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A Mycological Survey on Feed Ingredients and Mixed Animal Feeds in Ghom Province, Iran

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Abstract: Feed contamination to fungi can lead to nutrient losses and detrimental effects on animal health and production. The purpose of this study was to investigate the mycoflora of 32 feed samples representing 10 types of animal feed ingredients, which included corn seed, corn silage, cottonseed meal, dried bread, barely, straw, hay, bran, mixed-feed and concentrate, in Ghom province, Iran during a one-year study. The most dominant species isolated of animal feed samples belonged to the genera *Aspergillus* (56%), *Mucor* (17%), *Penicillium* (15%), *Fusarium* (6%), *Cladosporium* (2%) and yeast (4%). From *Aspergillus* genus, three species were identified and *Aspergillus flavus* was the most frequent (48%). The highest fungi were detected from barely (17.6%). In all samples, the toxigenic (*Aspergillus*, *Fusarium*, *Penicillium*) and non-toxicogenic fungi were prevailed in 67% and 33%, respectively, representing significant difference between two groups ($p < 0.05$). Regarding to presence of highly toxigenic fungi on the feeds, it should be considered to plan a program for identifying fungi in order to hygienic control of fungi on feeds into the future.

Key words: Mycoflora, animal feeds, toxigenic fungi, *Aspergillus flavus*

Introduction

Fungal contamination of animal feeds, with the consequent mycotoxin production, is one of the major threats to human and animal health (Castillo *et al.*, 2004). Livestock feed quality may however be affected by various microorganisms such as bacteria and fungi growing in different parts of the worlds. Cereals, concentrate, hay and the other animal feeds have been reported as substrates for fungal growth and mycotoxin production (Scudamore and Patel, 2000). The contamination may occur during processing and storage of harvested products and feeds whenever environmental conditions are appropriate for spoilage fungi. Moisture content and ambient temperature are key factors affecting fungal colonization and mycotoxin production in grains and compound feeds (Okoli, 2005). Mycotoxins are secondary metabolites that have adverse effects on human, animals and crops, resulting in illness and economic losses (Hussein and Brasel, 2001). They include aflatoxins, fumonisins, trichothecenes and zearalenone that produce by some toxigenic fungi such as *Aspergillus*, *Penicillium* and *Fusarium* in tropical or warmer parts of the world (Abarca *et al.*, 2001; Rosa *et al.*, 2002). In farm animals, mycotoxins have negative effects on feed intake, animal performance, reproductive rate, growth efficiency and immunological defense as well as been carcinogenic, mutagenic, teratogenic, tremorgenic, damage the central nervous system, liver and kidneys (Ratcliff, 2000; Abbasa *et al.*, 2006). It is well established that contamination with mycotoxins of animal feeds may induce sanitary disturbances and mortality among the

various animals and secondary contamination of the human consumer via eggs, meat and milk (Nyamongo and Okioma, 2005). Assessing the significance of toxigenic fungi in animal feeds remains a difficult and challenging problem. Overall, for quality control, the identification of the contaminating mycoflora is essential because it provides data on the potential production of its toxins and is helpful indicator to determine a feeds hygienic quality, but few studies on fungal contamination of animal feeds are available in Iran. Therefore, the aim of this paper is to determine the toxigenic and non-toxicogenic mycoflora occurrence in animal feeds in a tropical climate in Ghom province, Iran.

Materials and Methods

Sampling method: About 450 g different samples of animal farms of suspected or potential mycotoxicosis were collected in Ghom province, Iran from May 2006 to May 2007. These samples included corn seed, corn silage, cottonseed meal, dried bread, barely, straw, hay, bran, mixed-feed and concentrate (32 samples from 32 husbandries). The samples were kept in plastic bags, transferred to Mycology Research Center of University of Tehran and stored at 4°C until analysis.

Culture and fungal identification: One gram of each sample was added to 10 mL sterile distilled water and kept constantly at room temperature for approximately 60 min. Then, 100 µL of supernatant solution was spread on the surface of four solid media: Sabouraud glucose agar, Dichloran rose-bengal agar (Sigma, St. Louis, USA), Potato dextrose agar and Czapek-dox agar. The

Table 1: The frequency of isolated fungi from animal feeds during 2006-2007 in Ghom province, Iran

Ingredient	Fungal isolates									
	<i>A. flavus</i>	<i>A. fumigatus</i>	<i>A. niger</i>	<i>Aspergillus</i> spp	<i>Fusarium</i>	<i>Penicillium</i>	<i>Cladosporium</i>	<i>Mucor</i>	Yeast	Total
Corn seed	6 (2.8)	0 (0)	3 (1.4)	0 (0)	10 (4.7)	0 (0)	0 (0)	5 (2.3)	0 (0)	24 (11.5)
Cottonseed meal	3 (1.4)	3 (1.4)	2 (1)	1 (0.5)	0 (0)	5 (2.3)	0 (0)	3 (1.4)	0 (0)	17 (8)
Dried bread	7 (3.3)	2 (1)	3 (1.4)	3 (1.4)	0 (0)	4 (1.9)	0 (0)	4 (1.9)	0 (0)	23 (11)
Barely	3 (1.4)	2 (1)	2 (1)	2 (1)	4 (1.9)	0 (0)	0 (0)	4 (1.9)	20 (9.5)	37 (17.6)
Straw	3 (1.4)	6 (2.8)	3 (1.4)	1 (0.5)	0 (0)	5 (2.3)	0 (0)	3 (1.4)	0 (0)	21 (10)
Hay	4 (1.9)	5 (2.3)	1 (0.5)	2 (1)	0 (0)	3 (1.4)	0 (0)	5 (2.3)	0 (0)	20 (9.5)
Bran	3 (1.4)	3 (1.4)	3 (1.4)	1 (0.5)	0 (0)	0 (0)	0 (0)	3 (1.4)	10 (4.5)	23 (11)
Mixed-feed	6 (2.8)	0 (0)	0 (0)	0 (0)	0 (0)	4 (1.9)	0 (0)	6 (2.8)	0 (0)	16 (7.7)
Corn silage	5 (2.3)	0 (0)	0 (0)	0 (0)	5 (2.3)	5 (2.3)	0 (0)	0 (0)	0 (0)	15 (7)
Concentrate	5 (2.3)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1.4)	3 (1.4)	3 (1.4)	0 (0)	14 (6.7)
Total	45 (21.5)	21 (10)	17 (8)	10 (4.7)	19 (9)	29 (14)	3 (1.4)	36 (17)	30 (14.5)	210 (100)

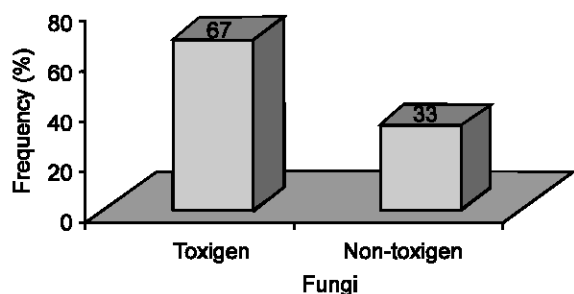


Fig. 1: The frequency of toxic and non-toxic fungi from animal feeds during 2006-2007 in Ghom province, Iran

cultures were incubated for 5 to 14 days at 25°C. Taxonomic identification of the different genera and species was made according to microscopic criteria in accordance with appropriate keys (Pitt and Hocking, 1997). All chemicals used, unless otherwise stated, were obtained from Merck Company (*Darmstadt, Germany*).

Statistical analyses: Unpaired Student's t test was performed using SPSS software (Version 13). A P value less than 0.05 were considered significant.

Results

The mycoflora associated with 32 animal feed samples collected from different husbandries of Ghom province was shown in Table 1. Among the isolated fungi, five filamentous genera were obtained. *Aspergillus* genera (56%) were the most prevalent, followed by *Mucor* (17%), *Penicillium* (15%), *Fusarium* (6%), *Cladosporium* (2%) and yeast (4%) species. There was a significant difference between *Aspergillus* species and the other fungi ($p < 0.05$). Among the *Aspergillus* species, *A. flavus* (48%), *A. fumigatus* (23%), *A. niger* (18%) along with some unidentified *Aspergillus* species (11%) were characterized. The frequency of isolated fungi in different feed ingredients were as follows: barely (17.6%), corn seed (11.5%), dried bread (11%), bran (11%), straw

(10%), hay (9.5%), cottonseed meal (8%), mixed-feed (7.7%), corn silage (7%) and concentrate (6.7%). Filamentous fungi were mostly isolated from concentrate, corn silage and dried bread samples, whereas yeasts were detected abundantly from barely and bran. As shown in Fig. 1, the toxic (*Aspergillus*, *Fusarium*, *Penicillium*) and non-toxic fungi were prevailed in 67% and 33%, respectively, representing significant difference between two groups ($p < 0.05$).

Discussion

Quality livestock feed is necessary for the maintenance of physiological functions and animal defense systems against diseases and parasites. Traditionally, feed quality has been specified on basis of the nutritional value of every individual feed component (Fink-Gremmels, 2004). In the present investigation, the total of 320 samples collected from tropical region of Ghom province representing different popular fungi were determined for the first time. The expression of about six 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 (Bankole and Kpodo, 2005). These results are similar to those obtained by other researchers (Domsch and Gams, 1980; Kurata and Ueno, 1984; Marsilio and Spottie, 1987; Bragulat *et al.*, 1995; Accensi *et al.*, 2004). *Aspergillus* species increased over all studied sampling periods. This finding is also in agreement with Pitt and Hocking (1997) and Zimmerli and Dick (1996), who had earlier established *Aspergillus* genera predominance over other genera in tropical environments. In addition, *A. flavus* predominates in all kinds of feed ingredients under any storage conditions in our tropical climate. This result agrees with the findings of Adebajo *et al.* (1994), Dalcero *et al.* (1998), Magnoli and Dalcero (2002) and Accensi *et al.* (2004) obtained the same *A. flavus* isolation frequencies. 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 (Lebars-Bailly *et al.*, 1999). In a previous report, Lacey and Magan (1991) showed that the ideal temperature concerning growth and mycotoxin production ranges from 22 to 28°C for *F. moniliforme* strains and 25 to 35°C for *A. flavus* strains; temperature values recorded in the our region about 30 to 40°C, indicating favorable condition for *Aspergillus* growth. Fungal contamination is undesirable because of the potential for mycotoxin production. In this regard, spores from toxigenic fungi such as *A. flavus* and *Fusarium* species may be inhaled or consumed by animals with deleterious effects termed mycotoxicosis. Also, the other isolated fungi such as *Mucor* and *Cladosporium* species may cause mycotic abortion and allergy in animals and human as a result of systemic and respiratory transmissions, respectively (Rippon, 1988). Present results indicated that fungal contaminations were found in a variety of feeds. In this case, barely (17.6%), corn seed (11.5%) and dried bread (11%) were highly infested with toxigenic fungi. It seems that activity water (aw), pH and the nature of the substrate (higher in carbohydrate contents) have a selective influence on the mycoflora of the above-mentioned ingredients (Jay, 1986; Adams, 1987). In this regard, two routes have been postulated for barely and corn contamination with toxigenic fungi. According to the reports, spores originating from the previous harvest, weeds and grasses could be colonized on the above ingredients in the presence of adequate moisture and temperature as well as insects and birds would damage the grains and fungal spores brought by wind would fall upon the exposed ingredients, thereby promoting contamination (Abamuslum *et al.*, 2003). In the latter case, moisture is a less important factor. Present study suggests that the second route is the most likely one and also to lesser extent these contaminations could be due to long-term storage of feeds in the poor environmental conditions in barns. Although the detection of toxigenic fungi in a substrate does not necessarily indicate that mycotoxins are naturally occurring in the field, it alerts to the potential risk of contamination (Pitt, 2000). So, the present study warrants the need for constant monitoring of fungal growth with special reference in major feed and crops such as corn, hay, etc., which is harvested, processed and stored in the same agro-climatic conditions and need to routinely inhibit their growth.

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