Influence of Processing on *Fusarium* Mycotoxins in Contaminated Grains

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**Abstract**: Fusarium mycotoxins survive processing and tend to concentrate in products generally used for animal feeds. Mycotoxins are secondary metabolites produced by fungi colonizing cereal grains in the field and in storage and are harmful to both humans and animals. During adverse weather conditions, such as the unusually wet spring and summer of 1993 and 1994 in the upper Midwest, cereal grain crops such as corn, wheat, and barley may become contaminated with harmful levels of the *Fusarium* mycotoxins: zearalenone and deoxynivalenol (vomitoxin or DON). Conversely, under drought conditions which sometimes occur in the Midwest and Southwest, another type of *Fusarium* mycotoxin, the fumonisins can be a problem.

The effects of these mycotoxins on animals have been well documented. Zearalenone, a phytoestrogen, has been shown to cause the estrogenic syndrome in swine and "false heat" in breeding stock. Deoxynivalenol, a trichothecene, causes feed refusal, vomition, and immune suppression in swine and other animals. The fumonisins, a closely related family of mycotoxins, have been shown to cause leukoencephalomalacia in equine, pulmonary edema in swine, and liver cancer in rats.

The fungi which colonize cereal grains and produce these compounds are commonly found throughout the United States and in most countries around the world. Zearalenone and deoxynivalenol are produced primarily by *Fusarium graminearum* species whereas the fumonisins are produced by *Fusarium moniliforme* and *Fusarium proliferatum* species.

During normal growing seasons, damage to cereal crops by these fungi and the accumulation of these mycotoxins are not a significant problem for the grower or processor of cereal grains. Also, the U.S. Dept. of Agriculture Grading System provides a safeguard since the mycotoxins are generally found in lower grade grains which are not selected by the industry for human food products. However, adverse weather and improper storage of grains have led to levels of these toxins which exceed the levels of concern and thus pose a potential health threat to animals and humans should contaminated grains inadvertently be used for foods and feeds. The following is a review of the effects of processing on corn and wheat which were naturally contaminated with the *Fusarium* mycotoxins—zearalenone, deoxynivalenol, or fumonisins.

**Wet Milling**

Wet milling is the major process used to prepare corn products for human consumption. It has been shown to have a segregating effect on the levels of zearalenone and fumonisins in the chemically diverse products resulting from the process.

**Zearalenone**. Laboratory-scale studies have been conducted on naturally contaminated corn to determine the fate of zearalenone (Fig. 1) during processing (Bennett et al., 1978). Three lots of corn at 0.9, 4.1, and 9.4 ng/g zearalenone were wet milled. These lots of corn were USDA Grades 2, 4, and Sample Grade, respectively. The wet milling process followed the commercial procedure and the determination of zearalenone in wet milled products was by thin layer chromatography with a detection limit of 0.05-0.10 ng/g. The distribution of zearalenone in products from wet milling was in the order of gluten > milling solubles > fiber > germ and is summarized in Table 1.

The starch fractions, which account for 65-71% of milled products, were free of detectable zearalenone. Milling solubles contained one to four times the levels of zearalenone in the starting corn and gluten fractions (the most highly contaminated fraction) contained two to seven times the levels in starting corn. Although these fractions represent 14-19% of the corn, they account for 72-75% of the zearalenone. Fiber and germ fractions contained one to three times the levels of zearalenone in the starting corn and these fractions account for 15-16% of the milled corn. Clearly, if a zearalenone contaminated lot of corn is processed by wet milling, toxin free starch is produced; however, other products generally used in animal feed have much higher levels of toxin than the starting corn.

**Fumonisins**. The fumonisins (Fig. 2) are recently discovered mycotoxins produced by *F. moniliforme*, *F. proliferatum*, and other related species which readily colonize corn.
Influence of Processing on *Fusarium* (Continued)

Table 1—Zearalenone Distribution in wet-milled corn fractions (From Bennett et al., 1993)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Lot 1</th>
<th>Lot 2</th>
<th>Lot 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zearalenone</td>
<td>Zearalenone</td>
<td>Zearalenone</td>
</tr>
<tr>
<td></td>
<td>µg/g</td>
<td>% of Com</td>
<td>µg/g</td>
</tr>
<tr>
<td>Corn as milled</td>
<td>0.9</td>
<td>6.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Germ</td>
<td>6.9</td>
<td>9.0</td>
<td>7.7</td>
</tr>
<tr>
<td>Fiber</td>
<td>2.7</td>
<td>-0.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Gluten</td>
<td>6.7</td>
<td>9.1</td>
<td>13.4</td>
</tr>
<tr>
<td>Solubles</td>
<td>67.8</td>
<td>NDa</td>
<td>99.6</td>
</tr>
<tr>
<td>Starch</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>99.6</td>
</tr>
</tbody>
</table>

a=None detected

around the world. Limited research has been conducted on this family of toxins due to the infrequent outbreaks and the fact that toxic effects only arise mostly from heavily infected corn and corn screenings. Wet milling of two lots of naturally contaminated corn have been completed (Bennett et al., 1996) and results from laboratory scale milling are shown in Table 2.

These data were obtained from duplicate 800-g samples that were milled by the traditional process using lactic acid-sulfurous acid steeping prior to milling. No toxin could be detected in any fraction, except steep water, obtained from corn containing 1.0 µg/g fumonisin B₁. In the fractions from corn containing 13.9 µg/g toxin, a significant portion (22%) of recoverable fumonisin B₁ was found in the steep and process waters. Other fractions contained fumonisins in the order of gluten > fiber > germ. The gluten and fiber from corn contaminated at 13.9 µg/g could pose a risk because they contain toxins at levels considered to be harmful to certain animals (Ross et al., 1991). No toxin could be detected in starch fractions from this corn.

Dry Milling

Dry milling is a process to separate grain components by grinding the corn or wheat into various particle sizes in roller mills. The different fractions (grits, germ, flour) retain much of the characteristics of the original corn or wheat. This section contains information on the effects of dry milling on zearalenone, deoxynivalenol, and fumonisins.

Zearalenone. Three lots of naturally contaminated corn (0.93, 3.50, and 7.80 µg/g zearalenone) were dry milled by both a laboratory scale and a commercial process (Bennett et al., 1976). Dry cleaning (screening) of the corn did not significantly reduce toxin levels in the corn lots. All mill fractions from both procedures were contaminated with zearalenone. The highest levels of toxin were in the hull and high-fat fractions. Prime product mix (grits, low-fat meal, and flour, which account for 57–63% of product yield) contained about 20% of the zearalenone from the starting corn samples. Levels of zearalenone in fractions from U.S. Grade 2 corn (0.93 µg/g) were high enough (1.2 µg/g) to be of concern because they are greater than levels that elicit

Table 2—Distribution of Fumonisins B₁, B₂, and B₃ in wet milled corn fractions of contaminated corn (From Bennett et al., 1994)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>FB₁ µg/g</th>
<th>FB₂ µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber</td>
<td>5.7</td>
<td>2.1</td>
</tr>
<tr>
<td>Germ</td>
<td>2.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Gluten</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Starch</td>
<td>3.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Starch</td>
<td>5.8</td>
<td>4.7</td>
</tr>
<tr>
<td>Starch</td>
<td>5.1</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Levels of fumonisin B₁ in starting corn, 13.9 µg/g

ND = Level below detection limit of 0.1 µg/g
an estrogenic response in pigs (Kuiper-Goodman et al., 1989). Zearalenone has been detected in cereal grain products for human consumption although the levels were generally low (Warner et al., 1987).

**Deoxynivalenol.** Deoxynivalenol (Fig. 3) contamination in wheat appears to be a continuing problem with localized outbreaks occurring almost annually. Since the outbreak in Ontario wheat in 1980, scientists in the U.S. and Canada have conducted numerous cleaning, milling, and baking studies on contaminated wheat. Although significant quantities (ca. 25%) of toxin can be removed by cleaning and polishing, the toxin remains in wheat flour at levels ranging from 60–60% of toxin levels from the starting wheat (Seitz et al., 1986; Trenholm et al., 1991).

Another study on wheat samples from several states showed much less deoxynivalenol in flour from spring wheat that had been cleaned by aspiration prior to milling into flour (Bennett et al., 1992). Wheat samples that contained high levels (>10 μg/g) yielded flour that was above the recommended level for wheat products destined for human consumption: 1.0 μg/g (Abouzied et al., 1991). Baking does not destroy or significantly reduce levels of deoxynivalenol.

**Fumonisins.** No specific dry milling studies have been conducted on fumonisin contaminated grains. However, assays on corn-based foods have shown that the toxin is present in human foodstuffs, i.e., corn meal and corn grits (Sydenham et al., 1971; Stack and Eppley, 1992). The significance of the levels found (mean value 1.0 μg/g and 0.6 μg/g for meal and grits, respectively) remain to be determined. Surveys of corn for fumonisins show that low levels (<1 μg/g) normally occur and high levels are associated with drought conditions (Murphy et al., 1993). Notably, most episodes of animal toxicoses associated with fumonisins have arisen from use of contaminated corn screenings which contained very high levels of toxin.

**Ethanol Fermentations.**

Ethanol fermentations is a major processing operation used to produce a value-added product, gasohol, from corn. Since the grade of corn has little effect on the ethanol yield, this process could be used to utilize poorer quality corn not desirable for human consumption. This section describes the effect of ethanol fermentations on zearalenone, deoxynivalenol, and fumonisins.

**Zearalenone.** Utilization of zearalenone contaminated corn for ethanol production was investigated by Bennett et al. in 1981. Two lots of corn, one naturally contaminated at 8.0 μg/g and one from field-inoculated corn at 33.5 μg/g toxin, were used in fermentation procedures generally used in the fermentation industry. The presence of toxin had no apparent effect on ethanol yield and there was no carry-over of zearalenone to distilled ethanol. However, the fermentation process did not destroy the toxin and recovered solids contained 2 to 2.5 times the level of zearalenone in the starting corn. Solubles from naturally contaminated corn also contained slightly higher levels of toxin than the starting corn.

The levels of zearalenone in recovered solids from these fermentations would increase the potential for animal disorders in the event they were used for feed for swine, the most sensitive animal.

Traditional fermentations for brewing of corn beer have shown 51% carry-over of zearalenone into the finished product (Okeere, 1978). Also, fermentations by *Saccharomyces cerevisiae* of wort containing zearalenone resulted in conversion of 69% of the toxin to beta-zearalenone, a metabolite with less activity than the parent compound (Scott et al., 1992). A recent survey of Canadian and imported beers for *Fusarium* mycotoxins revealed no beta-zearalenone of zearalenone in the samples tested (Scott et al., 1993).

**Deoxynivalenol.** Because this toxin is produced by many of the fungal species that produce zearalenone, assays have been developed to detect both toxins simultaneously. Deoxynivalenol was found in 29 of 50 samples of Canadian and imported beers (Scott et al., 1993). Nine samples contained greater than 5 ng/mL toxin. Conflicting data on toxin stability has been reported for certain steps in the beer-making process. During the fermentation process in barley, El-Bana (1989) reported that 77% of added deoxynivalenol was destroyed in 5 days. On the other hand, naturally occurring deoxynivalenol (as well as zearalenone) has been reported in malt (Lee et al., 1985). Data is dependent upon accurate analytical methodology, which can detect the parent toxin and possible metabolic product which may arise from processing.

**Fumonisins.** The fate of fumonisins in naturally contaminated corn during ethanol fermentations follows the pattern determined for zearalenone. Two lots of corn, contaminated at 15 and 36 μg/g, respectively, and a control lot were used for ethanol production (Bothast et al., 1992). Although pure fumonisins exhibit considerable water solubility, residues of toxin remain in the distillers' dried grains (Table 3). This fermentation product accounts for 31 to 51% of the total fumonisin B₁ in the starting corn. Facilitated by solubility in water, 51–54% of fumonisin B₁ in starting corn was extracted into whole stillage. No toxin could be detected in distilled ethanol even after a 10-fold concentration increase. Unfortunately, the fermentation process did not destroy the fumonisins and 85% of the toxin could be recovered in the products. Products from ethanol fermentations generally used as animal feeds could be detrimental if consumed by pigs or horses, animais sensitive to rel-

![Fig. 3—Deoxynivalenol Structure](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control Corn</th>
<th>Contaminated Corn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting corn</td>
<td>ND</td>
<td>15</td>
</tr>
<tr>
<td>Fermented mash (72 hr)</td>
<td>ND-0.4</td>
<td>1.8-2.7</td>
</tr>
<tr>
<td>Distillers aned grains</td>
<td>4.0-5.0</td>
<td>19.2-25.3</td>
</tr>
<tr>
<td>Thin stillage</td>
<td>ND-0.6</td>
<td>1.5-1.7</td>
</tr>
<tr>
<td>Centrifuge solids</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Distillers solubies</td>
<td>ND-0.2</td>
<td>1.3-1.7</td>
</tr>
<tr>
<td>Distilled ethanol</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table 3—Distribution of Fumonisin B₁ in products from ethanol fermentations of contaminated corn (From Bothast et al., 1992)
Influence of Processing on Fusarium (Continued)

More Research Needed

The *Fusarium* mycotoxins—zearalenone, deoxynivalenol, and fumonisins—survive most processing methods and ethanol fermentations. The stability of the fungal metabolite are of concern because some products, such as gluten from wet milling and distillers and dried grains from fermentation of contaminated grains, contain higher levels of toxin than found in the original grain. The use of such products for animal feed would present a potential for toxicoses in some animals. However, beneficial aspects of processing are realized by the production of toxin-free starch from wet milling of zearalenone and fumonisin contaminated corn.

Considerable research remains to be done on processes which could reduce the level of toxin contamination in milling and fermentation by-products. Removal of damaged grains by density segregation or by aspiration prior to processing are avenues under investigation. Additional treatments of products, such as washing to remove water-soluble toxins, may be viable techniques to lower toxin levels to acceptable concentrations. In addition to these strategies, additional research must be done to improve reliability of analytical methods for processed products. Procedures for raw grains generally are not reliable for products arising from various processing techniques.

Although the levels of *Fusarium* mycotoxins found in foods would not present an acute toxicity risk to humans (Forsell et al., 1987), it is imperative to keep such exposure to a minimum to reduce total exposure to these and other environmental toxins.

References


Updated April 1996 from a paper presented during the IFT Toxicology and Safety Evaluation Division symposium, "Influence of Processing Upon Residues in Food," at the Annual Meeting of the Institute of Food Technologists, Atlanta, Ga., June 25-29, 1994.

—Edited by James H. Giese, Associate Editor