

Isolation and identification of common fungal spp. from commercial broiler feeds available in market of Sylhet District, Bangladesh

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ABSTRACT

The present study was designed to investigate the mycological contamination of commercial broiler feeds used in poultry establishments in sylhet, Bangladesh. The feed samples of commercial broiler feed (Starter, Grower and Finisher) were collected from the different areas of Sylhet district. A total of 189 commercial broiler feed samples where 63 Starter, 63 Grower and 63 Finisher were collected from the different areas of local market in Sylhet. The selected areas were Kadamtali, Shibjong, Khadim, Kamal Bazar, Dakshin Surma, Fenchugonj. From the feed samples analyzed for the presence of fungal agents, 144 (76.2%) were found positive for one or more fungal species. Fungal isolates were found among 36 (57%) of the 63 Starter feed samples, 45 (71.4%) of the 63 Grower feed samples and 63 (100%) of the 63 Finisher feed samples. The fungal agents isolated from Broiler Starter Feeds, *Aspergillus* spp. 51 (70.8%) has the highest frequency of occurrence, followed by *Fusarium* spp. 12 (16.7%) and least is *Rhizopus* sp. 9(12.5%). Similarly, in case of Broiler Grower Feeds, *Aspergillus* spp. 66 (68.8%) has the highest frequency of occurrence, followed by *Fusarium* spp. 18 (18.7%) and least is *Rhizopus* sp. 12(12.5%). In case of Broiler Finisher Feeds, *Aspergillus* spp. 90 (69.8%) has the highest rate of occurrence followed by *Fusarium* spp. 24 (18.6%) and least is *Rhizopus* sp. 15 (11.6%) respectively.

Keywords: Commercial Broiler feed (Starter, Grower and Finisher), Isolation, *Aspergillus* spp., *Fusarium* spp., *Rhizopus* spp.

INTRODUCTION

The advancement of poultry industry in Bangladesh is interrupted by a number of constraints of which the major one is the outbreak of diseases causing about 30% mortality of chickens in every year [3]. The major etiological agents are the microorganisms (Bacteria, Virus, Fungus etc.), parasite and deficiency of mineral and vitamins. Poultry feed may serve as a carrier for a wide variety of microorganisms including pathogenic fungal species (The genera included *Aspergillus*, *Fusarium*, *Rhizopus* etc. spp.) [5]. Funguses are adapted to the low amount of available moisture and grow actively within stored seeds and grains [15]. Fungus can affect feed quality negatively of reducing dry matter and nutrients, causing musty or sour odours, causing caking of the feed and most importantly producing mycotoxins [15]. Mycotoxins are harmful substances produced by fungi in various feeds are estimated to affect as much as 25% of the world's crop each year [12]. Most of these mycotoxins belong to the three genera of fungi: *Aspergillus*, *Penicillium* and *Fusarium*. Due to the diversity of their toxic effects and their synergetic properties, mycotoxins are considered as risky to the consumers of contaminated feeds [27]. Talha (1999) detected *Aspergillus* in 4.20%, *Aflatoxicosis* in 0.52% in poultry in Mymensingh district of Bangladesh. In another survey conducted on breeding, commercial broiler and layer flocks of major poultry raising belts in and around Dhaka and Gazipur districts of Bangladesh and recorded *Aspergillus* in 1.5% birds examined [21]. *Aspergillus* spp is the most common fungi found in air or litter of poultry houses [9, 14, 22, 26]. Spores are widely distributed in nature and birds of almost all species and ages may be affected where

the commonest route of infection probably by inhalation [6]. Fungi grow well between 21°C and 32°C temperature and above 70% relative humidity. Bangladesh is a tropical country with a predominant hot humid environment and the environment is much favorable for the propagation of fungi on feed and feed materials. To prevent economic losses in poultry flocks isolation and identification of birds being affected by fungal infection needs to be determined. Such studies on commercial broiler feed sample in Bangladesh is not well reported. Thus, the present study is designed to investigate the mycological contamination of commercial broiler feeds used in poultry establishments in Sylhet, Bangladesh. In view of above considerations, the present study is proposed with the objectives of isolation of fungal species from Commercial broiler feed samples and identification of the common fungal species from isolates.

MATERIALS AND METHODS

The research work was conducted in the laboratory of Microbiology and Immunology, Sylhet Agricultural University, Sylhet from July to December 2013.

Study area

The feed samples of commercial broiler feed (Starter, Grower and Finisher) were collected from Kadamtali, Shibjong, Khadim, Kamal Bazar, Dakshin Surma and Fenchugonj, Sylhet.

Experimental design

The total study was performed in three (3) steps. The first step included feed sample collection, transportation and preservation. The second step of the experiment was isolation of fungal genera from commercial broiler feed sample and the third step

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was identification of common fungal spp. from pure culture.

Collection of samples

The Commercial Broiler feed samples were collected from different farms and dealers for the experimental study. A total number of 189 field samples were aseptically collected into sterile plastic bag from different spots and carried to the laboratory for the isolation and identification of common fungal Species.

Isolation of fungal organisms

Firstly, Ten-fold serial dilution of 1g of feed with distilled water then 0.1ml of the dilution was cultured by spread plate technique into Potato dextrose agar (PDA) supplemented with chloramphenicol at 40 µg/ml and Gentamycin at 500 µg/ml and incubated for 5 to 14 days at room temperature. Pure culture of the different colonies (based on morphology) was obtained by sub-culture of the isolates on potato dextrose agar plates and sabouraud's dextrose agar plates. The fungal isolates were identified to the genus/species level based on macroscopic and microscopic characteristics of the isolates obtained from pure cultures.

Nutrient broth (NB)

Nutrient broth was prepared by dissolving 13 gms of dehydrated nutrient broth into 1000 ml of distilled water and was sterilized by autoclaving at 121°C under 15 pounds pressure per square inch for 15 minutes (1kg/cm²). Then the broth was dispensed into tubes (10 ml/tube) and stored at 4°C in the refrigerator until used.

Phosphate buffer saline (PBS)

For preparation of phosphate buffer saline, 8 grams of sodium chloride (NaCl), 2.89 grams of disodium hydrogen phosphate (Na₂HPO₄.12H₂O), 0.2 grams of potassium chloride (KCl) and 0.2 grams of potassium hydrogen phosphate (KH₂PO₄) were suspended in 1000 ml of distilled. The solution was heated to dissolve completely. The solution was then sterilized by autoclaving at 121°C maintaining a pressure of 15pounds per sq. inch for 15 minutes (1kg/cm²) and stored at 4-8°C until used. The pH of the solution was measured by a pH meter and maintained at 7.0-7.2^[6].

Potato Dextrose Agar

A total of 64 gms of media were properly mixed with 1000 ml distilled water and boiled to dissolve the medium completely. It was sterilized using autoclaving at 15 lbs pressure (121°C) for 15 minutes (1kg/cm²) and mixed well before dispensing to start aseptically and with proper care so that any contamination be avoided.

Sabouraud's Dextrose Agar

A total of 65 gms of media were properly mixed with 1000 ml distilled water and boiled to dissolve the medium completely. It was sterilized using autoclaving at 15 lbs pressure (121°C) for 15 minutes (1kg/cm²) and mixed well before dispensing to start aseptically and with proper care so that any contamination be avoided.

Culture of samples

An inoculum is prepared from the sample and streaked on the SDA media and incubated at room temperature. The different plate media were examined for the fungal growth every day until growth is found after 3 to 4 days of plating. Pure culture was prepared from this initial culture^[18].

Preparation of pure culture

In order to make a pure culture, spores from initial culture was transferred to media containing petridishes by sterilized inoculating loop for avoiding any contamination with other fungus and incubate for 3-4 days at room temperature until the fungal growth is found.

Identification of fungus

From pure culture, fungal colony was taken with the help of an inoculating needle on a fresh glass slide containing two drops of lactophenol cotton blue. The fungal colony was covered with a cover slip and the slides were examined under the microscope. The fungus was identified on the basis of its cultural and morphological characteristics^[18].

RESULTS

It was observed that ,from 189 feed samples analyzed for the presence of fungal agents, 144 (76.2%) were found positive for one or more fungal species. Fungal isolates were found among 36 (57%) of the 63 Starter feed samples, 45 (71.4%) of the 63 Grower feed samples and 63 (100%) of the 63 Finisher feed samples (Table 1).

Of the fungal agents isolated from Broiler Starter Feeds, *Aspergillus* spp. (70.8%) has the highest frequency of occurrence, followed by *Fuserium* spp. (16.7%) and least is *Rhizopus* spp (12.5%) (Table 2). Similarly, in case of Broiler Grower Feeds, *Aspergillus* spp. (68.8%) has the highest rate of occurrence, followed by *Fuserium* spp. (18.7%) and least is *Rhizopus* spp. (12.5%). And, in case of Broiler Finisher Feeds, the rate of occurrence is high in *Aspergillus* spp. (69.8%) followed by *Fuserium* spp. 24(18.6%) and *Rhizopus* spp. (11.6%) respectively (Table 2).

DISCUSSION

In the present study, fungal contamination was present in a high proportion of the commercial broiler feed samples (76.2%) (Table 1) which is reported from many parts of the world^[7, 16, 19, 10, 11, 20]. Different genera of contaminating fungi in the present study ranked according to their isolation frequency, where *Aspergillus* spp. (69.7%) appeared to be the most prevalent, followed by *Fuserium* spp. (18.3%) and the least is *Rhizopus* spp. (12.1%). Similar results have been documented by other investigators^[1, 7, 17, and 23] but differ from other investigators^[13] who stated that there is more chances of contamination by Mycotoxins. It may be stated that, *Aspergillus*, *Fuserium* and *Rhizopus* are the typical fungal genera inhabiting poultry feed mixtures in the study area. In fact, they are very

Table 1: Percentage of culture positive feed sample

Sample Feed positive	Type Number	Tested Number For Fungi	Positive (%)
Broiler Starter	63	36	57.00
Broiler Starter	63	45	71.40
Broiler Starter	63	63	100.00
Total	189	144	76.20

Table 2: Percentage distribution of fungal isolates in poultry feeds

Species	Rate of occurrence(%)			Total	%
	Starter feed Positive(%)	Grower feed Positive(%)	Finisher feed Positive(%)		
<i>Aspergillus spp</i>				207	69.5%
<i>A.fumigatus</i>	30	39	57		
<i>A.flavus</i>	6	66(68.8%)	9	90(69.8%)	
<i>A.parasiticus</i>	6	9	18		
<i>A.ochraceous</i>	9	12	6		
<i>Fuserium spp</i>				54	18.3%
<i>F.proliferatum</i>	9	9	15		
<i>F.verticillioides</i>	3	18(18.7%)	0	24(18.6%)	
<i>F.graminearum</i>	0	3	9		
<i>Rhizopus spp</i>				36	12.1%
<i>R.stolonifer</i>	9	12	15	15(15.6%)	
Total	72(24.2%)	96 (32.3%)	129 (65.5%)	297	100

important contaminants being renowned for their ability to form a huge number of various types of toxic extrolites- mycotoxins^[13].

In the present study, high temperature and humidity might be responsible for higher frequency of isolation of *Aspergillus* spp. especially *A. fumigatus* in commercial Broiler Poultry Feeds compared with other species of *Aspergillus* which might be due to their high temperature tolerance character^[4]. Fungal contamination frequency was higher in Broiler Finisher Feeds (100%) as compared with Broiler starter (57%) and Broiler Grower (71.4%) feeds. A possible reason for low fungal contamination frequency in Broiler Starter Feeds might be inclusion of antifungal agents to prevent fungal growth. As the chicks are very sensitive to fungus oriented disease (eg. Brooder Pneumonia) and mycotoxicosis, so, antifungal inclusions are strongly used for prolonged and varied storage conditions at farms and dealer level, whereas in Broiler Grower and Broiler Finisher Feeds such inclusions might be less strongly used due to less chance of disease occurrence in case of aged birds by fungal agents.

Among the *Aspergillus* spp. isolated from feed samples, *A. fumigatus* were the predominant species followed by *A. parasiticus*, *A. ochraceous* and *A. flavus*. This results differ from some reports describing *A. niger* as the most predominant followed by *A. flavus*^[20] also *A. flavus* as the predominant species followed by *A. niger* aggregates^[2, 19, 24].

CONCLUSION

From the study it may be concluded that, in starter feed, the company used antifungal inclusions and less used such things in grower and finisher feeds. As fungal diseases are sensitive for commercial broilers that's why different feed company use high level of fungistat during feed processing. But, this practice is harmful for public health. The use of strong antifungals in every level of feeds (Starter, Grower and Finisher) may reduces the fungal contamination of feeds when storage at dealer and farm levels; On the other hand, the feed mill manufacturing process should be maintained properly and post processing contamination should be strictly avoided. Therefore, the occurrence of fungi in commercial broiler feeds may be due to pre and post processing contamination of feed ingredients, bad manufacturing process, contamination of the feed by handlers in the farm, bad feed storage facilities in the farm among others. Due to this fact, regular microbiological and mycotoxicological analysis should be performed for maintaining quality and safety of poultry feed.

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