The investigation of pathogenic fungi in poultry feed in some selected poultry farms in Sohag Governorate, Egypt

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ABSTRACT

Mould occurrence and growth in poultry farms is one of the major threats to poultry economy and health. The aim of this study was to investigate the mycological contamination of poultry feeds used in poultry farms in Sohag Governorate, Egypt. A total of 160 samples of poultry feed were collected from 10 farms for mycological analysis. The isolated fungi from these samples were cultured in Sabouraud Dextrose Agar and Potato Dextrose Agar at 25°C and 37°C for two weeks. Positive cultures were identified through standard methods. The mycological examination revealed the isolation of 8 fungal species and 102 (63.7%) of poultry farms in the locality had mycotic contamination. The most prevalent fungi species were Fusarium solani (24.5%) and Aspergillus flavus (17.65%), followed by Aspergillus niger (14.71%), Penicillium chrysogenum (11.76%), Mucor species (9.8%), Rhizopus species (8.82%), Cladosporium species (7.84%) and the least frequency species were from genus Alternaria species (3.92%). These findings therefore suggest that poultry feed provides a good ecology for fungi thereby posing a great public health threat to humans.

Key words: Pathogenic fungi, contamination, poultry feeds, Sohag.

INTRODUCTION

Poultry feed quality is important prerequisite for achieving of optimal production results, as well as preservation of health condition of the animal, especially in intensive industrial production, therefore it is necessary to control raw materials and finished forage mixtures, constantly.

Food is the essence of life as well as its safety, yet the majority of people give little thought to ensure that food is safe to eat. When food safety issues are raised, it is normally the perceived problems of pesticides or other man-made chemicals. However, natural toxins produced by a range of microbes, are potent toxins and carcinogens and therefore of equal or a greater threat to food safety as manmade chemicals[1].

Poultry feed may serve as a carrier for a wide variety of microorganisms including pathogenic fungal species [2].Some of these microorganisms are adapted to the desiccated and relatively nutrient-deficient conditions of the soil and survive in similar niches on growing crops [2]. Gastrointestinal pathogens can be introduced into the food chain by animals defecating in the farm environment or by fertilization of crops with manures; also microorganisms are introduced during storage [3]. In general, the amount of available water in the feed matrix determines whether a microorganism will grow or survive. Some microorganisms, primarily moulds, are adapted to the low amount of available moisture and grow actively within stored seeds and grains.

Food safety is an imperative in food production worldwide. Poultry meat, eggs, and poultry products derived from them are crucial in safe food chain. As far as safety is concerned, special attention is directed towards possible contamination of food and poultry feed with fungi and to the risk of mycotoxin contamination[4].
Fungi and mycotoxins contamination of the raw materials occur during the pre-harvest (field produced fungi) and/or the post-harvest (storage-produced fungi) periods. During these periods, temperature and humidity play an important role in the growth of fungi and mycotoxins contamination. In poultry production, feed is the key vector for introducing mycotoxins into flocks. Other routes of exposure include litter. Mycotoxin potential residues may greatly influence on meat and eggs production[5].

The intake of very low levels of mycotoxins causes overt mycotoxicosis but also leads to the impairment of immune and acquired resistance to infections causing health problems which lead to economic losses in the form of decreased productivity [6]. Mycotoxins can also cause vomiting, abdominal pains, pulmonary edema, convulsions, coma, carcinogenic effects, immunosuppression, gene toxicity, immunological cytotoxicity, mutagenic effects, low appetite, weight loss, faintness, depression and death[7,8]. There should be some standards for suitable storage of food because these products may get contaminated which endanger human health; therefore, it is crucial to pass some regulations to reduce mould contamination[9,10].

Of numerous mycotoxins, most attention in the world is directed to aflatoxins, ochratoxins, zearalenones, toxins from the group of trichothecenes and fumonisins. Aflatoxins are the most spread, most dangerous and most studied mycotoxins. Main producers of these mycotoxins are fungi of genus Aspergillus, although also some other species of fungi from genera Penicillium and Rhizopus produce them.

The Ochratoxins are produced by Aspergillus ochraceus, Aspergillus niger, Penicillium viridicatum and Penicillium verrucosum. The Ochratoxin is the most important mycotoxin which may cause disorder endocrine, chronic and acute toxicity, immune toxicity and carcinogenic in human [11,12,13]. Fusarium genus is the most prevalent one producing toxin. The most important toxin of this genus is Zearalenone which is produced by Fusarium graminearum[14].The most important species producing Fumonisin are Fusarium moniliforme, Fusarium nymgamai and Alternaria alternaria[11]. on the other hands, many fungi produce trichotheccenes, the most important are certain species of genus Fusarium.

Thus the aim of the study was to investigate the mycological contamination of poultry feeds used in poultry establishments in Sohag Governorate, Egypt.

MATERIALS AND METHODS

A total 160 samples of feed intended for nutrition of poultry of all categories obtained from 10 different poultry in Sohag Governorate, Egypt were investigated for determination of total fungi count and the frequency of fungi genera. Investigation of total fungi count was done using standard microbiological methods according to valid regulations on hygiene standard and quality of animal feed. From each visited farm, feed present in the farm were sampled by selecting 20 to 25% of the bags present in the farm by simple random sampling. For each sample, 5 to 10 g of feed was collected in a polyethylene bag and then collectively transported to the Veterinary Microbiology laboratory for immediate processing. In some cases the feed samples were stored at room temperature (22 to 25°C) for a maximum of 24 hour prior to inoculation onto culture media. Isolation of special fungi genera was done by planting of samples on selective media for fungi, Sabouraud dextrose agar (Oxoid, England)[15]containing 2% chloramphenicol to inhibit bacterial growth and Potato Dextrose Agar (Oxoid, England)[16].

Identification of all fungi isolates:
The inoculated plates were incubated at 25°C and 37°C for 5 to 14 days. Pure culture of the different colonies (based on morphology) was obtained by sub-culturing of the isolates on nutrient ager and SDA plates respectively. The fungal isolates were identified to the genus/species level based on macroscopic and microscopic characteristics of the isolates obtained from pure cultures. The rapidity of growth, colour and reverse site of the culture plate, shape, texture and consistency of the growth were observed, while the microscopic examination was done using lactophenol blue stain. A drop of the stain was placed on a clean grease-free slide. A small portion of the fungal culture was emulsified on the slide and covered with a coverslip, avoiding bubbles. The slide was thereafter viewed under the microscope. A slide culture of the fungal isolate was also prepared and examined under the microscope. Such characteristics include; septation of hyphae, shape, size, texture and arrangement of the conidia. The fungi were identified according to methods of[17,18,19,20]. The fungal isolates were also sub-cultured on SDA slants, incubated at 27°C for 10 days and stored in refrigerator for future studies[21].

RESULTS

Fungal counts observed by analysis of investigated poultry feed samples on malt extract agar ranged from $0.1 \times 10^2$ to $3.55 \times 10^3$ cfu.g$^{-1}$ of sample, with an average $1.8 \times 10^2$ cfu.g$^{-1}$ of sample.
A total of 102 fungal agents were isolated from 160 poultry feed samples obtained from different poultry farms investigated. This put the prevalence rate of mycotic infection of poultry farms in the locality at 63.7%.

By microbiological analysis of the investigated poultry feed samples, eight fungi genera have been isolated and identified: *Fusarium*, *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Cladosporium* and *Alternaria*. (Table 1)

The most prevalent fungi species were *Fusarium solani* (24.5%) and *Aspergillus flavus* (17.65%), followed by *Aspergillus niger* (14.71%), *Penicillium chrysogenum* (11.76%), *Mucor species* (9.8%), *Rhizopus* sp. (8.82%), *Cladosporium* sp. (7.84%) and the least frequency species were from genus *Alternaria* sp. (3.92%). (Table 2, Figure.1).

Table 1: Pathogenic fungi isolated from poultry feed.

<table>
<thead>
<tr>
<th>Mycotic Agent</th>
<th>Number</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus species</td>
<td>33</td>
<td>32.35</td>
</tr>
<tr>
<td><em>Fusarium</em> species</td>
<td>25</td>
<td>24.5</td>
</tr>
<tr>
<td><em>Penicillium</em> species</td>
<td>12</td>
<td>11.76</td>
</tr>
<tr>
<td><em>Mucor</em> species</td>
<td>10</td>
<td>9.8</td>
</tr>
<tr>
<td><em>Rhizopus</em> species</td>
<td>9</td>
<td>8.82</td>
</tr>
<tr>
<td><em>Cladosporium</em> species</td>
<td>8</td>
<td>7.84</td>
</tr>
<tr>
<td><em>Alternaria</em> species</td>
<td>4</td>
<td>3.92</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>102</td>
<td>63.7</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of Mycotic Agents Isolated from poultry feed.

<table>
<thead>
<tr>
<th>Mycotic Agent</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fusarium solani</em></td>
<td>23</td>
<td>24.5</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>18</td>
<td>17.65</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>15</td>
<td>14.71</td>
</tr>
<tr>
<td><em>Penicillium chrysogenum</em></td>
<td>12</td>
<td>11.76</td>
</tr>
<tr>
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<td>63.7</td>
</tr>
</tbody>
</table>

Fig. 1: Prevalence of Mycotic agent isolated from poultry feed.
Fig. 2: Macroscopic and microscopic characteristics of *Fusarium solani*. On right: S.D.A containing *Fusarium solani*; the colony is cottony or woolly with a delicate lavender, rose colour. On left: Microconidia abundant and characteristic (bean shape).

Fig. 3: Macroscopic and microscopic characteristics of *A. flavus*. On right: colony features on SDA; on left: microscopic globose vesicle and the streigmate over almost the entire surface and conidophore.

Fig. 4: Macroscopic and microscopic characteristics of *A. niger*. On right: colony features on SDA; on left: conidial heads, vesicle and coarsely roughened conidia.
DISCUSSION

Poultry feed contamination with fungi can lead to nutrient losses and detrimental effects on animal health and production. More than 100,000 fungal species are considered as natural contaminants of agricultural and food products [22]. Researchers have previously highlighted that farm inputs such as feed and water are likely routes of poultry farm contamination [23,24].

Besides their negative impacts on nutritional and organoleptic properties, moulds can also synthesize different mycotoxins. Decreasing fungal growth and Mycotoxins formation in food and feed are essential since it is consumed by both human and animals. The fungi and mycotoxins have serious effects upon the growth rate and health of human being and animals, as some mycotoxins had been found to be hepatotoxic, carcinogenic, tremorgenic, haemorrhagic and dermatitic.[25]

Total fungi count is one of the criteria in evaluation of hygienic quality and it is very important for orientation in lower or higher probability that the feed contains mycotoxins. According to the regulations on maximal quantities of harmful substances and components in livestock feed Službeni [9] and Oliveira[26]discussed that mixtures and raw materials for animal feed are not in compliance with standards of the hygiene quality if they contain above 300,000 cfu g$^{-1}$ of forage mixture for older animal categories or 50,000 cfu g$^{-1}$ for younger animals.

Fungal counts observed by analysis of investigated poultry feed samples on malt extract agar ranged from $0.1 \times 10^2$ to $3.55 \times 10^7$ cfu.g$^{-1}$ of sample, with an average $1.8 \times 10^5$ cfu.g$^{-1}$ of sample. Similar results concerning the fungal
counts in poultry feed mixtures from Turkey have been reported by Heperkan and Alperden[27], from Spain by Bragulat[28], from Slovakian by Roman and Dana [29] and from Argentina by Dalcero[6,30].

A total of 102 fungal isolates were isolated from 160 poultry feed samples collected from popular farms with a prevalence rate of 63.7%. These result is in line with the reports from previously studies [5,9,21,30,31,32,33,34,35]. These result higher than Šefer[36] established that from 160 analyzed samples of forage mixtures used in poultry nutrition only 57 (35.6%) were in accordance with provisions of the Regulation on quality of livestock feed. Krnjaja[5] stated that from a total of 230 samples of poultry feeds in 2007 and 235 samples in 2008, the incidence of fungi were 38.26% and 45.53% in 2007 and 2008, respectively.

Different genera of contaminating fungi in the present study ranked according to their isolation frequency were Fusarium solani (24.5%) and Aspergillus flavus (17.65%), followed by Aspergillus niger (14.71%), Penicillium chrysogenum (11.76%), Mucor species (9.8%), Rhizopus sp. (8.82%), Cladosporium sp. (7.84%) and the least frequency species were from genus Alternaria sp. (3.92%), these result nearly similar to Krnjaja[5] who stated that the most prevalent fungi genera isolated from poultry feed in Serbia were Fusarium (56.09%) and Aspergillus (54.35%), followed by Rhizopus (40%), Penicillium (30.87%), Mucor (30.04%) and the least frequency species were from genus Alternaria (3.48%). Similar result also has been reported by[27,28,30], they stated that Aspergillus, Penicillium and Fusarium are the typical fungal genera inhabiting poultry feed mixtures. In Argentina, the species belonging to Aspergillus (85%) and Fusarium (70%) genera were the most frequent in 130 investigated poultry feed samples [6]. In Egypt [37,38,39,40,41] stated that the most predominant genus was Aspergillus which was isolated from all samples. Other moulds were isolated but in low frequency as Penicillium, Fusarium, Cladosporium and Alternaria. Fusarium and Aspergillus were found in food storage places which produce mycotoxin at suitable moisture and temperature, [42]. In fact, Aspergillus, Penicillium and Fusarium are the typical fungal genera inhabiting poultry feed mixtures, they are very important contaminants being renowned for their ability to form a huge number of various types of toxic extrrolites-mycotoxins such as ochratoxins, zearalenones and aflatoxins. [43,44,45].

These result not consistent with that of Heperkan and Alperden [27] whose found in their work dealing with Turkish poultry feed mixtures and feed ingredients, Penicillium aurantiogriseum was the main fungal contaminant, being encountered in 59% of samples, followed by Aspergillus flavus (48%) and A. niger (23%). Also my result not agree with that of Pacin [46] in Ecuador, Abdul-wahab [47] in Saudi Arabia, Dalcero [29] in Argentina, Šefer [24] in Iraq and Salihoy [48] in Nigeria their isolation frequency were Aspergillus sp. appeared to be the most prevalent, followed by Penicillium sp., Rhizopus sp., Mucor sp. and the least the Fusarium sp.

While, Šefer [35] stated that the most frequent isolated fungi species are of Mucor genus (76.5%), followed by species of genera Aspergillus (58.3%), Penicillium (29.6%) and Fusarium (15.6%). All investigated mixtures were contaminated with two mycotoxins, zearalenone and ochratoxin. Oliveira [9] showed that Penicillium spp. (41.26%) and Aspergillus spp. (33.33%) had the highest isolation frequencies followed by Fusarium spp. (20.63%).

CONCLUSION

Feeds and feedstuffs are excellent media for the growth of fungi and so a very high standard of hygiene is necessary to avoid feed contamination which can lead to disease of mycological origin traceable to livestock feed. A total of 102/160 poultry feed samples in the locality had mycotic contamination with a prevalence rate of 63.7%. The mycological examination revealed the identification of 7 fungal genera (Fusarium, Aspergillus, Penicillium, Mucor, Rhizopus, Cladosporium and Alternaria) of which genera Aspergillus and Fusarium had the highest presence (32.25%) and (24.5%) respectively, and genus Alternaria the lowest (3.92%). It must be pointed out that some fungi species like Fusarium and Aspergillus which are the most frequent producers of different and very dangerous mycotoxins (zearalenones, triothecenes, fumonisins, aflatoxins, ochratoxins) in poultry feed. Due to the high fungal recovery of fungal species of public health concern in this study which indicate a potential hazard to both animals, and humans, regular microbiological but also mycotoxicological analysis should be necessary methods for determination of quality and safety of poultry feed as well as a great attention must be done for the storage methods employed by the poultry farmers, livestock feed manufacturers, distributors and the retailers.

REFERENCES


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