Summary

Short article

NATURAL ZEARALENONE CONTAMINATION OF WHEAT FROM GOLESTAN PROVINCE, NORTHERN IRAN

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Zearalenone contamination of 175 wheat samples produced in 2004 in Golestan province, northern Iran was determined. The 10-20 kg samples collected at different stages, including pre-harvest, before and after storage for 2 months in local silos. Samples were ground and sub-sampled with an analytical Romer mill. Each sample extract was cleaned-up using an immunoaffinity column. Zearalenone levels were determined by high-performance liquid chromatography (HPLC) with UV detection at 275 nm. The linearity of the calibration curve in the range 12.5-750 ng/ml standard solutions of zearalenone was 0.997. The limit of detection (LOD) of the method was 30 ng/g. Mean recovery of the method was 85.5 % and 79 % at spiking levels of 100 and 200 ng/g, respectively. Fifteen samples out of 175 samples (8.6%) were contaminated. Mean level for contaminated samples was 72 ng/g and the range of contamination was 39-104 ng/g. The proportion of contaminated samples was low and the levels were lower than the advisory levels for zearalenone in wheat.

Key words: Natural contamination, Zearalenone, Wheat, IAC + HPLC, Iran

Zearalenone is a widely distributed mycotoxin produced by Fusarium species, especially F. graminearum, F. culmorum and F. crookwellense (Jimenez et al.1996) which colonize maize, sorghum, wheat, barley, oats and other cereal grains (Mirocha et al. 1977). Zearalenone is an estrogenic mycotoxin that causes vulvovaginitis and estrogenic responses in swine; it also induces hyperestrogenism in cows (Diekman et al.1992) and in both male and female swine (Mirocha et al. 1974). Physiological responses in swine occur when the zearalenone level in feed corn exceeds about 1 mg/kg (Kurtz and Mirocha, 1978). Reduction of the sex drive, infertility, fetal mummification and abortion are some other effects of

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Zearalenone can be transmitted to piglets via sows’ milk, causing estrogenism in the young pigs. Some countries have specific regulations for zearalenone levels in foods and feeds with maximum tolerated levels ranging from 30 to 1000 ng/g (Boutrif & Canet, 1998). In Iran the Maximum Advisory Level for zearalenone in wheat is 200 ng/g (ISIRI, 2002). During 1990-92 because of the favorable environmental conditions for Fusarium head blight disease in Iran, wheat scab became epidemic and caused considerable losses. *F. graminearum*, *F. culmorum*, *F. semitectum* and *F. proliferatum* were isolated from infected plants (Forutan, et al. 1993; Golzar 1993; Zamani-Zadeh & Forutan 1992). All the *F. graminearum* isolates, which were the dominant species and were obtained from 99 samples of wheat kernels collected from Mazandaran province, produced zearalenone in the range 46.6-400 mg/kg, whereas fourteen *F. culmorum* isolates could only produced zearalenone on corn seeds (20-80mg/kg). However, 37 samples of wheat naturally infected with *F. graminearum* were contaminated with the average of 3-42.6 mg/kg zearalenone (Zamani-Zadeh & Khoursandi, 1995). The potential for zearalenone production by 59 *F. graminearum* isolates, which were randomly recovered from scabby wheat samples collected from different provinces in Iran, was studied by thin layer chromatography and high-performance liquid chromatography (HPLC). Ninety-three percent of the isolates produced 70.4-2073 mg/kg zearalenone (Golzar et al. 2000). Antibody-based immunoaffinity columns (IACs) for cleaning up and pre-concentration of the analyte combined with HPLC is a useful technique for the determination of mycotoxins in food and feedstuffs (Scott et al. 1999).

Materials and methods

Wheat samples were collected from different locations in Golestan province in northern Iran during 2004. Each 10-20 kg sample was the mixture of many subsamples from different places in the lot and was finely ground by Romer mill series II TM, (MO, USA). 100 ml acetonitrile-water (75: 25; v/v) was added to 20 g sample accompanied with 2 g NaCl and blended at high speed for 3 min. After filtering through Whatman No.1 filter paper, 10 ml of the filtrate was diluted with 40 ml deionized water and filtered through a GH/A filter. Then, 10 ml was passed through EASI-EXTRACT® ZEARALENONE immunoaffinity column at a flow rate of about 1-2 drops/second. The column was rinsed with 10 ml deionized water at the same flow rate and then dried for 1 minute. Zearalenone was eluted with 1.5 ml methanol, collected in a 4 ml cleaned dark glass vial, diluted with 1.5 ml deionized water, and homogenized on a vortex for 1 second. Chromatographic separation and detection was
performed using a Waters (Milford, MA, U.S.A) 616 pump, automatic injector (Waters 717) and a Waters 486 tunable absorbance detector set at 275 nm. Data were processed using the Millennium software (version 3.2). The reversed-phase column was a Waters Nova-pak® C-18, 3.9 mm×250 mm, 4µm particle size. An acetonitrile: ethanol: water mixture (46:8:46; v/v) was used as isocratic mobile phase at 1.0 ml/min flow rate. The mobile phase was degassed through a vacuum – degassing device (Waters). The injection volume was 200µl (equivalent to 0.027g sample). Zearalenone was quantified by measuring the area under the chromatogram at the zearalenone retention time and comparing with the relevant calibration curve obtained from the same experiment. Standard stock solution was prepared by dissolving the solid standard (Sigma) in methanol (1 mg/ml). More diluted standard solutions for calibration (12.5, 25, 50, 100, 200, 250, 500 and 750 ng/ml standard solutions) or spiking purposes were prepared by evaporation of aliquots (under a stream of N₂) followed by appropriate dilution with the mobile phase. All solvents and reagents used were analytical or HPLC grade (Merck, Darmstadt, Germany) and immunoaffinity columns for zearalenone (EASI-EXTRACT® ZEARALENONE) were from R-Biopharm Rhone Ltd (Glasgow G20 0SP, Scotland UK). Filter papers (Whatman No.1) and glass microfibre filters (GF/A) were from Whatman (Maidstone, UK.). The blank ground wheat material used for recovery experiments was obtained from Marjaan Khatam Institute (Tehran, Iran). To prepare 100 and 200 ng/g spiked wheat samples, an appropriate amount of zearalenone standard solution (10µg/ml) was added to 20 g of the blank wheat samples.

**Results and discussion**

Natural contamination of zearalenone was determined in wheat from Golestan province, where the climatic conditions are favorable for epidemics of wheat scab disease (*Fusarium* head blight), growth of *Fusarium* species and zearalenone production. Average recoveries at levels 100 and 200 ng/g in spiked samples were 85.5 % and 79 %, respectively. The linearity of the standard curve for 12.5-750 ng/ml was $R^2=0.997$. The limit of detection (LOD) was 30 ng/g. The mycotoxin analysis of 175 samples revealed that only 15 samples contaminated with zearalenone in the range 38.9-104.2 ng/g (Table I).

The results obtained from this research indicated that the proportion of zearalenone contaminated samples (15:175) from this high-risk area for the incidence of Fusarium head blight was low (0, 12.5 and 14.8% at the various stages of harvest and storage investigated). The mean of contamination levels was approximately constant including 72.1 and 72.4 ng/g before and after silo storage respectively and the contamination levels (38.9-104.2ng/g) were
Table 1. Zearalenone natural contamination (ng/g), proportion and percent of contaminated wheat samples, through different stages.

<table>
<thead>
<tr>
<th>Location</th>
<th>At harvest</th>
<th>Before silo</th>
<th>After silo</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Proportion of contaminant</td>
<td>Proportion of contaminant</td>
<td>Proportion of contaminant</td>
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<tr>
<td></td>
<td>range(mean)</td>
<td>range(mean)</td>
<td>range(mean)</td>
</tr>
<tr>
<td>Gonbad</td>
<td>0/20</td>
<td>1/17</td>
<td>4/14</td>
</tr>
<tr>
<td></td>
<td>58.5-97.2</td>
<td>(75.6)</td>
<td>58.5-97.2</td>
</tr>
<tr>
<td>Kalaleh</td>
<td>0/15</td>
<td>-</td>
<td>0/13</td>
</tr>
<tr>
<td>Gorgan</td>
<td>0/16</td>
<td>2/24</td>
<td>1/14</td>
</tr>
<tr>
<td></td>
<td>0/13</td>
<td>38.9-104.2</td>
<td>0/16</td>
</tr>
<tr>
<td></td>
<td>(75.6)</td>
<td>(71.5)</td>
<td>(75.6)</td>
</tr>
<tr>
<td>Ali-Abad</td>
<td>0/14</td>
<td>4/15</td>
<td>3/15</td>
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<td></td>
<td>58.5-97.2</td>
<td>58.5-97.2</td>
<td>58.5-97.2</td>
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<tr>
<td></td>
<td>(72.4)</td>
<td>(72.4)</td>
<td>(72.4)</td>
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<tr>
<td>Total</td>
<td>0/65</td>
<td>7/56</td>
<td>8/54</td>
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<tr>
<td></td>
<td>38.9-104.2</td>
<td>38.9-104.2</td>
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<tr>
<td></td>
<td>(72.1)</td>
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Percent of contamination

|          | 0%         | 12.5%       | 14.8%       |
|          | 0%         | 12.5%       | 14.8%       |

under the advisory level (Table 1). These results are in contrast with previously published results on zearalenone contamination in different years in which samples had been collected from fields with serious scab disease (Zamani-Zadeh et al. 1995). The exceptionally favorable environmental conditions for occurrence of wheat scab during 1992-1995 could be another reason. In addition, using HPLC coupled with a clean-up step with IAC to measure zearalenone is more accurate (Scott & Trucksess, 1997) than TLC, gas chromatography or HPLC without a specific clean-up step used in previous works. On the other hand, F. graminearum wheat isolates collected from wheat fields throughout various Iranian provinces possessed great variation in their ability to produce toxin (Golzar et al. 2000).

See Persian text for figurees.

References

Karami Osboo and Mirabolfathy. Natural zearalenone contamination


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