Natural Occurrence of Fumonisin B₁ in Broiler Feeds in Ninevah Governorate

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ARTICLE INFO

Article history:
Received 25 April 2014
Received in revised form 8 May 2014
Accepted 20 May 2014
Available online 17 June 2014

Keywords:
ELISA, Fumonisin B₁, mycotoxin, survey.

ABSTRACT

Background: Contamination of broiler feeds with different mycotoxins is one of the major problems associated with poultry sector. Ninevah governorate/Iraq as any other Middle East countries claimed from the contamination of poultry feeds with different fungi and mycotoxins. Objective: A survey was conducted in Ninevah governorate/Iraq to assess the level and extent of Fumonisin B₁ contamination in commercial broiler feeds. A total of 60 broiler feed samples randomly collected from five different localities in Ninevah governorate/Iraq during January to March 2014 were analyzed by a competitive enzyme-linked immunosorbent assay (ELISA) for the detection of fumonisin B₁. Results: Naturally occurring fumonisin B₁ was found in 50/60 (83.33%) in all tested broiler feed samples with an average of 286.5± 56.69 µg/kg and a range of 102 to 1200 µg/kg. Conclusion: no sample was detected exceeded the accepted FDA level of 50 mg/kg feed for fumonisin B₁.

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INTRODUCTION

The dominant natural fungal flora associated with feeds are belonged to three genera: Aspergillus, Fusarium, and Penicillium (Murphy, 2006). Fumonisins are a group of mycotoxins produced by many member of Fusarium genus. These toxins are naturally found as contaminants of feeds and feed ingredient worldwide and are mostly found in corn (Jackson and J. Jablonski, 2004). F. verticillioides and F. proliferatum are considered the most dominant Fusarium species that produce fumonisins in feeds and foods, beside other fusarium species including F. nygamai, F. acutatum, F. begoniae, F. brevicatenulatum, F. phyllophilum, and F. napiforme which also produce fumonisin, but to a lesser extent (Howard et al., 2001; Fotso et al., 2002). Fumonisin-producing Fusarium have been isolated from feed ingredients normally used in animal feed formulation like corn, barley and wheat (Desjardins et al., 1997, 2000).

Fumonisins were reported with other mycotoxins in broiler feeds implicated for potential mycotoxicosis here in Ninevah governorate (Shareef, 2010). Feeding Fumonisin B₁ to broiler chicks was reported to reduce weight gain, increase mortality, and induced lesions in several internal organs (Javed et al., 1992a,b). More recently, a worldwide surveys covering many mycotoxins including Aflatoxins, Fumonisins, Ochratoxin, Zearalenone, Deoxynivalenol were published in the world Nutrition Forum, 2012 (Naehre, 2012b; Rodrigues and Naehre, 2012). The FDA guidelines for acceptable fumonisins level in broiler feed is 50 ppm. (Food and Drug Administration, 2001).

The aim of this survey was to study the level of natural fumonisin B₁ occurrence in broiler feeds in Ninevah governorate, and the categorization following the general FDA guidelines in finished commercial broiler feed.

MATERIALS AND METHODS

Sampling:

From January to March 2014, a total of 60 samples of pelleted boiler (starter and finisher) feed samples were analysed for the detection of their natural contamination with fumonisin B₁, using competitive Enzyme-linked immunosorbent assay. Samples were collected from different 15 broiler farms occurred within 5 localities in Ninevah governorate (3 fams/locality). These localities were as follow: Kog-galy (Locality A); Al-Hamdana (Locality B); Bartila (Locality C); Al-Rashidia (Locality D) and Al-Abasias (Locality E). Four kilograms of feed/ broiler farms were collected using the dynamic sampling method (Codex Alimentarius, 2004) as illustrated in (Fig. 1). According to the Regulation of European Commission Regulation (EC) No

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401/2006 (2006), 4 incremental samples of 1 kg each, were taken from each lot of 50-300 kg feed. Samples were thoroughly mixed to make a representative sample. All Samples were analysed at the department of Veterinary Public Health laboratories in the College of Veterinary medicine, Mosul University, Mosul-Iraq.

Fig. 1: Dynamic sampling method for collecting feed samples from a conveyor belt.

**Extraction of fumonisin B$_1$ from broiler feed samples:**
A commercially available competitive enzyme-linked immunosorbent assay plate kit, (Shenzhen Lushiyuan Biotechnology Co., Ltd, Green spring version: 2012-02 ), was applied for the quantification of fumonisin B$_1$. Mycotoxin extraction and testing were performed according to the manufacturer's instructions. Five grams portion of each sample were extracted by thoroughly shaking for I min. with 25 ml of sample extracted solution (4 parts of methanol with one part of deionized water) and then centrifuged at above 4000 r/min for 5 min . From the supernatant 100µl was taken and 300µl of 1XFB1diluent (provided with the kit) was added. Fifty µl were taken for test (Dilution factor:30).

**ELISA procedure:**
Fifty µl of enzyme conjugate were dispensed into each well of ELISA plate, then 50 µl of standards and samples were added to the appropriate test wells. Thereafter, Fifty µl of antibody solution was added and the mixture was incubated at 37°C for 20 min. in the dark. After incubation the mixture was dispensed, and 250 µl of wash solution was used to rinse each well. Rinsing with wash solution was repeated three times. Fifty µl of substrate A solution and 50 µl of substrate B solution were added to the wells and incubated at 37°C for 10 min. in the dark. Finally 50µl of stop solution was added to each test well. Absorbance was read at 450nm. using Epson LQ-300+II reader. Software for result analysis of ELISA was used.

**Statistical analysis:**
Fumonisin B$_1$ concentration in each sample was calculated using the Software supplied with the kit. Data were analyzed using Sigma stat 3.1program. Probability of 0.05 or less was considered significant.

**Results:**
**Percentages and level of fumonisin B$_1$ contamination in broiler feeds:**
The percentages of fumonisin B$_1$ contamination in all broiler feed samples for the 15 broiler flocks within 5 localities in Ninevah governorate are shown in table 1 below. It was found that 83.33% (50/60) of all broiler feed samples were contaminated with fumonisin B$_1$. The mean level of fumonisin B$_1$ in the positive and total broiler feed samples are shown in table 2. The mean fumonisin B1 concentration was 337.0± 69.68 µg/kg in the positive samples and 286.5± 56.69 µg/kg in the total broiler feed samples. There was no significant differences between fumonisin B1 concentration in the 5 localities in Ninevah governorate. The range of fumonisin B$_1$ level in the positive sample was between 102-1200 µg/kg.

**Food and Drugs Authority maximum accepted level of Fumonisin B$_1$ in broiler feed samples:**
Distribution of the fumonisin B1 levels across the concentration range is shown in figure 2. From Figure it is evident that 41.66 % of fumonisin B$_1$ levels were occurred between 0 and 200 µg/kg. In the second order (18.33%) were fumonisin B$_1$ concentrations ranging between 201 and 400 µg/kg. In the third order (15%) were fumonisin B$_1$ concentrations that occurred between 401 and 600 µg/kg. In the forth order (3.33%) were the concentration of 601-800 and 801-1000 µg/kg of fumonisin B$_1$ at a rate of (3.33%). In the last order were the...
concentration of ≥1200 µg fumonisin B₁/kg feed samples at a percentage of (1.66%). From FB₁ distribution results illustrated in figure 2, it is clear that all broiler feed samples (100%) had levels below the Food and Drugs Authority maximum accepted level in poultry feeds (<50 mg/kg) (Table 3).

<table>
<thead>
<tr>
<th>Locality</th>
<th>Number of broiler flocks</th>
<th>Total feed Samples</th>
<th>Number of positive feed samples</th>
<th>% +Ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>12</td>
<td>10</td>
<td>83.33</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>12</td>
<td>10</td>
<td>83.33</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>E</td>
<td>3</td>
<td>12</td>
<td>10</td>
<td>83.33</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>60</td>
<td>50</td>
<td>83.33</td>
</tr>
</tbody>
</table>

Table 2: Mean ± SEM for positive and total Fumonisin B₁ levels and Maximum of fumonisin B₁ positive broiler feed samples.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Number of broiler flocks</th>
<th>Total feed Samples</th>
<th>Mean ± SEM for +ve feed samples (µg/Kg)</th>
<th>Mean ± SEM for total feed samples (µg/Kg)</th>
<th>Maximum +ve Feed samples (µg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>12</td>
<td>358.333 ± 119.409</td>
<td>268.750 ± 94.830</td>
<td>1200</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>12</td>
<td>333.917 ± 63.522</td>
<td>333.917 ± 63.522</td>
<td>915</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>12</td>
<td>329.700 ± 57.109</td>
<td>274.750 ± 59.969</td>
<td>624</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>12</td>
<td>330.200 ± 46.150</td>
<td>275.167 ± 53.187</td>
<td>546</td>
</tr>
<tr>
<td>E</td>
<td>3</td>
<td>12</td>
<td>372.444 ± 62.211</td>
<td>279.375 ± 66.912</td>
<td>804</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>60</td>
<td>337.0 ± 69.68</td>
<td>286.5 ± 56.69</td>
<td>1200</td>
</tr>
</tbody>
</table>

Fig. 2: Distribution of the Fumonisin B₁ levels in broiler feed samples across the concentration range.

Table 3: Food and Drugs Authority maximum accepted level of Fumonisin B₁ (50 mg/kg).

<table>
<thead>
<tr>
<th>Locality</th>
<th>Number of broiler flocks</th>
<th>Total feed Samples</th>
<th>Level of fumonisin B₁ Contamination (%) of the total broiler feed samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>12</td>
<td>Recommended level (&lt;50 mg/Kg)</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>12</td>
<td>Medium contamination (50-100 mg/Kg)</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>12</td>
<td>High contamination (&gt;100 mg/Kg)</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>12</td>
<td>Recommended level (&lt;50 mg/Kg)</td>
</tr>
<tr>
<td>E</td>
<td>3</td>
<td>12</td>
<td>Medium contamination (50-100 mg/Kg)</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>60</td>
<td>Recommended level (&lt;50 mg/Kg)</td>
</tr>
</tbody>
</table>

Discussion:

Fumonisins are widely distributed geographically, and their natural occurrence in feeds has been reported in many areas of the world including the Middle East (Naehrer 2012a). A high percentage of fumonisin B₁ contamination (83.3%) in broiler feeds was reported here in this study. This is not a surprising result, since most corn-containing feeds are contaminated with fumonisins at detectable levels and even sound, whole corn
kernels may contain up to 10 µg/g fumonisins (Rheeder et al., 1992). A higher percentage of Fumonisin
recovery (72%) was the result of analysis carried out in the last 14 years at the Laboratório de Análises
Micotoxicológicas (LAMIC) and found that 75% of corn samples and 72% of feed samples were contaminated
with fumonisins (Mallmann, 2011). The percentage of fumonisin B1 recovered was higher than that reported
previously (51.15%) here in Ninevah governorate (Shareef, 2010). This increase in fumonisins recovery
percentage through these years was in the same trend of the results reported by Rodrigues (2012) in his survey
about the contamination of wheat (used as an alternative feed ingredient) through the years 2009-2110-2111,
which were 0.9 and 39% respectively.

The average concentration of fumonisin B1 in this study was 286.5 µg/kg, which was also higher than that
reported here in Ninevah governorate (127 µg/kg) before five years (Shareef 2009). The increase in fumonisins
concentration was also noticed through the years 2010-2011 from 217 µg/kg to 305 µg/kg in Asia, from 0
µg/kg to 1407 µg/kg in Americas respectively (Rodrigues, 2012). The increase trend in fumonisin recovery and
concentration in different parts of the world may be related to the recent global climatic changes making annual
monitoring mycotoxin programs an urgent issue.

However, the average level of fumonisin B1 here was in the line of that referred by Naehrer (2012a) and
Naehre, and Hofstetter (2012), who found that the average of fumonisins contamination in feeds and feed
ingredients in the Middle East through their two worldwide surveys were 290 µg/kg and 298 µg/kg
respectively.

Anyhow, these fumonisins concentration levels were <0.5 ppm, and so nearly about 100 fold less than the
level of fumonisins recommended by FDA for broiler feed (50 ppm) (FDA, 2001), and in the mean time less
than 200 fold the effective fumonisins level for broiler chickens (Ledoux et al., 1992; Brown 1992;
Espada1994, 1997). Fumonisin B1 concentrations obtained in this survey were in the same line of that referred
by Mallmann (2011), who stated that contamination levels in maize from different parts of the world are
normally below 5 ppm.

The low concentrations of fumonisins mycotoxins in broiler feed samples in the five locations of Ninevah
governorate, may be traced to the environmental conditions under which these toxins are formed. Fumonisins
are usually formed in corn only before harvest or during the early stage of drying by F. verticilloides and F.
proliferatum which grow over a wide range of environmental temperatures and at relatively high water activities
(above about 0.9), and the toxin concentration do not increase during grain storage except under extreme
conditions (Marin et al., 1995; Ono et al., 2002). The climatic conditions in Iraq during Jan.-Feb.-Mar. (Winter
months during which samples were collected) are characterized by an environmental temperature of about
~15°C and a relative humidity between 0.6-0.65. (El-Kuwaz, 2007), which were not optimal environmental
conditions for F. verticilloides and F. proliferatum growth and fumonisins production. This fact could be
supported by a wider range of fumonisins concentration in countries of more favorable tropical environmental
conditions were higher temperature and relative humidity are prevalent like India. In this country, Jindal et al.,
(1999), for example, found that poultry feed samples collected from Haryana contained fumonisin B1 at a range
of 20 µg/kg to 280 mg/kg.

Moreover, new constructed local feed mills in Ninevah governorate produce high quality pelleted finished
feeds supplemented with antifungal and antimycotoxin additives, aiding in more safer storage of feeds in poultry
farms with less chance of fungal growth and mycotoxins production.

It should be stressed that our findings did not mean that broiler sector in Ninevah governorate/Iraq is far
from fumonisin contamination risk, but further survey in indeed needed to be done regularly for monitoring
fumonisin recovery percentage and concentrations in addition to other mycotoxins in broiler feeds and other
feeds, which in case of their presence may affect poultry health and performance through their synergistic or
additive effects.

REFERENCES


reduction of Aflatoxins contamination in peanuts. FAO, Rome.


