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The Frequency of the Most Potentially Toxigenic Fungi in Broiler Feeds in Kermanshah Province, West of Iran

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Abstract: The objectives of this study were to investigate the occurrence and identification of the most potentially toxigenic fungi in broiler feeds in Kermanshah province, west of Iran. From April 2008 to March 2009, a total of 100 broiler feed samples were aseptically collected from different broiler farms located in the province. Samples were transported to the laboratory, homogenized, quartered to obtain a one 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^{-1} (dilution 1). Then this mixture was serially diluted to 10^{-2} (dilution 2), 10^{-3} (dilution 3) and 10^{-4} (dilution 4). From each dilution, 25µL of the mixture was deeply inoculated on *dichloran rose-bengal-chloranphenicol agar* (DRBC) and incubated at 25°C for 5 to 15 days. During the incubation period, gross and microscopic features of fungal colonies were studied. Statistical analyses of the data were done using *SPSS* software (Version 16). *Aspergillus Spp.* were the most prevalent; they could be isolated from 92 samples (51.1%) in dilution 1, 76 samples (59.3%) in dilution 2, 56 samples (66.6%) in dilution 3 and 40 samples (83%) in dilution 4. *Fusarium spp.* Were isolated from 52 samples (28.8%) in dilution 1, 28 samples (21.8%) in dilution 2, 20 samples (23.8%) in dilution 3 and 8 samples (16%) in dilution 4. *Penicillium spp.* Could be isolated from 36 samples (20%) in dilution 1, 24 samples (18.7%) in dilution 2, 8 samples (9%) in dilution 3 and 0 samples (0%)) in dilution 4. The results of this study indicate the high rate of contamination of broiler feeds to common toxigenic fungi in western parts of Iran.

Key words: Toxigenic fungi · *Mycotoxins* · Broiler feed · Kermanshah province

INTRODUCTION

Mold and mycotoxin contamination of mixed 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 [5]. Fungal contamination is undesirable because of the potential for mycotoxin production [6]. Poultry are highly susceptible to mycotoxicosis and mycosis [7-10]. Fungal toxins can be stored in poultry meat and egg and finally transferred to human beings[11].

Most species of *Aspergillus* and *Penicillium* are able to grow on a wide range of organic substrates. They are

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essentially saprophytic and are particularly associated with stored moldy plant products [12]. Members of *Aspergillus* and *Penicillium genera* have been implicated in the production of a wide range of *mycotoxins*, *Aspergillus* genera is the most important toxigenic fungi [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]. 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* [10].

Several *Penicillium and Fusarium spp.* associated with field contamination and development are responsible for the production of a number of other mycotoxins, the most important being *cyclopiazonic acid, patulin, citrinin, penicillic acid deoxynivalenol* (DON), *zearalenone* and the *fumonisins* [16-21].

Feeds and feedstuffs are excellent media for the growth of fungi and so, very high standard of hygiene is necessary to avoid feed contamination. One of the best ways to control feed contamination and mycotoxin problem is to investigate frequency of fungi genera in feeds. Few studies on fungal contamination of poultry feeds are available in Iran, so in this study the incidence of most potentially toxigenic fungi (*Aspergillus spp, fusarium spp* and *penicillium spp*) in broiler feeds was investigated.

MATERIALS AND METHODS

In this study from March 2008 to April 2009, a total of 100 feed samples were taken randomly from commercial broilers 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. Ten g from 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. 25µL of each dilution was deep point inoculated on dichloran *rose-bengal-chloranphenicol agar* [22, 23] and incubated at 25°C for 5 to 15 days. Fungal colonies were selected for identification, according to the methods proposed for the genus [24]. The distinct colonies were picked, subcultured for purification and characterized using standard techniques [25]. 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 [23]. Identification of *Aspergillus* species was made as per Raper and Fennell [29]. Statistical analysis of data was performed using SPSS software (Version 16) with 95 percent accuracy. A P-value less than 0.05 were considered significant.

RESULTS

The count of isolated genera fungi was defined as the percentage of samples in which each fungus was present. The frequency of isolated fungi from broiler feed samples in Kermanshah province is shown in Table 1. *Aspergillus Spp.* were the most prevalent; out of 100 feed samples, they could be isolated from 92 samples (51.1%) in dilution 1, 76 samples (59.3%) in dilution 2, 56 samples (66.6%) in dilution 3 and 40 samples (83%) in dilution 4. *Fusarium spp.* Were isolated from 52 samples (28.8%) in dilution 1, 28 samples (21.8%) in dilution 2, 20 samples (23.8%) in dilution 3 and 8 samples (16%) in dilution 4. *Penicillium spp.* Could be isolated from 36 samples (20%)

Fig. 1: *Fusarium spp* isolated from broiler poultry feeds in Kermanshah province, Iran

Fig. 2: *Aspergillus niger* isolated from broiler poultry feeds in Kermanshah province, Iran

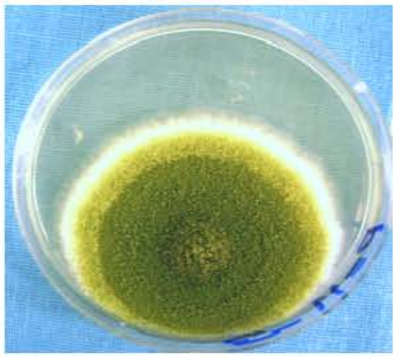

Fig. 3: *Aspergillus flavus* isolated from broiler poultry feeds in Kermanshah province, Iran

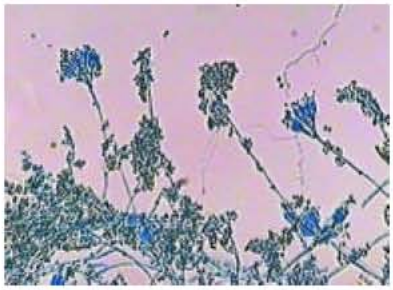

Fig. 4: *Penicillium spp* isolated from broiler poultry feeds in Kermanshah province, Iran

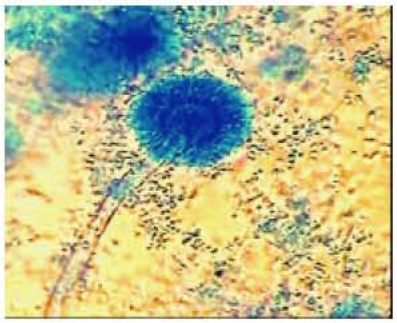

Fig. 5: *Aspergillus flavus*, isolated from compounded broiler feeds in west of Iran

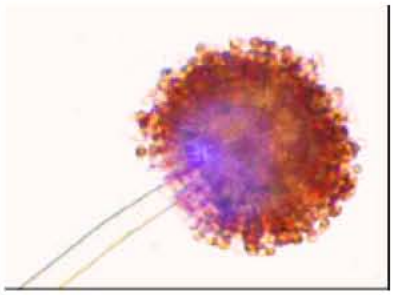

Fig. 6: *Aspergillus niger*, isolated from compounded broiler feeds in west of Iran

Fig. 7: *Aspergillus fumigatus* isolated from compounded broiler feeds in west of Iran

in dilution 1, 24 samples (18.7%) in dilution 2, 8 samples (9%) in dilution 3 and 0 samples (0%) in dilution 4. Among *Aspergillus spp* in all dilutions the highest contamination rate belonged to *Aspergillus flavus*, followed by *Aspergillus niger* and *Aspergillus fumigatus* (Table 2).

DISCUSSION

The present study revealed that *Aspergillus spp, Fusarium spp* and *Penicillium spp* were the most common moulds growing in commercial poultry feeds in Kermanshah province west of Iran. Similar results relating to presence of certain fungi genera in animal feed are

Table 1: Frequency of isolated potentially toxigenic fungi from broiler feed during 2008-2009 in Kermanshah province, west of Iran

	Aspergillus spp.		*Fusarium spp.*		*Penicillium spp.*		Total	
Dilutions	Number	%	Number	%	Number	%	Number	%
1	92	51.1^{a}	52	28.8^{a}	36	20^{b}	180	100
2	76	59.3^{a}	28	21.8^{b}	24	18.7^{b}	128	100
3	56	66.6^{a}	20	23.8^{b}	8	9^{c}	84	100
4	40	83^{a}	8	16^{b}	0	0^{c}	48	100

^{a,b,c} Figures with different superscripts within rows are significantly different ($p<0.05$)

Table 2: Frequency of *Aspergillus species*. isolated from broiler feeds during 2008-2009 in Kermanshah province, west of Iran

	A. flavus		*A. niger*		*A. fumigatus*		Total	
Dilutions	Number	%	Number	%	Number	%	Number	%
1	68	37.7^{a}	20	11.1^{b}	4	2.2^{c}	92	51.1
2	52	40.6^{a}	20	15.6^{b}	4	3.1^{c}	76	59.3
3	44	52.3^{a}	8	9.5^{b}	4	4.7^{b}	56	66.6
4	28	58.3^{a}	8	16.6^{b}	4	8.3^{b}	40	83

^{a,b,c} Figures with different superscripts within rows are significantly different ($p<0.05$)

stated by other authors, mainly these are genera *Aspergillus*, *Fusarium* and *Penicillium* [26-28]. Many studies have shown that most feeds have species from *Aspergillus, Fusarium* and *Penicillium* genera as predominant flora [32-35]. Economically, the presence of these fungal genera has been reported to overwhelmingly affect the viability of some animal husbandry undertaking and agriculture in general [30, 31].

Aspergillus and *Fusarium* were the most prevalent genera isolated; these results were similar to those reported by other researchers [36]. However, they are in contrast with others who found *Fusarium* as the predominant genus, followed by *Aspergillus* and *Penicillium* [37]. Isolation of *Fusarium* with species adapted to a wide range of habitats throughout the world is very important, because *fusarium spp.* are potentially toxygenic species and the most frequent producers of different and very dangerous mycotoxins (*zearalenons, trichothecens, fumonisins*) in animal feed [38].

There was a significant difference between the occurrence of *Aspergillus* species and *Fusarium* species ($p<0.05$). These genera were found the most frequently in animal feeds [39 -41] ~" and had the highest individual counts [42]. The expression of about three different fungal species, representing both field and storage fungi and the occurrence of species of *Aspergillus*, *Fusarium* and *penicillium* in higher percentage is particularly important, because these are known to be toxin producers [13].

Aspergillus genera were the most prevalent in dilution 1 (%51.1) and in dilution 4 (%83), this finding is in agreement with Pitt and Hocking [44] and Zimmerli and Dick [52] who had earlier established *Aspergillus* genera predominance over other genera in tropical environments. The occurrence of *Aspergillus* species in broilers 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 [45, 46].

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 [5], Accensi *et al.* [32] Adebajo *et al.* [33], Magnoli *et al.* [34], *Dalcero et al.* [47], Heperkan and Alperden [19], Dalcero *et al.* [14], and Khosravi *et al.* [49]. Within *Aspergillus* species in dilutions 1, 2, 3 and 4, the highest contamination rate belonged to *Aspergillus flavus* (37.7, 40.6, 52.3 and 58.3%, respectively), followed by *Aspergillus niger* (11.1, 15.6, 9.5 and 16.6%, respectively) and *Aspergillus fumigatus* (2.2, 3.1, 4.7 and 8.3%, respectively). There was a significant difference between the contamination rate to *Aspergillus flavus* and the other isolated *Aspergillus s*pecies ($p<0.05$). It is suggested that the majority of this genus representatives such as *A. flavus* are thermophilic and thermo-resistant and distribute abundantly in tropical to subtropical climates [50]. Lacey and Magan [12,15] showed that the ideal temperature concerning growth and mycotoxin production ranges 25 to 35°C for *A. flavus* strains. The average annual range of temperatures in western parts of Iran varies from 21 to 28°C, but is generally more than 24°C. Badripour [51], indicating favorable condition for *A. flavus* growth.

The results of this study showed that the broiler feeds in Kermanshah province were highly contaminated to *Aspergillus* species in dilution 1 (51%) and dilution 4 (83%), *Fusarium* spp. in dilution 1 (28.8%) which are the most common toxigenic fungi found in feeds. This study reveals the mycobiota present in poultry feed samples and the natural occurrence of common toxigenic fungi and warrants the need for analyzing the samples for fungi mycotoxins, especially, *aflatoxin, zearalenone, trichothecens* and *fumonisins* and also to design effective management strategies to prevent contamination of poultry feeds to potentially toxigenic fungi. The study highlights a potential risk of poultry feeds getting contaminated with hazardous toxic compound, thus making it for further analysis and continual monitoring and evaluation of poultry feeds.

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