ISOLATION AND CHARACTERIZATION OF FUNGI AND MYCOTOXINS (DEOXYNIVALENOL AND ZARALENONE) IN FISH FEED FROM BAGHDAD CITY.

Dalia K. Abdual-shahid* Oday S. Abbas** ZahidE. Mohammad***

*College of Veterinary Medicine–University of Baghdad .
** Iraqi Center for Cancer and Medical Genetics Research–University of Al-Mustansiriyah
*** College of Veterinary Medicine–University of Diyala.

ABSTRACT
This research provides a brief review of approaches for the early detection of fungi and its metabolites in feed of fish from some Baghdad farms. During a mycological analysis of complete feed mixes (15 samples), a total of five genera of moulds were identified. *Penicillium* spp. was present in considerably more samples than any other genus 36.4%, followed by the genera *Fusarium* spp. 24.5%. Other fungi from the genera *Aspergillus* spp. 20%, *Mucor* spp. 11.1% and *Alternaria* spp. 8% were represented in a smaller amount. The mycotoxins deoxynivalenol and zearalenone were detected. Deoxynivalenol was detected in 10 samples in the concentration range 0.25–2.5 mg/kg. Zearalenone was detected in 8 samples in the concentration range 0.2–5.0 mg/kg. These findings indicate that there may be a risk for animal exposure to mycotoxins through the consumption of moldy infected feeds.

Key words: *Fusarium* mycotoxins, Deoxynivalenol, Zearalenone, Fungal.

INTRODUCTION
The contamination of agricultural commodities with fungi able to produce toxic metabolites is a worldwide concern. Discoloration, quality deterioration, reduction in commercial value and mycotoxin production has been linked to moldy contaminated foods and feeds (Pardo et al., 2005). Mould contamination not only generates great economic losses, but also represents a threat to human and animal health, particularly through the synthesis of mycotoxins (Mishra and Sopori, 2012).

Mycotoxins are a structurally diverse group of mostly small molecular weight compounds, produced by the secondary metabolism of fungi that contaminate the whole food chain, from the harvested products to the plate of consumers. Mycotoxins occur sporadically both seasonally and geographically (Pestka, 2011). The main mycotoxin classes of concern produced by fungi in the genera *Aspergillus,* *Penicillium* and *Fusarium* include the aflatoxins, ochratoxin A, trichothecenes and fumonisins (Danicke et al., 2011). Environmental factors
such as nutrients, light, temperature, pH and water activity (aw), either as single factors or in combination, are known to control mycotoxin production in many filamentous fungi (Georgianna and Payne, 2009). These environmental factors typically exert their influence on mycotoxin biosynthesis at the level of gene transcription (Mostrom and Raisbeck, 2012). Limited information exists regarding the effects of low levels of multiple mycotoxins in fishes. It has been suggested that combination of mycotoxins at low concentrations may have negative effects on animals, even though the concentrations of individual mycotoxins are well below concentrations reported to cause negative effects. Therefore, rapid and specific detection of mycotoxigenic moulds is important for ensuring both microbiological quality and safety of both feed and food (Sultana and Hanif, 2011). Deoxynivalenol (DON) is one of the most common Fusarium based mycotoxins found in human foodstuffs. It is also called vomitoxin, because of its impact on farm animals consuming contaminated feed. DON is much more frequently found in barley, corn, sunflower, wheat, and compound animal feeds. The fungal pathogens producing DON cause ear rot in corn and head blight in wheat, two of the most common sources of DON in the feed supply. Rain during the flowering period in small grains clearly increases the risk of DON contamination (Mosse, 2012). Zearalenone (ZON) is a secondary metabolite of Fusarium species. Unlike other mycotoxins, zearalenone is virtually non-toxic to mammals following acute ingestion (Shekhany, 2008). Nonetheless, it is extremely potent in other ways, since it resembles a key hormone produced by human ovaries, 17β-estradiol, and as a result, can disrupt the human endocrine system (Dombroski, 2012). As little as 0.0001 ppm of zearalenone has been shown to create a detectable, hormone-related uterogenic response in females (Bennett and Klich, 2003). In other words, zearalenone exposure has been shown to impact reproductive processes at a dose that is 100 million times less than the lethal dose in a mice LD50 study (Mosse, 2012). Therefore, the aims of this research were to: Isolation of fungi and detection of mycotoxins (Deoxynivalenol and Zearalenone) in fish feeds.

MATERIAL AND METHODS

Sample collection
The research materials consisted of 15 representative fishfeed samples which were collected directly at fish farms from different parts of Baghdad city during a 2 month period. The samples (each about 1 kg) were stored at -4°C and analyzed the day after collection.

Material

Standards of zearalenone (ZON) and deoxynivalenol (DON) were purchased from Sigma. All others solvents and reagents were analytical grade.
Isolation and identification of fungal strains

Isolation and identification of fungal strains were done on solid media using the potato dextrose agar (PDA). Plates were incubated at 25°C for 7 days. Each isolated mould colony was observed microscopically for morphological characterization and identification to genera/species level. This was done by their macro- and micro morphology features using appropriate identification keys (Samson et al., 2002).

Extraction and Cleanup of Mycotoxins

The procedure described by Sreenivasa et al., (2009) was used for extraction and clean up of mycotoxins.

Mycotoxin analysis

Analysis of two mycotoxins in all samples was performed by High Performance Liquid Chromatography (HPLC). The optimized instrumental conditions are summarized in Table 1. A standard chromatogram of the Fusarium mycotoxins of major interest is shown in Figure 1. The mycotoxin concentration in the samples was calculated by comparing the area of chromatographic peak of the samples with that of the standard calibration curve by densitometry analysis.

Table 1. Instrumental conditions of HPLC.

<table>
<thead>
<tr>
<th>Instrumental conditions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LC Column</td>
<td>Shimadzu Shim 150 mm x 2 mm x 5 µm</td>
</tr>
<tr>
<td>Flow-rate</td>
<td>0.1 mL/min</td>
</tr>
<tr>
<td>Detection wavelength</td>
<td>230 nm</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 µL</td>
</tr>
<tr>
<td>Column temperature</td>
<td>40 °C</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>10 mM ammonium acetate – methanol</td>
</tr>
<tr>
<td>Time (minutes)</td>
<td>10 mM ammonium acetate %</td>
</tr>
<tr>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 1. Standard chromatogram of the deoxynivalenol (DON) and zearalenone (ZON) mycotoxins.

Statistical analysis
Differences in the mean levels of mycotoxin contamination across the two groups of positive samples were calculated by a Student’s t-test.

RESULTS AND DISCUSSION
The results obtained from the mycoflora analysis and incidence of mycotoxins contamination in the samples originating from the region where the samples were collected is presented in Table 2 and Figure 2.

Mycoflora analysis
The most frequently isolated fungus was Penicillium spp., a 36.4%. Other frequently isolated moulds included Fusarium spp. 24.5%, Aspergillus spp. 20%, Mucor spp. 11.1% and Alternaria spp. 8% were represented in smaller amount.

Figure 2. Percentage of moulds contamination fish feed.
Occurrence of mycotoxins in samples

The results obtained from the analysis of mycotoxins in the fish feed samples are presented in Table 2. The predominant mycotoxin for all analyzed samples was DON. The incidence of DON and ZON in all the samples was 51.7% and 48.3%, respectively figure 3. No significant differences were found between the median DON and ZON contents for all feed items (P 0.05).

Table 2. HPLC data for occurrence of mycotoxins in fish feeds.

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Positive samples (Incidence %)</th>
<th>Rang (mg/kg)</th>
<th>mean ± SD* (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DON</td>
<td>10 (51.7%)</td>
<td>0.25-2.5</td>
<td>0.78 ± 0.85a</td>
</tr>
<tr>
<td>ZON</td>
<td>8 (48.3%)</td>
<td>0.2-5.0</td>
<td>0.85 ± 1.416a</td>
</tr>
</tbody>
</table>

* SD Standard deviation
Means with same letters within the same column are no significantly differences.

Isolated species in our case are mostly storage contaminants, implicating that the high number of contaminated feed is most probably the result of manipulative mistakes (storage duration, temperature and humidity levels, etc.), during storage of feedstuffs or feed. Fungal colonization, growth and synthesis of toxins, results from the complex interaction of several factors (water availability, temperature and incubation time) and therefore, an understanding of each factor involved is essential for understanding the overall process and predicting fungal spoilage in agricultural and food products (Pardo et al., 2005). Improper storage accompanied by too high a temperature and elevated moisture content in the grain favour further mycotoxin production and lead to reduction in grain quality (Ramirez et al., 2004). It is well known that cereal infection with moulds and toxin production depends strongly on environmental conditions (damp climate, cool temperatures). However, these data must be interpreted with caution, as they were calculated from a limited number of
samples. The results of the mycoflora analysis carried out in this study are similar to previous results found by other authors (Stankovic et al., 2007; Milicevic, 2008). This shows that the incidence of these various species was important, as the produce was stored for prolonged periods of time. The main advantages of the HPLC technique include its general applicability to a broad range of compounds, high sensitivity and outstanding selectivity. Several methods already have been reported for the simultaneous determination of mycotoxins, which offer significant advantages over conventional techniques (Scarlett et al., 2012).

REFERENCES


Exploration of this investigation was carried out to identify various species and their mycotoxins produced in the food industry and animal feed. A total of 180 samples were collected from various sources in Baghdad city. An identification of mycotoxins was carried out using high performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS). The results showed that the most prevalent mycotoxins were deoxynivalenol, zearalenone, and fumonisins. The detection limits were found to be 0.1 mg/kg for deoxynivalenol and 0.2 mg/kg for zearalenone. The levels of fumonisins were found to be below the limit of detection.