds so it may be due to an inadvertent increase in some possible antinutrient that may impact feed intake.

Conclusion

Feeding high DON wheat did not impact broiler performance, organ weights or villi parameters. Sorting of the wheat into low DON fractions did not improve performance nor health parameters.

Growth performance

Table 4. Growth performance (g) of broilers fed diets containing sorted or unsorted DON contaminated wheat in graded levels.

	14 d					21 d				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*	Low sorted	High unsorted	Control	SEM	*P*
15	549.6	537.1	-	8.95	0.339	1079.5	1076.4	-	14.17	0.88
30	526.3	526	-	7.67	0.976	1047	1048.2	-	13.39	0.949
45	539.2	529.1	-	5.74	0.23	1067.8	1050.5	-	12.64	0.347
60	513.2	524.1	525.9	6.25	0.319	1025.4	1039.5	1049	11.24	0.342
SEM	7.17	7.33				12.37	13.23			
P	0.006	0.61				0.02	0.246			

	28 d					35 d				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	P	Low sorted	High unsorted	Control	SEM	*P*
15	1792.2	1794.2	-	24.97	0.955	2505.5	2504.1	-	47.69	0.983
30	1735.7	1738.7	-	23.76	0.929	2450.4	2434.3	-	29.62	0.704
45	1779	1734	-	20.39	0.136	2497.9	2437.6	-	39	0.288
60	1730.8	1715	1710.7	22.39	0.801	2479.7	2401.2	2401.8	42.14	0.332
SEM	23.23	23.2				42.06	37.52			
P	0.173	0.11				0.797	0.283			

Significance indicated at P<0.05, means with different superscripts in rows are significantly different.

P<0.05 in columns indicates significant regression

Birds were fed a commercial starter from hatch to 7 d, then started on treatment diets.

Table 5. Feed intake (g) of broilers fed diets containing sorted or unsorted wheat in graded levels.

	14 d					21 d				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*	Low sorted	High unsorted	Control	SEM	*P*
15	428.3	425.1	-	5.99	0.707	700.9	701.8	-	9.3	0.945
30	410.5	412.9	-	6.28	0.789	686.2	684.5	-	8.92	0.894
45	429.7	429.4	-	7.49	0.975	710.4	686.7	-	9.31	0.089
60	417.3	417.3	419.4	5.77	0.955	685.7	693.4	691.8	9.08	0.819
SEM	5.01	7.05				8.07	9.14			
P	0.028	0.353				0.102	0.542			

	28 d					35 d				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*	Low sorted	High unsorted	Control	SEM	*P*
15	1059.8	1069.1	-	22.97	0.778	1255.6	1288.2	-	35.65	0.525
30	1042.9	1053.7	-	17.25	0.664	1256.2	1230.7	-	17.27	0.309
45	1081.9	1032.5	-	22.17	0.132	1276	1289.3	-	35.57	0.794
60	1030.4	1024.8	1033.4	17.47	0.94	1279.5	1214.9	1230.5	29.42	0.286
SEM	19.33	21.68				27.84	33.16			
P	0.278	0.468				0.89	0.272			

	0 to 35 d				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*
15	3445	3483	-	67.1	0.681
30	3396	3381	-	43.83	0.823
45	3498	3438	-	64.44	0.517
60	3413	3350	3375	51.44	0.691
SEM	53.89	60.90			
P	0.561	0.425			

Significance indicated at P<0.05, means with different superscripts in rows are significantly different.

Birds were fed a commercial starter from hatch to 7 d, then started on treatment diets.

P<0.05 in columns indicates significant regression

Table 6. Feed conversion of broilers fed diets containing sorted or unsorted DON contaminated wheat in graded levels.

	14 d					21 d				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*	Low sorted	High unsorted	Control	SEM	*P*
15	1.28	1.28	-	0.01	0.86	1.54	1.53	-	0.01	0.721
30	1.28	1.27	-	0.01	0.575	1.52	1.53	-	0.01	0.65
45	1.25	1.28	-	0.01	0.088	1.5	1.53	-	0.01	0.157
60	1.24	1.25	1.28	0.01	0.014	1.49	1.5	1.56	0.01	0.02
SEM	0.01	0.009				0.01	0.01			
P	0.026	0.286				0.02	0.129			

	28 d					35 d				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*	Low sorted	High unsorted	Control	SEM	*P*
15	1.69	1.72	-	0.02	0.321	2	1.99	-	0.03	0.879
30	1.66	1.65	-	0.01	0.366	1.95	1.98	-	0.01	0.248
45	1.64	1.68	-	0.02	0.199	1.96	1.96	-	0.03	0.991
60	1.71	1.67	1.74	0.02	0.28	1.94	1.98	2.03	0.04	0.477
SEM	0.01	0.02				0.02	0.03			
P	0.031	0.092				0.293	0.887			

	0 to 35 d				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*
15	1.63	1.63	-	0.01	0.909
30	1.61	1.61	-	0.01	0.813
45	1.59	1.61	-	0.01	0.203
60	1.6	1.60	1.65	0.02	0.103
SEM	0.01	0.01			
P	0.122	0.360			

Significance indicated at P<0.05, means with different superscripts in rows are significantly different.
P<0.05 in columns indicates significant regression

Organ weights

Table 7. Organ weights (g) of broilers fed diets containing sorted or unsorted DON contaminated wheat at graded levels.

	Liver (g)					Spleen (g)				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*	Low sorted	High unsorted	Control	SEM	*P*
15	58.7	57.2	-	1.43	0.455	2	2.1	-	0.08	0.739
30	56.1	52.7	-	1.13	0.034	1.9	1.9	-	0.07	0.462
45	55.1	54.1	-	1.28	0.56	2.1	1.9	-	0.08	0.185
60	56.9	54.4	56.2	1.31	0.355	2.1	2	1.98	0.08	0.677
SEM	1.25	1.3				0.08	0.08			
P	0.215	0.112				0.257	0.65			

Significance indicated at P<0.05, means with different superscripts in rows are significantly different.
P<0.05 in columns indicates significant regression

Table 8. Empty intestinal weights of broilers fed diets containing sorted or unsorted DON contaminated wheat in graded levels.

		Duodenum (g)			
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*
15	10.0	10.1	-	0.70	0.906
30	10.4	10.3	-	0.61	0.888
45	10.6	10.1	-	0.61	0.625
60	10.3	9.9	10.2	0.57	0.885
SEM	0.59	0.66			
P	0.921	0.985			
		Jejunum (g)			
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*
15	20.0	21.6	-	0.94	0.238
30	22.6	22.2	-	0.78	0.940
45	20.7	21.2	-	0.87	0.705
60	22.4	21.4	21.9	0.86	0.698
SEM	0.83	0.91			
P	0.071	0.881			
		Ileum (g)			
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*
15	18.4	20.1	-	0.81	0.125
30	19.2	19.4	-	0.78	0.888
45	18.8	19.1	-	0.70	0.853
60	19.3	18.4	19.4	0.67	0.506
SEM	0.74	0.75			
P	0.809	0.441			

Significance indicated at P<0.05, means with different superscripts in rows are significantly different.
P<0.05 in columns indicates significant regression

Table 9. Intestinal villi analysis of broilers fed diets containing sorted or unsorted DON contaminated wheat.

	Jejunum (g)					**Ileum (g)**				
	Villus height (µm)					Villus height (µm)				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*	Low sorted	High unsorted	Control	SEM	*P*
15	1366	1348	-	103.15	0.905	807	790	-	40.55	0.769
30	1266	1300	-	83.75	0.781	806	866	-	42.50	0.337
45	1338	1403	-	77.5	0.579	797	832	-	45.53	0.597
60	1241	1288	1320	54.46	0.603	815	804	729	35.09	0.180
SEM	83.25	85.92				43.10	43.19			
P	0.715	0.792				0.993	0.635			
	Villus width (µm)					Villus width (µm)				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*	Low sorted	High unsorted	Control	SEM	*P*
15	103	103	-	3.32	0.968	114	115	-	2.54	0.627
30	104	101	-	4.00	0.537	118	108	-	4.15	0.123
45	96	96	-	3.47	0.941	113	114	-	5.12	0.893
60	113[a]	104[ab]	100[b]	3.00	0.040	115	110	117	5.12	0.634
SEM	3.72	3.41				4.65	4.19			
P	0.03	0.475				0.848	0.607			
	Crypt depth (µm)					Crypt depth (µm)				
Treatment wheat included (%)	Low sorted	High unsorted	Control	SEM	*P*	Low sorted	High unsorted	Control	SEM	*P*
15	169	173	-	16.65	0.885	168	159	-	8.71	0.497
30	170	155	-	10.00	0.312	173	173	-	9.59	0.989
45	170	194	-	10.12	0.179	165	168	-	7.38	0.810
60	182	172	189	12.13	0.590	166	161	161	7.09	0.864
SEM	12.9	12.63				8.80	8.09			
P	0.920	0.254				0.892	0.599			

Significance indicated at P<0.05

P<0.05 in columns indicates significant regression

Reducing Deoxynivalenol in *Fusarium* infected barley using Near Infrared Transmittance individual kernel sorting technology, air fractionating aspiration, and ozonation.

Objectives

The overall objective of this portion of the project is to determine the most efficient and practical method for reducing DON levels in *Fusarium* infected barley.

i. Determine if *Fusarium* infected barley kernels can sort into fractions ranging in DON concentrations using NIT technology based on calculations developed to estimate the degree of *Fusarium* infection of individual kernels.
ii. Determine if cleaning chaff and light seeds from *Fusarium* infected barley prior to NIT sorting affects sorting efficiency and DON levels in sorted fractions.
iii. Determine if the density of *Fusarium* infected barley is related to the DON concentration and therefore the grain can be segregated into a range of DON levels by air fractionation.
iv. Determine if *Fusarium* is susceptible to abrasion and can be dislocated from the surface of barley during handling and therefore the levels of DON can be reduced if the grain is subject to abrasion prior to air fractionation.
v. Determine if DON can be oxidized during modified storage conditions post harvest using ozone pumped into *Fusarium* infected barley therefore resulting in reduced DON levels.

Hypothesis

1. Due to the chemical compositional changes as are result of *Fusarium* infection in barley, DON can be reduced by sorting the barley into fractions with NIT technology based on calibrations developed to estimate the degree of *Fusarium* infection in individual kernels.
2. Cleaning *Fusarium* infected barley prior to sorting with NIT technology will result in a more consistent grain size which will be more effectively sorted into fractions therefore resulting in more accurate sorting of individual kernels into fractions and greater segregation of grain based on DON concentration.
3. Barley kernels in *Fusarium* infected grain varies in DON concentration which is directly related to grain density which I a reflection of the severity of the infection and therefore the grain can be

sorted into high and low DON concentrations based on density using a fractionating aspirator which sorts by density.

4. The DON concentration in highly infected barley seeds can be reduced by oxidation during storage by injecting ozone in the aeration process during the first few hours of storage post-harvest. Humidification of the air during ozonation will enhance DON oxidation.

8.6 THE USE OF NEAR INFRARED TRANSMITTANCE (NIT) KERNEL SORTING TECHNOLOGY TO REDUCE DEOXYNIVALENOL (DON) IN *FUSARIUM* INFECTED BARLEY BASED ON PREDICTED *FUSARIUM* INFECTION CALIBRATIONS.

Introduction

Previous research demonstrated that NIT sorting based on CP can indirectly produce fractions with different DON levels (Kautzman, 2015). At the time of the research, the NIT technology was unable to sort based directly on *Fusarium* infection in individual kernels and different wavelength settings for the NIT had not been developed yet. This trial seeks to explore new technology available and further research the potential of sorting grains based on chemical compositions.

Material and methods

Barley source

A total of 428 kg of *Fusarium* infected barley was collected on November 10, 2017 at a Saskatchewan farm (51°31'06.8"N 106°48'03.3"W) that had an average DON concentration of 3.8 ppm. This barley was packaged into 11 bags, approximately 39 kg each and stored at the University of Saskatchewan Poultry Research Centre in a shipping container without temperature control.

BoMill IQ 1002 Quality Grain Sorter

Fusarium estimate calibrations were developed by BoMill with the assistance of Western Canada barley samples provided by the Canadian International Grains Institute (Cigi). This research focused on the BoMill IQ 1002 calibrations: MVK, *Fusarium* and HVK, *Fusarium*. These calibrations correspond to how hard, or vitreous, the grain is. Hard-vitreous kernels (HVK) are kernels that have a translucent colouring which is an externally visible sign of hardness, whereas kernels that have a starch spot are considered non-vitreous kernels. This harness impacts the NIT spectroscopy due to its effect on wavelength transmittance through the kernel. For this reason, three levels corresponding to the grain's hardness were developed, LVK (for non-vitreous kernels), MVK (an in-between), and HVK (for hard-vitreous kernels) The No. 26 disc was used for sorting. The disc has 200 oval shaped pockets measuring 9.3 cm long, 4.6 cm wide, and 2.9 cm deep each. Pockets have a single hole drilled through the center to allow the NIT spectroscopy to penetrate the kernel in the disc pocket without interference. Prior to

sorting, 1.5 kg of barley was analyzed by the BoMill IQ 1002. This allows the software to develop an expected distribution of the kernels and

Experimental Design

Table 0.1 *BoMill sorting experimental treatments.*

Treatment	Level of Cleaning Prior to Sorting	NIT Calibration
1	No cleaning	HVK
2	No cleaning	MVK
3	Cleaned	HVK
4	Cleaned	MVK

- Materials were ground with a Bunn G1 Coffee grinder on fine setting. This was chosen to consistently obtain a grind that allowed at least 95% of the ground material to pass through a U.S. No. 20 sieve. No. 20 sieves have a mesh opening of 0.841 cm.
- DON levels were measured by ELISA with a Vicam Lateral Flow Reader using the room temperature method.

Batches of *Fusarium* infected barley weighing 1.5 kg were sorted on the BoMill IQ 1002 grain quality sorter. Uncleaned barley batches were unaltered prior to sorting. Cleaned barley batches were hand sieved with a No. 6 slotted hand sieve with a pan attached. The hand sieve has slotted perforations 2.38 mm wide and 19.05 mm long. Barley that did not pass through the hand sieve was used for sorting. Materials that passed through the hand sieve were collected from the pan, weighed, ground, and analyzed for DON concentration. For all sorted batches, unscreened and screened, the fractions were set up as: 16.7% each for the first through fourth fractions, and 16.6% each for the fifth and sixth fractions. These settings represent the expected percentage of total kernels to be sorted into each fraction. Samples were taken prior to sorting, then ground and analyzed for initial DON concentrations. After sorting, all fractions were weighed, ground, and analyzed for DON concentration. Rejected kernels were also collected and analyzed for DON concentration. A diverter pipe of 1.5 cm diameter was fabricated and installed alongside the six fraction pipes. Instead of rejected kernels being ejected back

into the unsorted materials, rejected kernels were ejected into the diverter pipe and collected into a bag to remain separated from unsorted materials and trash materials. After sorting was completed, any kernels that were unable to be sorted (e.g. too large to fit in disc pockets or abnormally shaped), as well as nonbarley materials and dust were collected into the BoMill IQ 1002's trash bin using a soft brush and compressed air. These materials were then weighed, ground, and analyzed for DON concentration.

Results and Discussion

Mass recovery in to each of the 6 sorted fractions as well as the rejects are shown in Tables 2 and 3. The system was set up to in theory segregate the material into 6 equal fractions of 16.7%. The closer the values are to the set levels the more efficiently the system is working. It is inevitable that some of the grain will not either fit in the slot in the unit or be outside of calibration curve but the objective is to minimize the amount of unsorted seed. Ideally the remaining sortable seed is equally distributed within the 6 fractions. Seed was cleaned prior to sorting in attempt to reduce the rejects due to seed which do not fit in the singulation slots. Cleaning by sieving did not reduce the number of rejects or the efficiency of sorting. However, Vitreous kernel settings had a significant impact ($p<0.0001$) on sorting efficiency. HVK setting had less rejects than MVK setting, 14.8 vs 54.1% respectively. Vitreous kernel setting impacts is related to protein content of the seed. Canadian feed barley tends to be higher protein than some other regions and using the High vitreous kernel setting was most appropriate for the barley sorted in this experiment but if samples have lower protein it may be advisable to use medium vitreous kernel setting. Low vitreous kernel setting was tried in this experiment but there were so many rejects the system automatically shut down therefore making it impossible to use this setting.

When the HVK setting was used, sorting was efficient from a mass segregation perspective and approached the ideal levels of 16.7% in each of the six fractions. As expected based on the high levels of rejects, MVK did not result in effective sorting. However, the remaining seed was evenly distributed within the six fractions although markedly less than the theoretical 16.7% in each.

Table 2. Mass recovery (%) of fusarium infected barley samples sorted on a BoMill IQ using HVK and MVK vitreous kernel settings, with and without cleaning.

	Treatment					
Setting	**HVK**	**MVK**	**HVK**	**MVK**	**SEM**	**Interaction**
Cleaned	**No**	**No**	**Yes**	**Yes**		**P<**
Fraction	% of mass in Fraction					
1	14.3	9.1	16.6	7.6	2.13	0.520
2	12.5	7.1	15.1	7.1	1.93	0.605
3	16.6[a]	6.5[c]	14.7[b]	8.1[c]	0.42	0.002
4	15.2	6.3	14.6	8.1	0.55	0.052
5	13.7	6.1	13.9	7.7	1.06	0.520
6	9.1	5.9	11.4	6.4	1.57	0.571
Rejects	15.8	55.1	13.8	53.2	3.18	0.972

Levels not connected by the same letter are significantly different.

Table 3. Main effects of cleaning and vitreous kernel setting on mass recovery (%) in fractions of fusarium infected barley samples sorted on a BoMill IQ.

	Vitreous Kernel Setting		Cleaned			Main Effects P<	
Setting	**HVK**	**MVK**	**Yes**	**No**	**SEM**	**Setting**	**Cleaning**
Fraction	% Mass in fraction						
1	15.0[a]	8.4[b]	11.6	11.7	1.51	0.009	0.968
2	13.8[a]	7.3[b]	11.3	9.8	1.37	0.006	0.445
3	15.6[a]	7.3[b]	11.4	11.6	0.30	0.001	0.667
4	14.8[a]	7.2[b]	11.4	10.7	0.39	0.001	0.257
5	13.8[a]	6.9[b]	10.8	9.9	0.75	0.001	0.411
6	10.3[a]	6.1[b]	8.9	7.5	1.11	0.021	0.393
Rejects	14.8[b]	54.2[a]	35.5	35.5	2.25	0.001	0.550
SEM	1.09	1.32	3.21	3.35			

P<	0.025	0.001	0.001	0.001

[a-b]Means with in a row within a main affect sharing a common superscript are not significantly different (P<0.05)

Tables 4 and 5 shows the DON concentrations of the fractions created by the Bomill – IQ using two vitreous kernel settings with and without pre-cleaning. Cleaning significantly reduced the DON concentration prior to sorting. Small shriveled seeds that were removed by sieving were likely highly infected with barely and highlights the value of cleaning infected barley before marketing. BoMill IQ effectively sorted barley into low DON fractions. MVK without cleaning resulted in the highest DON level in fraction one but that does not mean this is the more effective sorting method. There were excessive levels of rejected grain using this setting so it is not practical in these circumstances. HVK setting results in the most effective sorting when both DON levels of fractions and mass recovery is considered. HVK sorting resulted in fraction 6 having less than 1 ppm DON resulting in a highly marketable fraction. When combined with cleaning Fractions 3 to 6 using HVK setting resulted in fractions with less than 1 ppm and would result in the highest return for the grain after cleaning. Interestingly the rejected grain was not excessively high levels of DON and was likely just not sorted to elevated protein in these seeds.

Table 4. DON concentrations (ppm) of fusarium infected barley samples sorted on a BoMill IQ using HVK and MVK vitreous kernel settings, with and without cleaning.

	Treatment					
Setting	**HVK**	**MVK**	**HVK**	**MVK**	**SEM**	**Interaction**
Cleaned	**No**	**No**	**Yes**	**Yes**		**P<**
Prior to Sorting	2.68	3.32	2.26	1.76		
Fraction						
1	9.7[b]	20.2[a]	5.6[b]	9.5[b]	1.03	0.008
2	2.3	3.4	1.7	2.2	0.43	0.485
3	1.6	2.4	1.0	1.5	0.23	0.388
4	1.1	1.7	0.8	1.4	0.11	0.97
5	1.0[b]	1.5[a]	0.9[b]	1.0[b]	0.06	0.004
6	0.8	1.0	0.7	1.0	0.08	0.525
Rejects	3.2[a]	2.0[b]	1.9[b]	2.0[b]	0.16	0.002

Means in a row that share a common superscript are not significantly different P<0.05.

Table 5. Main affects of cleaning and vitreous kernel settings on DON content (ppm) of fusarium infected barley samples sorted on a BoMill IQ using HVK and MVK vitreous kernel settings, with and without cleaning.

Setting	Vitreous Kernel Setting		Cleaned		Main affect P<		
	HVK	MVK	Yes	No	SEM	Setting	Cleaning
Fraction	DON (ppm)						
Initial	2.32	2.44	1.75	3.0	0.27	0.759	0.007
1	7.6[b]	14.8[a]	7.5[b]	15.0a	0.73	0.001	0.001
2	2.0	2.8	1.9	2.8	0.30	0.079	0.053
3	1.3[b]	2.0[a]	1.2[b]	2.0[a]	0.16	0.010	0.006
4	1.0[b]	1.5[a]	1.1[a]	1.4[b]	0.08	0.001	0.047
5	1.0[b]	1.2[a]	1.0[b]	1.3[a]	0.044	0.001	0.001
6	0.7[b]	1.0[a]	0.8	0.9	0.06	0.005	0.380
Rejects	2.5[a]	2.0[b]	2.0[b]	2.6[a]	0.12	0.014	0.002
SEM	0.34	0.87	0.30	0.86			
P<	0.001	0.001	0.001	0.001			

[a-b]Means with in a row and within a main effect sharing the same superscript are not significantly different (P<0.05)

Conclusions

The BoMill IQ effectively sorted fusarium infected barley when using the HVK setting and the fusarium calibration. Cleaning did not increase sorting efficiency, however, it did reduce the starting DON concentration of the grain and resulted in the greatest proportion of grain recovered with less than 1 PPM DON when combined with the HVK setting.

8.7 REDUCING DEOXYNIVALENOL (DON) IN *FUSARIUM* INFECTED BARLEY BY DENSITY SEPARATION AND ABRASION.

Introduction

Fusarium infection reduces the density of barley kernels (Nielsen, 2017) but kernels are not simply diseased or not, there is a range of infection (Kautzman, 2015) therefore it should be possible to segregate fusarium infected barley based on density. It was hypothesised that infected kernels can be separated using an air fractioning grain cleaner. In addition, antidotal reports from producers suggests that handling of grain using a grain tends to reduce the DON concentration of the barley. We hypothesised that the combination of applying abrasion to the seed prior to air fractionation would result in a further reduction of DON.

Material and methods

Batches of 5 kg of *Fusarium* infected barley were sent through the Karkov ISM-10 CSM (Impeller Separating Machine with Cyclone Sedimentary Chamber) Fractionating Aspirator at five different fan speeds, 40%, 50%, 60%, 70%, and 80% of capacity as indicated by readout on the variable frequency drive. Samples were taken prior to being sorted with the fractionating aspirator to determine initial DON levels. The fractionating aspirator separates the barley into seven fractions through 200 mm grain ducts based on density, with the lightest materials, primarily chaff, hulls and dust, collected from the cyclone. Heaviest materials fall into the first vessel and lighter materials fall into the seventh vessel. After sorting, the first and second fractions are combined due to the small volume of barley in the first fraction. Samples were taken from each fraction, ground and analysed for DON concentration. The same processes are performed after barley has been subjected to 10 minutes of abrasion in a small cement mixer. The material from the cyclone is collected and weighed. All samples are ground and DON levels are measured using the same specifications as trial one.

Experimental Design:

- 10 sorting treatments, replicated 3 times, 5 X 2 factorial (5 fan speeds X 2 levels of abrasion):
    - 40% fan capacity, not abraded

- 40% fan capacity, with 10 minutes of abrasion
- 50% fan capacity, not abraded
- 50% fan capacity, with 10 minutes of abrasion
- 60% fan capacity, not abraded
- 60% fan capacity, with 10 minutes of abrasion
- 70% fan capacity, not abraded
- 70% fan capacity, with 10 minutes of abrasion
- 80% fan capacity, not abraded
- 80% fan capacity, with 10 minutes of abrasion

- Sorting was performed with the Karkov ISM-10 CSM (Impeller Separating Machine with Cyclone Sedimentary Chamber) Fractionating Aspirator
- Materials were ground with a Bunn G1 Coffee grinder on fine setting so that at least 95% of the ground material passes through a U.S. No. 20 sieve.
- DON levels were measured by ELISA with a Vicam Lateral Flow Reader at room temperature.

Results and Discussion

Abrasion

Table 1 shows the DON level in fractions of Barley which without and with abrasion followed by separation using air fractionation. Air fractionation separated the grain into high and moderate levels of Don with Fractions 1 &2, 7 and cyclone containing the highest levels of DON. Abrading the barley prior to air fractionation did not reduce the DON level of the fractions as was hypothesized instead the levels appeared to increase, this is especially true of the fraction collected in the cyclone. Based on our hypotheis, it was expected that more of the DON would be found on the product collected in the cyclone as this will contain the chaff and hulls which tend to have higher levels of DON than the seed itself. How DON concentration appears to increase in all fractions with abrasion is not known at this time. Based on this experiment subjecting the grain to abrasion is not suggested however air fractionation does appear to reduce DON concentration in a number of fraction likely due to a combination of segregating low and high density kernels with a range of infection. Removing the chaff and dust through aspiration appears to reduce the load of DON in the final seed however the amount of material ending up in this cyclone fraction is a relatively small portion of the product being processed.

Table 1. Interaction of abrasion and Fraction on DON concentration (ppm) of Fusarium infected barley (intial DON = 3.1 ppm) fractionated at 80% air speed.

Fraction	Abraded	
	No	Yes
	DON (ppm)	
1&2	3.0[c]	3.5[c]
3	1.0[c]	1.6[c]
4	1.4[c]	2.0[c]
5	1.8[c]	1.8[c]
6	2.3[c]	3.8[c]
7	3.6[c]	5.1[c]
Cyclone	12.6[b]	25.5[a]
SEM	0.87	
Interaction P<	0.001	

- Means not sharing a common superscript are significantly different (p<0.05)

Air Fractionation and Fan Speed

A set of experiments was conducted to determine if segregation of fusarium infected barley could be enhanced by modifying the fan speed. Table 2 and Figure 1 shows the relative proportions of barley in each of the fractions as affected by airspeed. If the airspeed is too high, very little product goes into factions 1-3 and this is counterproductive from a fractionation perspective. Ideally the product should be segregated into a more normal curve representing the expected distribution of kernel density. Air speeds of 40, 50 and 60% appeared to spread the product out in a nice distribution, however at 60% air speed little product was in fractions 1 and 2 so may not be making efficient use of the fractionation capacity of the unit.

Table 2. The recovery (%) of fusarium infected barley (3.1 ppm DON) sorted by air fractionation

	Fan speed (% of maximum)					SEM	P<
	40%	50%	60%	70%	80%		
Fraction	% Mass recovery						
1&2	9.6[b]	4.6[c]	0.8[e]	0.3[d]	0.1[d]	0.62	0.079
3	43.8[a]	25.9[b]	9.4[d]	3.2[d]	1.3[d]	2.45	0.001
4	37.0[a]	45.9[a]	26.7[b]	11.8[c]	5.7[d]	2.22	0.001
5	7.2[b]	17.6[b]	35.2[a]	24.5[b]	14.7[c]	1.55	0.001
6	1.4[b]	3.8[c]	17.8[c]	27.8[ab]	24.5[b]	0.93	0.001
7	0.7[b]	1.8[c]	9.7[d]	31.1[a]	50.6[a]	1.42	0.001
Cyclone	0.2	0.3[c]	0.5[e]	1.3d	3.1	0.57	0.013
SEM[1]	2.12	2.12	0.84	1.30	1.85		
P<	0.001	0.001	0.001	0.001	0.001		

[a-d]Means within a column sharing a common superscript are not significantly different (P<0.05)

Figure 1. The impact of Air speed on the proportion of fusarium infected barley separating into fractions through air fractionation.

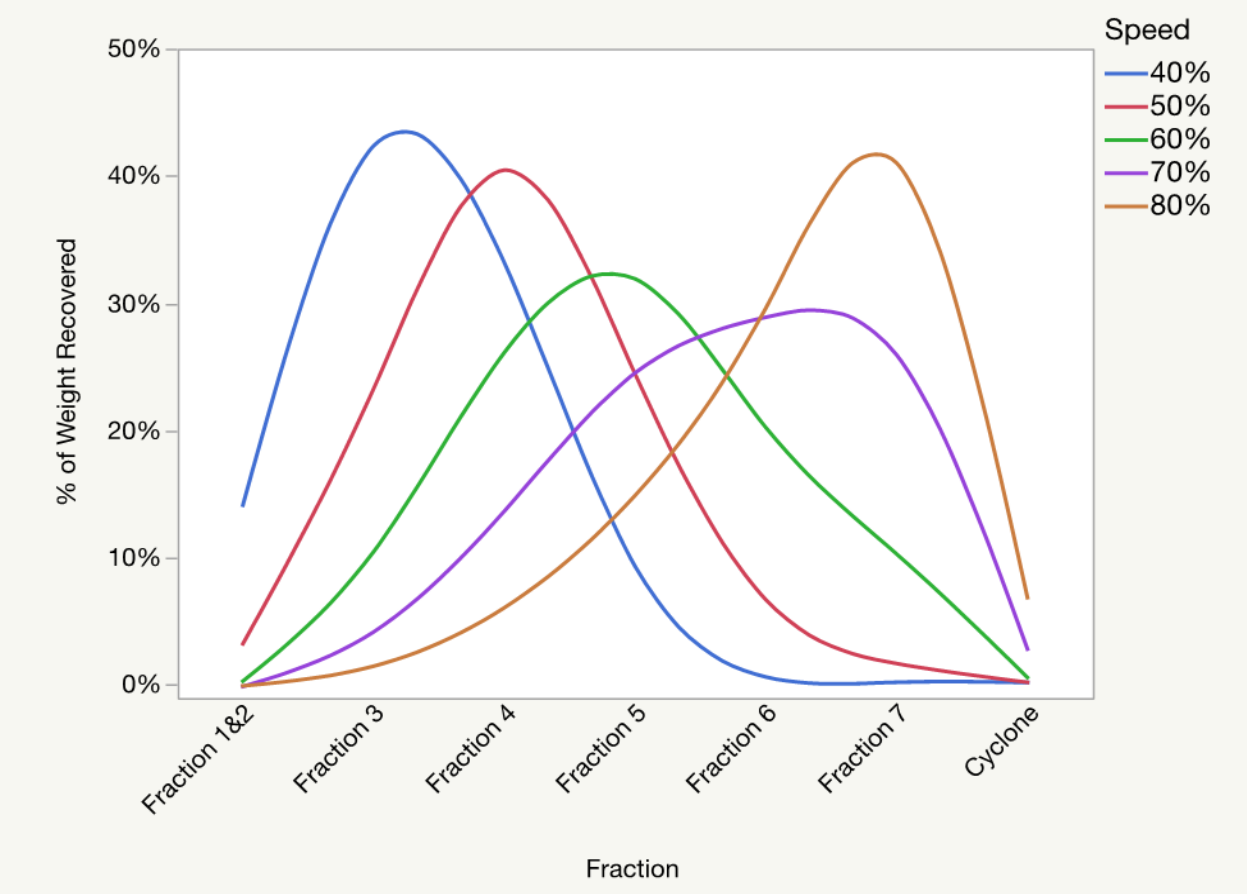


Figure 2 and table 3 show the DON concentration in each of the fractions produced by air fractionation as affected by fan speed. The higher the fan speed the further the contaminated grain was pushed through the system. As hoped, air fractionation did effectively separate the grain into low and high DON fractions of barley with the lowest being in the first fractions (most dense) and the highest levels in the lightest or later fractions. One exception to this is at 80% air speed where the fan speed may have been too high and caused some turbulence in the system causing some of the light kernels to return back into the system and end up in the first fraction.

Figure 2. The Impact of air speed on DON concentration in fractions of fusarium infected barley separated by air fractionation.

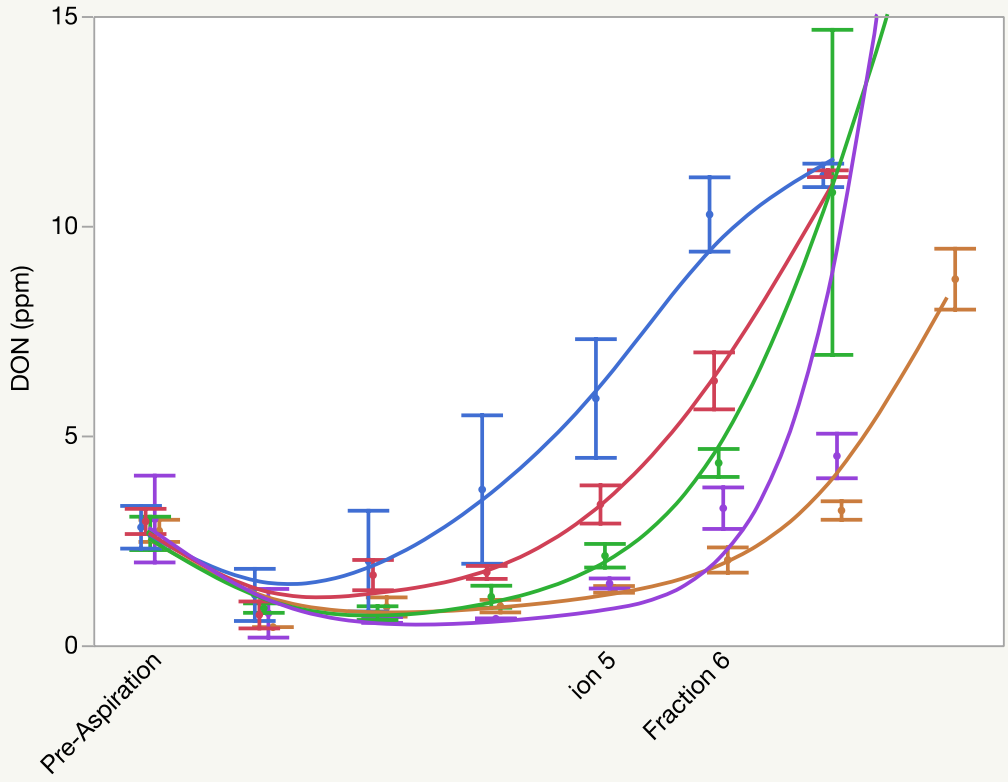


Table 3. The DON concentration (ppm) of fusarium infected barely (3.1 ppm DON) sorted by air fractionation

	Fan speed (% of maximum)					SEM	P<
	40%	50%	60%	70%	80%		
Fraction			DON (ppm)				
1&2	1.1^{d}	1.0^{c}	0.9^{c}	0.9^{b}	3.2^{b}	0.62	0.079
3	2.2^{d}	2.0^{c}	1.0^{c}	0.7^{b}	1.3^{b}	0.33	0.022
4	3.5^{cd}	2.4^{c}	1.5^{c}	1.0^{b}	1.7^{b}	0.46	0.009
5	6.9^{bc}	4.1^{bc}	2.3^{bc}	1.5^{b}	2.2^{b}	0.47	0.001
6	10.7^{ab}	7.6^{b}	4.3^{bc}	2.7^{b}	3.0^{b}	0.61	0.001
7	13.7^{a}	8.9^{ab}	7.8^{b}	3.7^{b}	4.3^{b}	1.41	0.001
Cyclone	12.7^{a}	13.8^{a}	17.6^{a}	25.8^{a}	19.0^{a}	2.53	0.029
SEM[1]	1.00	1.11	1.16	0.99	0.94		
P<	0.001	0.001	0.001	0.001	0.001		

[a-d]Means within a column sharing a common superscript are not significantly different (P<0.05)
[1]SEM is for the means of Fractions 1-7, cyclone was missing a data point for 1 rep so had a higher SEM with fan speeds 40-60% (SEM = 1.28, 1.28,1.27 for cyclone fraction from 40, 50, and 60% fan speed, respectively)

Table 4 and Figure 3 shows the density of the fractions separated by air fractionation. The differences in grain density were relatively small yet separating based on relative density demonstrated significant segregation of low and high DON fractions. The higher the airspeed the more effective the system appeared to be at separating the highest density barley from the lower density. With one exception, at 80% air speed the first fraction had a relatively low density and this may be related to internal turbulence and lighter materials bouncing back into fraction 1. Note however, that the amount of grain in Fraction 1 at 80% air speed is very low 0.1% of the mass so this is likely of little consequence.

The overall objective of this study was to determine the optimal conditions for recovery of usable barley from an infected source using air fractionation. Table 5 shows the recovery of grain below 1 and 2 ppm at each of the air speeds tested. Recovery of grain below 1 and 2 ppm DON was greatest at 40% airspeed, this would suggest slow airspeed is required to effectively separate grains with a narrow range of densities as affected by fusarium infection. The lowest DON concentration is in the highest density seeds and if the airspeed is excessive, these higher density seeds are intermingled with the lower density seeds effectively reducing the DON segregation capacity. We did not test feed rate into the machine. It may be possible if the machine was being fed at a higher rate that higher air speed may be required to compensate for the mass of material that is impeding the air flow.

Table 4. Density (kg/hl) of fusarium infected barley (67.5 kg initial density) sorted by air fractionation

	Fan speed (% of maximum)					SEM	P<
	40%	50%	60%	70%	80%		
Fraction	Density (kg/hl)						
1&2	69.4[a]	69.1	70.6	71.0[a]	67.9[bc]	0.46	0.025
3	68.4[a]	68.7	70.0	69.6[ab]	70.1[a]	0.36	0.025
4	67.6[ab]	68.1	68.6	68.9[ab]	69.4[ab]	0.52	0.268
5	66.5[ab]	66.9	67.6	68.0[bc]	68.6[abc]	0.84	0.460
6	62.7[b]	64.1	66.7	67.1[bc]	68.0[bc]	0.61	0.007
7	NA	59.7	65.8	66.2[c]	66.3[c]	2.45	0.299
Cyclone[1]	NA	NA	NA	NA	NA		
SEM	0.90	1.88	0.91	0.46	0.41		
P<	0.029	0.070	0.053	0.003	0.003		

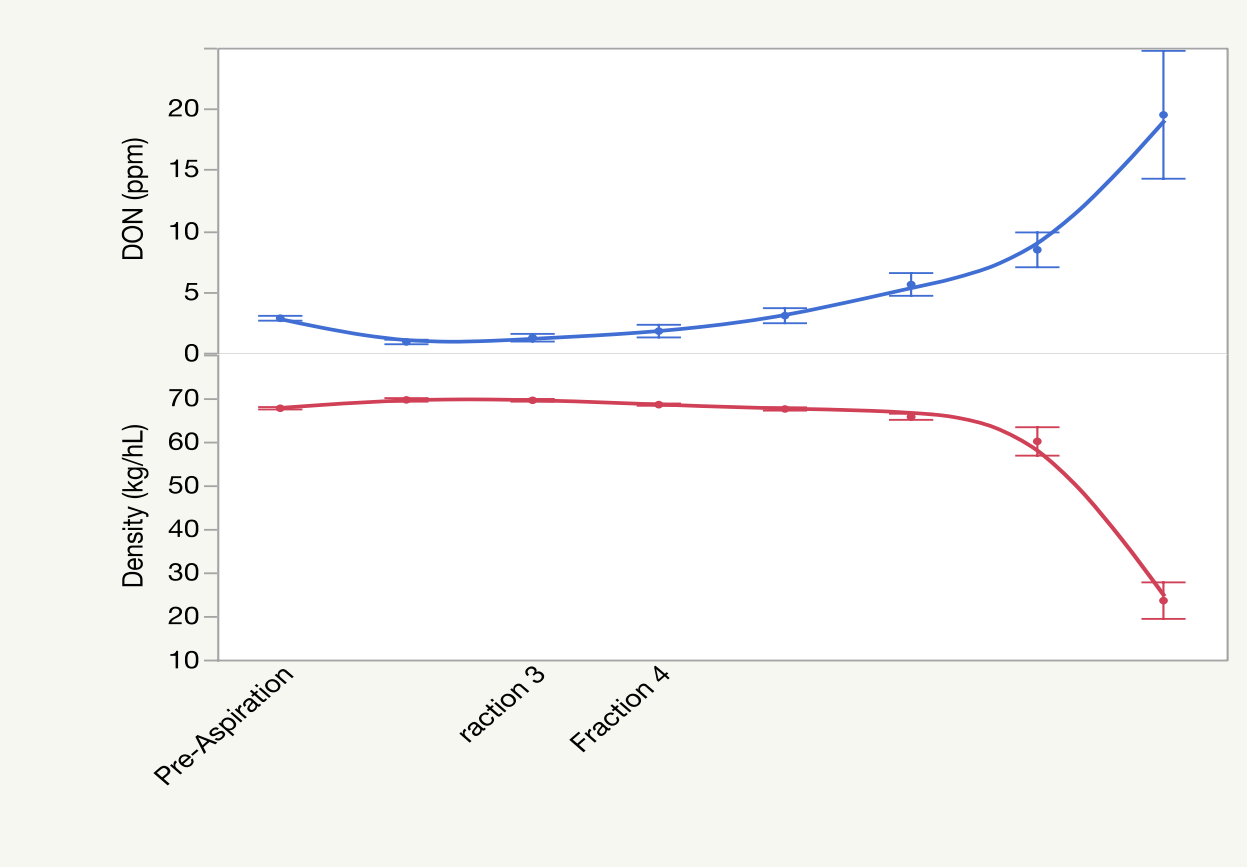
[a-d]Means within a column sharing a common superscript are not significantly different (P<0.05)

[1]Density of material from the cyclone was to0 low to measure accurately at approximately 20kg/hl

Table 5. The impact of fan speed setting on the recovery of barley containing less than 1 and 2ppm DON when separated using air fractionation.

Air Speed (% of max)	Grain recovery (%)	
	<1 ppm	<2 ppm
40	68.1%	99.4%
50	3.6%	78.2%
60	16.6%	76.4%
70	24.2%	49.0%
80	10.3%	38.5%

Figure 3. The density (kg/hL) and DON concentration in fractions of barley separated using air fractionation.



Conclusions

Air fractionation can be an effective method of separating fusarium barley prior to marketing. Removal of the chaff reduces DON concentration but in addition, the high density grain contains less DON than lower density grain. However, the differences in relative density were relatively small so it is separating based on relative density with a batch of grain. Abrasion of barley prior to air fractionation did not reduce DON concentration of the fractions created, the DON appeared to increase with abrasion. A large portion of barley could be recovered with less than 1 or 2 ppm DON if the slowest airspeed was utilized.

8.8 THE USE OF Ozone (O_3) TO REDUCE DEOXYNIVALENOL (DON) IN *FUSARIUM* INFECTED BARLEY.

Hypotheses

1. If *Fusarium* infected barley is treated with ozone for 24 hours, then DON will degrade at a higher rate than when exposed to air alone.

Objectives

1. Determine if 24-hour ozone exposure will degrade DON in *Fusarium* infected barley.

Introduction

Ozone is composed of three oxygen atoms (O_3). This third oxygen atom is readily available to split from the ozone molecule and attach with other molecules, this causes an oxidizing reaction. Ozone is currently approved by the US FDA to be used as an antimicrobial agent in processing and storage of food, water, certain juices, as well as ciders (Rice, Graham, & Lowe, 2002). Ozone quickly breaks down into O_2 with a short half life of only 20 to 50 minutes at standard room temperature. Ozone does not leave residues, nor does it require storage or disposal of any chemical containers (Rice et al., 2002). These advantages as well as already being widely used and accepted have made it a potential solution for toxin reduction during grain handling. In 2016, a study to explored the capabilities of ozone to degrade DON in wheat kernels with ozone exposure of 75 mg/L over 30, 60, and 90 minutes (Wang et al., 2016). Wang found that the initial DON levels of 1.69 mg/kg degraded by 26% to 53% (Wang et al., 2016). The longer the exposure, the higher the degradation. Wang also found that ozone treatment did not affect the protein content, fatty acid value, amino acid content, or starch content of the wheat (Wang et al., 2016).

Material and methods

Four Ozone Solutions (451 Black Forest Rd, Hull, Iowa, 51239 USA) HP-500 ozone generators were used to create a continuous flow of ozone during a 24-hour period. Each generator functions by using an electrical arc to split oxygen molecules (O_2), these single oxygen atoms then attach to other oxygen molecules to create ozone (O_3). The four ozone generators used have an expected output of 500

mg/hr. An external air system was attached to each ozone generator to maintain a constant air flow of 0.14 cubic meters per hour (m^3/h) through the ozone generator and a vessel containing 600 g of *Fusarium* infected barley. Vessel containing 600 g of barley were constructed of 51 cm long Poly Vinyl Chloride (PVC) pipes with an internal diameter of 5.7 cm. The ends of each pipe were closed with a threaded cap. The threaded caps were fitted with a 0.3 cm by 0.8 cm brass hose barb. The barb was used to attach the vessel to a silicone hose at each end of the vessel. One hose allowed the constant movement of air and any remaining ozone to flow out of the vessel. The other hose attached the vessel to the ozone generator. For barley that was not being treated with ozone, the vessel was still connected to the ozone generator in an identical manner except for the generator being unplugged to prevent any ozone being generated. These vessels of barley were still exposed to a continual flow of air at the same rate of 0.14 cubic meters per hour as vessels that were treated with ozone. This trial also investigated the impact higher moisture conditions had on the ability of ozone to degrade DON. Barley moisture content was altered by addition of water to the barley in water for a period of 24 hours to achieve the targeted moisture content of 17.5%.

Results

Figure 1 shows the main effect of ozone on Barley treated for 24 hours. On average DON was reduced from 3.0 to 2.1 ppm, this confirms that introduction of ozone into air being forced through barley can result in partial oxidation of the DON. Table 1 shows the impact of ozone, aeration, and increased moisture on DON concentrations. A control aeration treatment with no added ozone was added to the air stream was also included in the experiment. Interestingly, aeration alone decreased DON from 3.0 to 2.5 ppm. This would suggest, that some of the DON is readily oxidizable with just atmospheric oxygen at 20 C. Adding Ozone further reduced the DON concentration to 2.09 indicating the ozone with its strong oxidizing capacity is able to oxidize additional DON however, significant quanitites of DON still remained after 24 hours of treatment. Moisture was added back to the dry grain to mimic the conditions of drying grain through aeration. The DON concentration was further reduced in moist grain without added ozone to 2 ppm. Addition of Ozone further reduced this to 1.5 ppm DON.

Figure 1. Overall effect of aeration time on DON concentration in Fusarium infected grain.

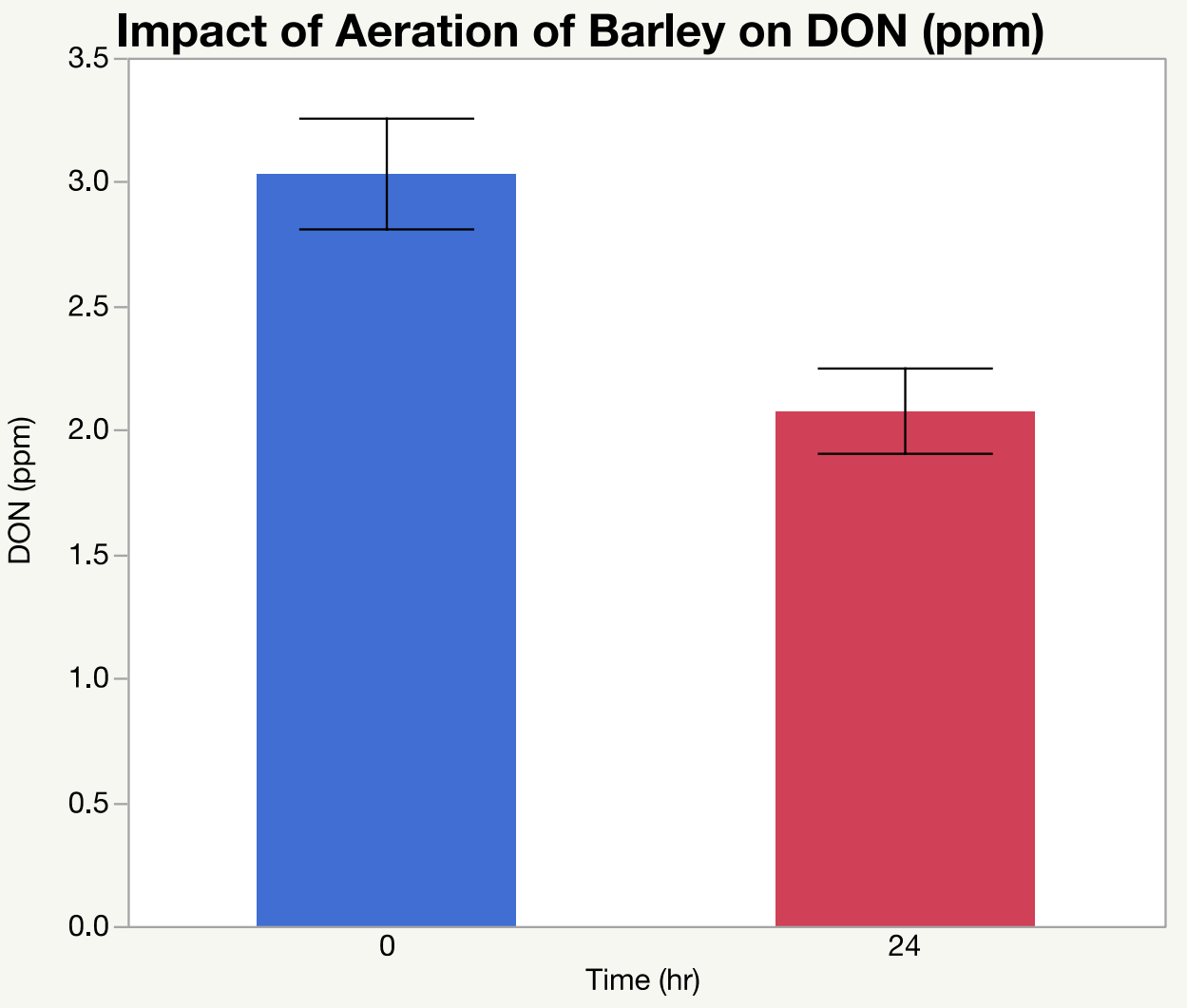

Impact of Aeration of Barley on DON (ppm)
DON (ppm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
24
Time (hr)

Table 1. The effect of Ozone treatment (24 hours 20ºC) and tempering (21.6% moisture) on DON concentration in Fusarium infected barley.

Treatment		DON (ppm)
Air		2.48[a]
Ozone		2.09[b]
SEM		0.12
P<		0.027
Dry		2.82[a]
Tempered		1.74[b]
SEM		0.12
P<		0.001
Ozone	Tempered	
no	no	3.01
no	yes	1.95
yes	yes	2.64
yes	yes	1.53
SEM		0.17
P<		0.89

[a-b]means sharing a common superscript are not significantly different (P<0.05)

Conclusions

In fusarium infected barley, DON can be partially oxidized during aeration at 20 C. Addition of Ozone to the aeration air enhances the oxidation. Increased moisture content increased the rate of DON oxidation. Addition of Ozone to aeration air during the drying of fusarium infected barley will potentially result in approximately a 50% reduction in DON concentration.

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Overall Discussion and Conclusions

Fusarium infection occurs sporadically in both wheat and barley crops in Saskatchewan and can cause significant downgrading of the product and in in some cases results in large portions of grain that are not marketable. This study investigated the potential for 3 technologies to recover a portion of this grain by either segregating the grain into high and low DON fractions and/or oxidizing the DON using Ozone during drying in an aeration bin. Both wheat and barley were effectively segregated into low and high DON fractions using an Near Infrared Transmittance singulator (BoMIll) and air Fractionating grain cleaner. The BoMill segregated the grains based on relative changes in chemical composition. The calibration and settings had significant effects on the efficiency of segregation. Fusarium calibration was the most effective calibration overall but segregating based on protein worked as well. The vitreous kernel setting had a significant impact on the sorting efficiency especially on the level of rejects. When sorting grain using the BoMill technology, it will be important to find the appropriate settings before

sorting significant quantities of grain. Cleaning wheat using sieving technology helped reduce sorting efficiency by reducing the number of kernels which do not fit in the singulator disc. It also increased efficiency by reducing the amount of chaff and other foreign materials which could interfere with the seed placement in the singulator device. BoMill is a highly specific technology that work well and this comes at a price, the units are relatively expensive given their sorting capacity (3 MT per unit) and the grain should be cleaned prior to sorting and of similar seed size and shape to fit in the slots in the drum.

In this project we also examined a low cost, simple process, air fractionating grain cleaner, to determine if it could be used to presort the grain prior to BoMill or be used as is for sorting the grain. In both wheat and Barley, the air fractionating system was able to sort the grain into relatively low and high DON fractions although not to the same level as the BoMill. Depending on the level of DON infection, we would reccomend using an air fractionator to clean the infected wheat and barley to determine if the DON levels can be reduced enough to make the product marketable. Unfortunately when sorting very high DON wheat, air fractionation resulted in a mid stream fraction which had moderate to high levels of DON but still contained high quality low DON seeds. In our study, this fraction of wheat was further sorted on a BoMIll and this technology was effective at sorting the high quality kernels from the low and therefore recovering additional marketable grain. In these experiments, we found that the combination of an air fractionation grain cleaner followed by BoMill sorting was the most effective method of sorting affected wheat.

A signficant portion of grain is dried by aeration following harvest by forcing air through the grain for a period of time. In our study we examined the potential to add an oxidizing agent to the air called ozone to promote oxidation of the DON. Based on these studies, grain temperature appeared to have little impact on oxidation of the DON but moisture content of the grain promoted oxidation. Air alone oxidized a portion of the DON but addition of Ozone to the air during drying reduced DON concentration by up to 50%. Ozone is inexpensive to produce as ozone generators are available that generate ozone by creating an electrical arc in an air stream. The ozone does not leave any residue in the grain and does not otherwise negatively affect the material. However, since the ozone has high oxidizing capacity it is important not to inhail significant quantities of the product so it needs to be handled in a safe manner. The half life is very short so once generation of Ozone is ceased the product does not linger in the

environment. One other potential downfall of the technology is it may promote oxidation of any exposed iron products. Most products in grain bins are protected by galvanization so this should protect the steel but corrosion does need to be considered.

In conclusion, when fusarium infection occurs and grain is either of low value or not marketable, the technologies (BoMill sorting, Air Fractionation, Ozone oxidation) examined in this study can be used to effectively recover grain that can be marketed.

9. **List any technology transfer activities undertaken in relation to this project:**
The results of these studies were shared with academia and industry on a number of occasions. This includes a hands on workshop as well as industry events and conferences. They include:

Newkirk, R.W., 2020. Feed Processing For Optimal Animal Health and Performance. Animal Nutrition Conference of Canada. Presentation given June 9, 2020 via Webinar.

M. E. Taylor and **R. W. Newkirk**, 2020. Sorting efficiency and grain recovery from DON contaminated wheat by near-infrared transmittance sorting (BoMill) is impacted by calibration type and HVK setting. Animal Nutrition Conference of Canada. Conducted online due to Covid. June, 2020

M.E. Taylor and **R.W. Newkirk**, 2020. Sorting of heavily Fusarium infected wheat using a combination of air fractionation and near-infrared transmittance (BoMill) to improve grain recovery with low DON levels. Animal Nutrition Conference of Canada. Conducted online due to Covid. June, 2020

Newkirk, R.W., 2019. Post-Harvest Grain Sorting
an On-Farm Strategy. Session: From Farm to Fork: A Systemic Approach to Reducing Post-Harvest Losses Throughout the Supply Chain. IFT19 Conference, New Orleans, LA, June 2-5, 2019.

Taylor, M.E. and **Newkirk, R.W.** 2018. Partitioning of wheat contaminated with deoxynivalenol from low to high concentration by air fractionation. 9th Canadian Workshop on Fusarium Head Blight and 4th Canadian Wheat Symposium. Winnipeg, Mb, Nov 19-22, 2018.

Garew, T.S. and **Newkirk, R.W.** 2018. Deoxynivalenol reduction in *Fusarium* infected barley by density sorting. 9th Canadian Workshop on Fusarium Head Blight and 4th Canadian Wheat Symposium. Winnipeg, Mb, Nov 19-22, 2018.

Newkirk, R.W. 2018. Impact of agronomic practices on commodity end uses and values. Top Notch Farming Workshops. Melfort, SK, Feb 13, Humbolt, SK, Feb 14th, Davidson, SK, Feb 15th.

Newkirk, R.W. 2018. Fusarium damaged grains….options. Manitoba Ag Days, Brandon, MB, January 17th, 2018.

Newkirk, R.W. 2017. Mitigation strategies for Fusarium, NARF Fusarium Head Blight Management Field Day, Melfort, Sk, July 25, 2017

The intention was also to hold in person workshops on or near farms with producers infected grains. However, since there was no appreciable levels of fusarium present during the final years of this study it was not possible to conduct those workshops. Instead a webinar has been created and posted on the CFRC website for producers to access when the issue arises again. The link to the webinar is : https://usask.cloud.panopto.eu/Panopto/Pages/Viewer.aspx?id=c6a4c1fa-1f10-4ac1-a8dd-abf90117d3b5

10. **Identify any changes expected to industry contributions, in-kind support, collaborations or other resources.**

One of the primary challenges we faced in this study was the lack of fusarium infection. Prior to starting this study Saskatchewan producers had several years of severely infected grain. But fortunately for producers but unfortunately for our study, this did not occur again during this study. The intention of the study was to do on farm testing of the technologies examined but since there was no appreciable amounts of infected wheat or barley in Saskatchewan for the past 3 years this was not possible and all the studies were conducted at the CFRC or in the Lab using material collected from previous years.

The project was supported in-kind by both BoMill and Flaman Grain Systems. BoMIll provided an IQ model unit at no cost for the duration of the study and Flaman allowed us to use their air fractionation systems at either no cost or very low rental rates.

SaskBarley also supported this project with a $25,000 contribution to the project.

11. **Appendices:**