International Scholars Journals

African Journal of Environmental and Waste Management ISSN 2375-1266 Vol. 8 (1), pp. 001-006, January, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Occurrence of aflatoxigenic fungi in cow feeds during the summer and winter season in Hamadan, Iran

S. A. Ghiasian and A. H. Maghsood

Department of Medical Parasitology and Mycology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.

Accepted 17 October, 2020

Aflatoxins are secondary toxic metabolites produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* that contaminate food and feed. Due to the high incidence of AFM_1 in milk that have been reported at the Hamadan region in Iran, a study was conducted to identify the cow feed mycoflora with special respect to aflatoxigenic fungi. 186 cows feed samples from traditional- and industrial-dairy farms of Hamadan region were examined using dilution plating technique in summer and winter seasons. The predominant fungi isolated were *Aspergillus* species (37.4%) (*Aspergillus clavatus, A. flavus, Aspergillus fumigatus, Aspergillus* nidulans, *Aspergillus niger, Aspergillus ochraceous, A. parasiticus, Aspergillus* terreus, *Aspergillus* ustus**) followed by, *Penicillium* (23.7%), *Fusarium* (17.5%), *cladosporium* (9.1%), *Alternaria* (4.3%), *Rhizopus* (3.9%) and *Mucor* species (3.4%) . The concentrate feed was the most contaminated feed, for which the mean colony counts for *A. flavus* and *A. parasiticus* were 7.25×10^2 and 7.50×10^2 cfu/g, respectively. The most contaminated feed with these two Aspergilli was concentrate feed (80%) in summer and wheat bran (45%) in winter. The mean colony count of aflatoxigenic fungi in industrial-diary farms was significantly higher than traditional-dairy farms. Moreover, the mean of aflatoxigenic fungi colony count in winter was significantly greater than those in summer seasons ($p < 0.00001$). The present study tested the hypothesis that differences exist between fresh forages used in summer and the ensiled form used in winter in terms of contamination with aflatoxigenic fungi.**

Key words: Animal feed, *Aspergillus*, aflatoxigenic, food contamination, mycoflora, Iran.

INTRODUCTION

The presence and growth of fungal propagules in food and feed (spoilage) is a matter of great concern, because this spoilage may be pursued by difficulties such as allergy, human and animal infection and mycotoxin production. Depending on environmental factors, most of the foods and feeds have the potential for being contaminated with certain fungi and subsequently mycotoxins. Contamination of feed and the raw materials with mould fungi and mycotoxins can occur during the pre- and the post-harvest periods respectively by field fungi and storage fungi (Magan and Olsen, 2004). Species of *Aspergillus*, *Penicillium*, *Rhizopus* and *Cladosporium* are the major members of storage fungi (Maciorowski et al., 2007) . *Aspergillus* species are the main contaminants of food and feed of which *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius, Aspergillus tamarii* and *Aspergillus pseudotamarii* are the main species capable of producing aflatoxins and other mycotoxins, although some species of genera *Penicillium* and *Rhizopus* are also toxigenic and able to produce aflatoxins (Murphy et al., 2006).

The carcinogenicity of many mycotoxins, especially those commonly encountered, has been well recognized for numerous animal species and humans (IARC, 2002). Depending on age, sex, stress, species and health position of animals, the signs of the various mycotoxicoses are different and mostly include liver

*Corresponding author. E-mail: s.a.ghiasian@umsha.ac.ir. Tel. +98- 811-827-6298. Fax: +98- 811- 827-6299.

Abbreviations: AF, Aflatoxin; **TDF,** traditional-dairy farms; **IDF,** Industrial-dairy farms; **SC,** Sabouraud's dextrose agar containing chloramphenical; **cfu/g,** colony forming units per gram.

damage, immunosuppression with compromised resistance to infection, disease feed refusal and vomiting, reduced milk and egg production, impaired reproductive function, nephrotoxicosis, neurotoxicosis, hepatotoxicosis, abortions, cancer, embryo toxicity and death (IARC, 2002). The multiple diets of cattle, comprising conserved feeds, concentrates, and forages can be very important sources of diverse mycotoxins (Fink-Gremmels, 2008). If domestic animals are fed a contaminated diet with a particular mycotoxin, there is a high probability of the "carry-over" of mycotoxin into animal products, such as meat or milk, intended for human consumption (Fink-Gremmels, 2008). In many mycotoxins recognized, aflatoxins are the most deleterious and most surveyed mycotoxins, which the most attention is directed to them in the world (Magan and Olsen, 2004; Murphy et al., 2006).

The objective of the present study was to identify the fungi associated with feeds in an extensive region of the main cattle breeding area in Iran, to determine the species diversity of the dominant mycotoxigenic fungi and to compare the mycoflora at different husbandries as well as different seasons. There is no information available about fungally-contaminated feed in Iran and to the best of our knowledge; this is the first report on feed mycoflora and fungal contaminations in different dairy farms in Iran.

MATERIALS AND METHODS

Feed samples

A total of 713 samples from seven different feeds (wheat bran, dried bread, alfalfa, straw, molasses, barley and concentrate) were collected over two periods, in summer and winter, from 79 traditional -dairy farms (TDF) and 14 industrial-dairy farms (IDF) in Hamadan region and were examined in order to determine their mycoflora as well as to isolate aflatoxin-producing fungi. All samples were transported to the Mycology Laboratory of Medical School of Hamadan University within 24 h, stored at 4°C and protected against light until the day of analysis, which was never more than 7 days after collection. All of the samples were intended for animal consumption and did not contain any visible signs of mold contamination.

Mycological analysis

In order to quantify the fungal flora of the feed samples the dilution plating method, a simple, rapid and reproducible procedure, was used. In brief: one gram of finely ground feed was mixed thoroughly with 9 ml of sterile distilled water, followed by ten-fold serial dilutions up to 10^{8}. An aliquot (1 ml) of each dilution was spread over the surface of a petri dish containing 15 ml melted Sabouraud's dextrose agar containing chloramphenical. (SC). The dishes were agitated, allowed to set and incubated at 28°C for 5 - 7 days. The appropriate dilution factor was selected by choosing the petri dishes containing 10 - 30 colonies. In order to check the laboratory procedure and precision, all the serial dilutions and the cultures in SC petri dishes were prepared in triplicate and the mean of fungal colonies were considered. Totally, three petri dishes were plated out per sample. In cases where several fungi were isolated from a single sample, all the colonies were recorded and different species subcultured onto SC. The results were expressed as colony forming units per gram (cfu/g) of sample.

Identification of fungi

The fungi of primary interest were *Aspergillus* species because of their known ability to produce aflatoxins and their relationship with human and animal diseases. For the identification of *Aspergillus* species subcultures were made on SC and incubated for 5 - 7 days at 28°C. Final identifications were made following Raper and Fennell (1965) for *Aspergillus* species and Pitt and Hocking (1997) for *Aspergillus* and *Fusarium* species. The identification of other fungal species was based on the macroscopic and microscopic characteristics of the isolates according to methods of Watanabe (1994).

Statistical analysis

Statistical analysis was performed using SPSS version 9.0 (SPSS Inc. Chicago, Illinois). Analyses of variance (ANOVA) and Tukey's multiple comparison tests were used to compare the means of cfu/g counts. Student's t -test was applied to verify the effect of the season and type of dairy farm in cfu/g counts. The Chi Square test and, if needed, Fisher exact test was used to assess the possible differences in incidence of fungi in feeds, seasons and types of dairy farm. The significance level was set at $p < 0.05$ for all tests.

RESULTS

The results of this study conducted on feeds consumed from animals including cattle showed that the animals were exposed to consumption of aflatoxin-contaminated feeds. A total of 1542 fungal isolates were recovered from 713 feed samples collected from 93 dairy farms in Iran. Mycological analyses revealed that all the feed sample were contaminated by diverse fungi. *Aspergillus* species (including *A. clavatus, A. flavus, A. fumigatus, A. nidulans***,** *A. niger, A. ochraceous, A. parasiticus, A. terreus* and *A. ustus***)** were the most frequently isolated fungi representing 37.4% of isolates, followed by *Penicillium* (23.7%), *Fusarium* (17.5%), *Cladosporium* (9.1%), *Alternaria* (4.3%), *Rhizopus* (3.9%) and *Mucor* species (3.4%) and four other fungal genera (Table 1). The relative density of fungi species isolated from different feed samples showed that seven *Aspergillus* species other than *A. flavus* and *A. parasiticus* had the highest relative density. Generally, the highest relative density of *Aspergillus* species was related to straw and alfalfa respectively for summer and winter seasons (Table 2) . The lowest relative density was related to *Fusarium* species with 6.1% of the total isolations. In both seasons, alfalfa was the highly contaminated feed with 23.20% of the total isolations (Table 2). Based on frequency, concentrate showed a considerably highest frequency (61%) between feeds studied (Table 1).

Results were expressed as colony forming units (cfu)/g based on the average count of triplicate dishes set and as rate of incidence. The concentrate feed was the most contaminated feed with aflatoxigenic Aspergilli for which

Table 1. The incidence rate of *Aspergillus flavus* and *A. parasiticus* from different feed samples and seasons in Iran.

Animal feed	Samples examined			*Aspergillus flavus*		*Aspergillus parasiticus*		Total positive samples
	Total	Summer	Winter	Summer	Winter	Summer	Winter	
	No	No	No	No (%)	No (%)	No (%)	No (%)	No (%)
Wheat bran	186	85	101	20(24)	31 (30)	2 (2.4)	14 (14)	67 (36)
Dried bread	37	29	8	12(41)	2 (25)	ND*	ND	14 (38)
Alfalfa	186	90	96	12 (13)	8(8.3)	8 (8.9)	5 (5.2)	33 (18)
Straw	171	67	104	6(9)	11(10.6)	3 (4.5)	9 (8.7)	29 (17)
Molasses	31	11	20	3 (27)	4 (20)	1 (9)	1 (5)	9 (29)
Barley	84	36	48	8 (22.2)	10(20.8)	1 (2.8)	5 (10.4)	24 (29)
Concentrate	18	10	8	8 (80)	2 (25)	ND	1 (12.5)	11 (61)
Total	713	328	385	69(21)	68(17.7)	15 (4.6)	35 (9.1)	187 (26.2)

* ND, not detected.

Table 2. The relative density of fungi species isolated from different feed samples and seasons in Iran.

Animal feed	Aspergillus spp.Ψ		Cladosporium		Rhizopus		Mucor		Alternaria		Penicillium		Fusarium		Total positive samples	
	Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.	Win.
Wheat bran	13.90	23.22	11.50	9.49	9.83	12.33	17.46	16.03	ND*	8.13	10.91	18.98	ND	ND	7.95	20.00
Dried bread	9.40	6.56	ND	18.80	12.17	11.60	10.26	ND	19.34	1.88	4.34	24.57	ND	ND	6.71	10.58
Alfalfa	25.98	30.58	28.39	22.30	11.68	13.37	20.63	18.46	30.00	36.83	12.58	10.48	24.50	30.79	16.22	23.20
Straw	35.80	16.68	45.57	13.31	15.99	17.79	20.42	15.63	18.99	17.39	21.44	11.71	36.95	22.93	21.07	14.90
Molasses	9.95	2.52	ND	18.46	11.35	13.56	ND	15.30	ND	8.56	10.52	19.69	ND	46.29	30.03	12.40
Barley	4.96	9.78	14.54	11.81	17.96	21.72	31.22	19.51	31.66	27.22	16.37	14.57	ND	ND	10.64	12.80
Concentrate	ND	10.66	ND	5.83	21.01	9.63	ND	15.06	ND	ND	23.83	ND	38.54	ND	7.39	6.12
Total isolates	4050	8571	722	4394	1832	1630	945	1235	1153	2289	1796	4057	755	1374	11253	23550

Aspergillus spp. other than *A. flavus* and *A. parasiticus* Sum.= Summer; Win.= Winter* ND, not detected.

the mean colony counts were 7.25 × 10^2 and 7.50 × 10^2 cfu/g for *A. flavus* and *A. parasiticus*, respectively. Regarding to the seasons, the higher incidence of these two aflatoxigenic fungi was seen in winter than that of summer (103 vs. 84) (Table 1). Despite season and kind of dairy farm, 187 (26.2%) samples were contaminated with both aflatoxigenic fungi (Table 1) . The most contaminated feeds with these two Aspergilli in summer were concentrate feed (80%) and in winter was wheat bran (45%) (Table 1). Although, no significant difference in the incidence rates of aflatoxigenic fungi were observed among two types of the dairy farms examined, but the mean colony count of aflatoxigenic fungi in IDF (8.19 × 10^2 cfu/g) was significantly different ($p < 0.03$) from TDF (4.33 × 10^2 cfu/g) (Figure 1). Furthermore, statistically significant differences ($p < 0.00001$) were observed for the mean of aflatoxigenic fungi colony count in winter and summer seasons (Figure 1).

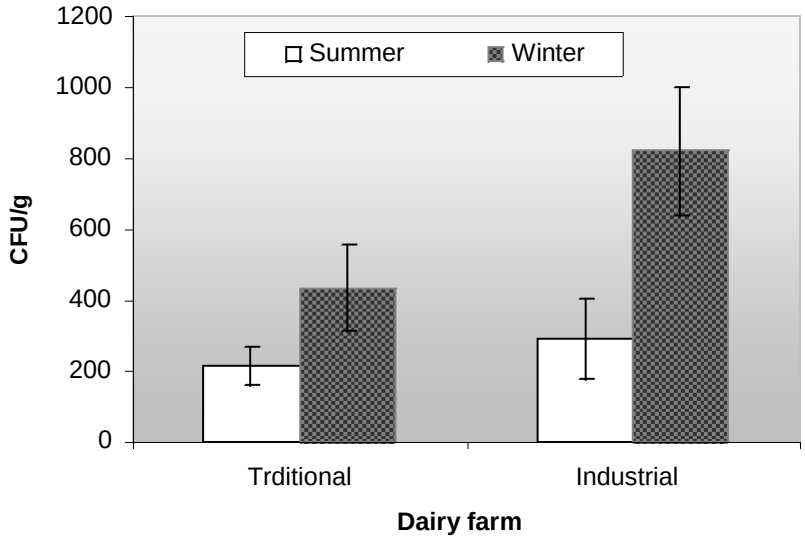

Figure 1. The mean colony counts of aflatoxigenic fungi in feed samples collected from different seasons and different dairy farms of Iran.

Regarding to contamination with different fungal genera isolated, straw and dried bread was the most contaminated feeds with 16 and 8 fungal genera, respectively.

DISCUSSION

A previous study on the occurrence of aflatoxin M1 in raw milk samples obtained from the Hamadan region showed that AFM_1 was detected in 119 out of 186 samples (63.97%)(Ghiasian et al., 2007). The mean concentration of AFM_1 in the contaminated samples was 43.4 ng/L and 14(11.76%) contaminated samples had AFM_1 in concentrations in excess of maximum levels specified in EU regulations, that is, 50 ng/L (Ghiasian et al., 2007). In the same study, the contamination ratio of milk reported was 56.5% and 71.7% in summer and winter months, respectively. In order to give an explanation for the occurrence of aflatoxins in raw milk, the present investigation was initiated to evaluate the occurrence of aflatoxigenic fungi in cow feeds in the aforementioned area.

Although, there are many papers describing the mycoflora of human foods, only a few data on mycotoxigenic fungi in feedstuff exist. The total fungal count, especially the aflatoxigenic ones, in animal feed samples is one of the most important criteria in evaluation of hygienic quality and the great probability of feed contamination with mycotoxins (Dalcero et al., 1998).

In our study, *Aspergillus* species, followed by *Penicillium* and *Fusarium* species were the main fungal genera isolated from the cow feeds sampled. Similar results were reported by Oliveira et al. (2006) who showed that *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. were three most frequent fungi isolated from poultry feeds of Brazil Likewise, in a study conducted on the mycoflora determination in poultry feed samples in Argentina, Dalcero et al. (2006) reported that the most dominant fungal species isolated, belonged to the genera *Aspergillus*. Williams and Bialkowska (1985) also reported that from 294 feed samples surveyed, *Penicillium* accounted for 53% of the fungal isolates, *Aspergillus* for 15%, *Cladosporium* for 8.9%, *Mucor* for 8.5% and *Rhizopus* for 4.8% of the isolates. According to Krnjaja et al. (2008a), 73.94 and 87.10% of animal feed samples examined in 2007 and 2008 in Serbia were positive for *Aspergillus* species. In support of our study, two recent studies of Krnjaja et al**.** (2008a,b) carried out on fungi-contaminated feed in Serbia revealed that *Aspergillus*, *Fusarium* , *Rhizopus, Penicillium, Mucor* and *Alternaria* have been six common fungal genera. But, in contrast to our finding, *Cladosporium* spp. were absent in their investigations. In another survey, Trenholm et al. (1982) reported that presence of fungal propagules in animal feeds of Canada was very low.

The higher rate of incidence of *A. flavus* over *A. parasiticus* in Iranian feeds (137 vs. 50) was comparable with a study in which a total of 198 *A. flavus* and 15 *A. parasiticus* isolates were identified out of 256 feed samples collected from different parts of Northern India (Dutta and Das, 2000). In our study, the predominance of *A. flavus* isolates in both warm and cold seasons suggests that it can be easily adapted in various geographical regions.

Another important point to consider is that this seed-borne and soil-borne fungus is a rapidly growing that can resist in low moisture levels (Maciorowski et al., 2007; Pitt and Hocking, 1997). Similar results about the adaptability of Iranian *A. flavus* isolates have also been reported in

Andaman and Nicober Islands in India (Banerjee and Shetty, 1992).

In the current study, the dominance (23.20%) of different fungal species in alfalfa in winter is most probably associated with its storage in wet conditions soon after harvesting, which has not allowed it to be dried. In such unhealthy situation, the majority of fungi especially *Aspergillus* species can grow and resulting in mycotoxin production. In accordance to our results, Chadd (2004) upon conducting a study on microbial flora of silages in European countries stated that *Penicillium*, *Aspergillus* and *Fusarium* have been the most frequently isolated fungal species. This observation is also in agreement with another study conducted by Bijeli et al. (2009). In the study carried out by or evi et al. (2003), the most frequently isolated species from alfalfa silage belonged to the genera *Penicillium*, *Cladosporium, Fusarium, Acremonium*, *Mucor*, *Botryotrichum* and *Strepromyces* and in contrast to out study, they did not isolated any *Alternaria* species.

The incidence rates of fungal contamination in both TDF and IDF samples were 70.9 and 66.2%, respectively and no significant difference was detected in terms of farm type. The mean colony count of aflatoxigenic fungi in IDF was significantly higher than in TDF. These results showed that because of the lack of hygienic regulation, which should be settled on industrial husbandries, there was no significant difference between these two husbandries in Hamadan region. Some studies have shown that the aflatoxigenic fungi in hygienic animal farms in some countries such as Canada (Trenholm et al., 1982) were much less than other countries which the regulations were not stabilized. In the current study, in order to find any probable seasonal influence, feed samples were taken in two different seasons with high temperature differences. Our previous results showed that the frequency of AFM_1-contaminated milk samples collected in winter were significantly higher than that of summer (71.7 vs. 56.5%) ($P < 0.02$) (Ghiasian et al., 2007). This finding was confirmed by statistically significant differences, observed for the mean of aflatoxigenic fungi colony count in winter and summer seasons. Generally, all of the surveyed feeds in winter were significantly contaminated to aflatoxigenic fungi higher than those analyzed in summer.

Among the Aspergilli genera, one of the important species identified was *A. niger*. The potential risk of *A. niger* in feedstuff because of mycotoxin production should also be considered, because studies have shown that occasional isolates of *A. niger* can produce ochratoxin (Abraca et al., 1994).

Furthermore, in this study, *Fusarium* species were ranked third among the isolated fungi. The occurrence of *Fusarium* spp. in food and animal feed has serious implications for human and animal health, because of their ability to produce many important mycotoxins especially fumonisins, a group of toxic and carcinogenic metabolites, which the International Agency for Research on Cancer has designated as Group 2B carcinogens (IARC, 2002). Based on our previous study on mycoflora determination of Iranian corn intended for animal consumption, members of the genus *Fusarium* were the most frequent fungal species encountered, as 38.5% of the 5584 fungal isolates were related to these very important fungal species (Ghiasian et al., 2004).

As regard to *Alternaria* species, this is a black saprophytic fungus widely distributed in the soil and most frequently found in outdoor air. The occurrence of these dematiaceous fungi in food and feed suggest that they may pose a hazard comparable to that of the well known toxigenic species of *Aspergillus*, *Fusarium* and *Penicillium.* The toxigenic species of *Alternaria* have strong potential for production of alternariol, altenuene, tenuazonic acid and other toxins (Maciorowski et al., 2007). In this study, although *Alternaria* spp. were not the predominant fungi isolated (4.3%), but their higher presence (20.75%) in afalfa as a main component of animal feed in Iran could be a potential threat and is not negligible. Similar to our results, Krnjaja et al. (2008a) reported that *Alternaria* spp. were the least frequent species (4.93%) of the six fungi genera identified in 2007. Similar results have also been found in the study conducted by Bijeli et al. (2010) that 3.6% of surveyed alfalfa was contaminated with this fungus.

In conclusion, the present study tested the hypothesis that differences exist between fresh forages used in summer and the ensiled form used in winter in terms of contamination with aflatoxigenic fungi. Although the detection of toxigenic fungi in a substrate does not necessarily indicate that mycotoxins are naturally occurring in the feed, it alerts to the potential risk of contamination. Furthermore, the high frequency of mycotoxin-producing fungal species in this study emphasizes the importance of continued research on feedstuffs contamination with toxigenic fungi in different parts of Iran.

ACKNOWLEDGEMENTS

The authors acknowledge with great thanks Ms. S. Karimkhani from Medical Parasitology and Mycology Department, School of Medicine, Hamadan University of Medical Sciences and Health Services for preparation and maintenance of feed samples.

REFERENCES

Abraca ML, Bragulat G, Cabanes FJ (1994). Ochratoxin A production by strains of *Aspergillus flavus* var. *niger*. Appl. Environ. Microbiol., 60: 2650-2652.

Banerjee A, Shetty HS (1992). Microbial load in poultry feed and detection of aflatoxin B1 using monoclonal antibody based enzyme linked immunosorbent assay. Lett. Appl. Microbiol., 15: 89–91.

Bijeli Z, Krnjaja V, Tomi Z, Stojanovi Lj, Ruži -Musli D (2009). Identification and quantification of fungi in Grass-Leguminous silage. Biotechnol. Anim. Husbandry, 25: 1209-1216.

Chadd S (2004). Mycotoxicological challenges to European animal production: a rewiev. 55th Annual Meeting of the EAAP, in Adamovi M., Bo arov-Stan i A., or evi N., Dakovi A., Adamovi I. (2005): Mycotoxins in the silage-causes of creating, aftermath and protection from acting. Zbornik Matice srpske za prirodne nauke., 108: 51-57.

Dalcero A, Magnoli C, Luna M, Ancasi G, Reynoso MM, Chiacchiera S, Miazzo R, Palacio G (1998). Mycoflora and naturally occurring mycotoxins in poultry feeds in Argentina. Mycopathologia, 141: 37-43.

or evi N, Aadamovi M, Grrubi G, Koljaji V, Bo arvostan i A (2003). Influence of min-a-zel plus on biochemical, microbiological and mycotoxicological parameters of lucerne silage. J. Agric. Sci., 48: 171-178.

Dutta TK, Das P (2000). Isolation of aflatoxigenic strains of *Aspergillus* and detection of aflatoxin B1 from feeds in India. Mycopathologia, 151: 29-33.

Fink-Gremmels J (2008). Mycotoxins in cattle feeds and carry-over to dairy milk: a review. Food Addit. Contam., 25: 172-180.

Ghiasian SA, Kord-Bacheh P, Rezayat SM, Maghsood AH, Taherkhani H (2004). Mycoflora of Iranian maize harvested in the main production areas in 2000. Mycopathologia, 158: 113-121.

Ghiasian SA, Maghsood AH, Neyestani TR, Mirhendi SH (2007). Occurrence of aflatoxin M1 in raw milk during the summer and winter seasons in Hamadan, Iran, J. Food Safety, 27: 188-198.

International Agency for Research on Cancer (2002). Aflatoxins, In IARC Monographs on the evaluation of the carcinogenic risks to humans: some traditional herbal medicines, some mycotoxins, Naphtalene and Styrene, International Agency for Research on Cancer, Lyon, France., 82: 301-366.

Krnjaja V, Stojanovi Lj, Cmiljani R, Trenkovski S, Tomaševi D (2008b). The presence of potentially toxigenic fungi in poultry feed. Biotechnol. Anim. Husbandry, 24: 87-93.

Krnjaja V, Stojanovi Lj, Trenkovski S, Bijeli Z (2008a). The Frequency of pathogenic fungi genera in animal feed. Lucr ri tiin ifice, 53: 341-344.

Maciorowski KG, Herrera P, Jones FT, Pillai SD, Ricke SC (2007). Effects on poultry and livestock of feed contamination with bacteria and fungi. Anim. Feed Sci. Technol., 133: 109-136.

Magan M, Olsen M (2004). Mycotoxins in food: detection and control. CRC Press LLC, Woodhead Publishing Ltd.

Murphy PA, Hendrich S, Landgren C, Bryant CM (2006). Food Mycotoxins: an update, J. Food Sci., 71: 51-65.

Oliveira GR, Ribeiro JM, Fraga ME, Cavaglieri LR, Direito GM, Keller KM, Dalcero AM, Rosa CA (2006). Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. Mycopathologia, 162: 355-362.

Pitt JI, Hocking AD (1997). Fungi and food spoilage. Second edition, Aspen publishers, Gaithersburg, Maryland, p. 593.

Raper K, Fennell D (1965). The Genus *Aspergillus.* Baltimore: Williams and Wilkins.

Trenholm HL, Friend DW, Hamilton MG, Thompson BK (1982). Vomitoxin and zearalenone in animal feeds. Minister of Supply and Services Canada Publication No. 1745/E, Ottawa.

Watnabe T (1994). Pictorial atlas of soil and seed fungi. In: Morphologies of cultured fungi and key to species. Lewis Publishers, Boca Raton, Boston, London, Washington D.C., p. 410.

Williams AP, Bialkowska A (1985). Moulds in mould spoiled foods and food products. Leatherhead Food R.A. Research report, p. 527.