Incidence of the Most Common Toxigenic Aspergillus Species in Broiler Feeds in Kermanshah Province, West of Iran

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INTRODUCTION

Mold and mycotoxin contamination of feed and feed ingredients occurs worldwide and because of the ubiquitous nature of these micro-organisms they cannot be totally eliminated from feeds and ingredients [1]. The presence of mold and mycotoxins in poultry feeds result from the raw material used in their production. Mold and mycotoxins contamination of the raw materials occur during the pre-harvest and/or the post-harvest periods. During these periods, temperature and humidity, as well as processing and handling of animal feed play an important role in the growth of fungi and mycotoxins contamination [2,3]. In general, the mixed feeds of poultry constitute corn and soybean as major ingredients, which represent an excellent substrate for growth and reproduction of numerous fungi, under favorable conditions such as high moisture and increased temperature [4]. When long-term physiological and environmental conditions for fungal growth are provided, mycotoxins are produced, which can not be removed from the feed completely [4]. Fungal contamination is undesirable because of the potential for mycotoxin production [5]. Fungal toxins can be stored in meat, milk and egg and finally transferred to human beings [6].

Most species of Aspergillus and Penicillium are able to grow on a wide range of organic substrates. They are essentially saprophytic and are particularly associated with stored moldy plant products [7, 8]. Aspergillus genera is the most important toxigenic fungi [9, 10].

Abstract: The objectives of this study were to investigate the occurrence and identification of Aspergillus species in broiler feeds in Kermanshah province, west of Iran. From April 2008 to March 2009, a total of 50 samples of broiler feeds were collected from different broiler farms located in Kermanshah province. All samples were aseptically transported to the laboratory, homogenized, quartered to obtain a 1 kg laboratory sample and were stored at 4°C for fungal analyses. Ten grams of each feed sample were homogenized in 90 ml sterile physiological saline for 30 minutes to obtain a concentration of \(10^0\) (dilution 1). This mixture was then serially diluted to \(10^{-1}\) (dilution 2), \(10^{-2}\) (dilution 3) and \(10^{-3}\) (dilution 4). From each dilution, 25 µL of mixture was deeply inoculated on dichloran rose-bengal-chloramphenicol agar (DRBC) and incubated at 30°C for 15 days. During incubation period, gross and microscopic features of fungal colonies were studied. Out of 50 feed samples, 46 samples (92%) in dilution 1, 38 samples (76%) in dilution 2, 28 samples (56%) in dilution 3 and 20 samples (40%) in dilution 4, were contaminated to Aspergillus species. The most prevalent species was Aspergillus flavus followed by Aspergillus niger and Aspergillus fumigatus. These results showed that a potential exists for the production of mycotoxins by the Aspergillus species. They suggest an association of mycotoxicosis with poultry feeds in western parts of Iran.

Key words: Aspergillus • Poultry feed • Broiler farms • Kermanshah province
Poultry are highly susceptible to mycotoxicoses and mycosis [10-12]. Aspergillosis is an increasingly common ubiquitous fungal infection of birds and occasionally other animals including man. *Aspergillus fumigatus* is the most commonly isolated species from the cases of aspergillosis, followed by *Aspergillus flavus* and *Aspergillus niger* [13].

At present, aflatoxins are considered to be one of the most toxic, carcinogenic compounds produced by several members of the *Aspergillus flavus* in foods and feeds [14, 15]. Therefore, throughout the world great attention is paid to investigation on *Aspergillus* species and elaborating means for controlling them [17]. Data on the microbiota and mycotoxins from poultry feeds in Iran are scarce. For this reason, our aim in this study was to isolate and identify the *Aspergillus* species found as contamination in broiler feeds.

**MATERIALS AND METHODS**

From April 2008 to March 2009, a total of 50 feed samples were taken randomly from commercial broiler farms in Kermanshah province. All samples were aseptically transported to the laboratory, homogenized, quartered to obtain a 1 kg laboratory sample and were stored at 4°C for fungal analyses [16]. Ten grams of each feed sample were homogenized in 90 ml sterile physiological saline for 30 minutes and serial dilutions of $10^{-1}$ (Dilution1), $10^{-2}$ (dilution 2), $10^{-3}$ (dilution 3) and $10^{-4}$ (dilution 4) were made. Then, 25 µL of each dilution was deep point inoculated on dichloran rose-bengal-chloranphenicol agar [17] and incubated at 30°C for 15 days. Fungal colonies were selected for identification, according to the methods proposed for the genus [18]. The distinct colonies were picked, subcultured for purification and characterized using standard techniques [19]. Wet mount smears and slide cultured colonies were stained with lactophenol cotton blue. Taxonomic identification of the fungi was made based on macroscopic and microscopic features in accordance with appropriate keys [17]. Identification of *Aspergillus* species were made as per Raper and Fennell [20]. Statistical analysis of data was performed using SPSS software (Version 16) with 95 percent accuracy. A P-value less than 0.05 was considered significant.

**RESULTS**

Mycological survey of 50 samples of broiler feed from western parts of Iran showed the presence of potentially toxigenic and infective *Aspergillus* species (Table 1, Figures 1-3). Out of 50 feed samples from each dilution, 46 samples (92%) in dilution 1, 38 samples (76%) in dilution 2, 28 samples (56%) in dilution 3 and 20
Table 1: The occurrence of Aspergillus species isolated from 50 broiler feed samples in west of Iran

<table>
<thead>
<tr>
<th>Dilution</th>
<th>A. flavus</th>
<th>A. niger</th>
<th>A. fumigatus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>34</td>
<td>73.9</td>
<td>10</td>
<td>21.7</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>68.4</td>
<td>10</td>
<td>26.3</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>78.5</td>
<td>4</td>
<td>14.2</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>70.0</td>
<td>4</td>
<td>14.2</td>
</tr>
</tbody>
</table>

Figures with different superscripts within rows are significantly different (p<0.05)

Aspergillus species (A. niger and A. fumigatus) in 4 dilutions (p <0.05), similar results obtained from mycological survey on feed ingredients and mixed animals in Ghom province (central parts of Iran) were reported by Khosravi et al. [26].

A. flavus was the most prevalent species. This result agrees with Adejumo et al. Dalcer et al. [27, 28]. Magnoli et al. [3] and Accensi et al. [29]. Dutta and Das [4] confirmed the predominance of A. flavus over A. parasiticus in poultry feeds. The majority of these genera representatives such as A. flavus are thermophilic and thermo-resistant and distribute abundantly in tropical to subtropical climates [30]. Lacey and Magan [21] showed that the ideal temperature concerning growth and mycotoxin production ranges 25 to 35°C for A. flavus strains [7, 8]. The average annual range of temperatures in western parts of Iran varies from 21 to 28°C, but is generally more than 24°C indicating favorable condition for A. flavus growth [31, 32].

Among the Aspergillus species isolated from broiler feeds, A. niger was the second most prevalent species. This is in agreement with Osho et al. [29] who reported that out of the 50 samples collected from various commercial poultry farms located in southwest Nigeria, A. niger was one of the common fungi found in the feeds [10]. Rhizopus spp. had the highest frequency of occurrence (44%), Fusarium spp. 42%, A. flavus 40%, occurrence and A. niger, (38%).

The occurrence of Aspergillus species in broiler feed is particularly important because there are known as the most toxigenic among the fungi. Most studies indicate that there is no correlation between the presence of a toxin and the producing fungus in the same substrate, but the presence of toxigenic fungi in feeds may be an indicative of their potentiality to produce mycotoxins. When the storage conditions are not appropriate and the toxigenic fungus is present, this may be able to produce a mycotoxin [33].

Mycological survey of 50 samples of broiler feed from western parts of Iran showed the presence of potentially toxigenic and infective Aspergillus species (Table 1). Many researchers have proved that the majority of feeds have species from Aspergillus and Penicillium genera as a predominant flora; Bragulat et al., (1995) reported a high frequency of Aspergillus species in mixed poultry feeds [21]. Magnoli et al. [25] reported a high frequency of Aspergillus group species found in poultry feeds from Argentina [3]. They reported that Aspergillus flavus and A. parasiticus, which are important aflatoxin producers, were the predominant species isolated. Glenda, et al. [11] reported that Aspergillus and Penicillium species had the highest isolation frequencies followed by Fusarium spp in poultry feeds in Brazil. Many studies have shown that most feeds have species from Aspergillus and Penicillium genera as predominant flora [21, 22].

In the present study, the main contaminating fungus appeared to be A. flavus, a potentially toxigenic species for the aflatoxins. Likewise, the species has shown high occurrence frequency in the studies of Labuda and Tancinova [3], Heperkan and Alperden [23], Magnoli et al. [24] and Dalcer et al. [25] and There was a significant difference between the rate of contamination to Aspergillus flavus and other isolated Aspergillus species (A. niger and A. fumigatus) in 4 dilutions (p <0.05).
The results of this study showed that the broiler feeds in Kermanshah province were highly (92% in dilution 1 and 40% in dilution 4) contaminated with *Aspergillus* species which are the most common toxigenic fungi found in feeds. This study warrant the need for analyzing the samples for *Aspergillus* mycotoxins, especially aflatoxins and also to design effective management strategies to prevent contamination of poultry feed to *Aspergillus species* and *Aflatoxin*. The study highlights a potential risk of poultry feeds getting contaminated with hazardous toxic compound and potentially infective *A. fumigatus*, thus making it for further analysis and continual monitoring and evaluation of feeds.

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**REFERENCES**


