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Investigation on Fungal Load and Their Abundance in Poultry Feed of Different Farms in Rajshahi Metropolis

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Abstract

The fungal load in feed samples of different poultry farms in Rajshahi Metropolis was assessed. The total viable fungi were found at a ranged from 1.01×10^4 to 4.01×10^8, 1.03×10^5 to 6.01×10^9 and 0.81×10^4 to 7.01×10^7cfu/g on PDA, Czapek's and Richard's media, respectively. Approximately 25-100% of the samples showed $>10^6$ cfu/g indicate the poor hygienic status of the farms. The feed samples of different poultry farms were significantly (P= 0.05) varied with the number of fungal colonies. Total six genera viz. *Aspergillus*, *Rhizopus*, *Penicillium*, *Alternaria*, *Fusarium* and *Paecilomyces* were identified from studied samples. The highest number of fungal colonies in respected medium was recorded as 215 (PDA), 316 (Czapeck's) and 228 (Richard's) in the feed of Juwel Poultry Farm. Maximum moisture (11.67%) with minimum pH (5.3) was recorded in the same sample. Among the samples the abundance of the fungal flora were recorded as 2.08- 29.71% in PDA, 2.10-21.11% in Czapeck's and 1.49-23.46% in Richards. *Aspergillus flavus* was dominating fungi and contributed 29.71, 21.72 and 23.465% in PDA, Czapek's and Richard's medium among the fungal flora. This remarkable incidence of fungi recovered from the feed samples can be indicated a potential hazard to the poultry.

Keywords: Poultry feed, fungal flora, cfu, abundance.

INTRODUCTION

Poultry is now a very important livestock industry in Bangladesh that committed to supply good quality nutritious animal protein to the nation (Shamsuddoha, 2011). Feed is one of the most important branches for the expansion of poultry farming (Alam *et al.*, 2001) and a total of 60.65% cost expanded in this purpose (Banergee, 1998). It is presume that feed may contain diverse microflora and may be contaminated at any time during growing, harvesting, processing, storage and dispersal which can be considered as potential threat to feed quality. Thus the poultry industry is keenly aware about its quality assessment (Brothers and Wyatt, 2000). The microbial diversity found in different feeds is depended on the water activity, oxygen tension, pH and nutrient composition of the feed matrix. Fungi are heterotrophic microorganisms, which are the major cause of deterioration and spoilage in stored feed or crops. A potential and more deadly hazard has been associated with the consumption of microbial toxins of bacterial and fungal origin in feed (Uwaezuoke and Ogbulie, 2008). Studies elsewhere have associated some animal feeds with toxigenic strains of fungi and bacteria of public health concern (Bilgram *et al*., 1995; White and Toreman, 1995). The poultry industry has extensively used the mold spore plate count to assess the mycological status of poultry feed (AFIA, 1999). This particular test can be less expensive than analyses for mycotoxin contamination, while yielding a number corresponding to the number of viable mold spores per unit of feedstuff. Some laboratories also furnish information regarding to identify of specific molds that were isolated from the feed and couple such information with the mold spore plate count. For these reasons, the mold spore plate count is commonly used by the poultry industry in quality

assurance programs (Tabib *et al.*, 1984). Therefore, the present investigation was undertaken to assess the fungal load covering characterization and abundance in the feed of different poultry farms of Rajshahi Metropolis.

MATERIALS AND METHODS

Sample collection

The feed samples were collected from different poultry farms of Rajshahi Metropolis during October 2013 to March 2014. The main composition of the formulated feeds were as only maize or maize with rice bran which were supplemented with Ca (3.7%), P (0.46%), lysine (0.8%) and metheonine (0.42%) as prescribed by Nilsagor Agro Ind. Ltd. The samples were collected in sterile polybag with proper labeling and brought to the laboratory, and stored at 4^0C for further experiment.

Determination of pH and moisture of the feed

The pH of each sample was determined by the digital pH meter (HANNA HI961070) following the method of Jackson (1973). For determination of moisture 100 g of each sample of poultry feed was kept at 60°C in an oven for 24 hours. The dried samples were weighted again and calculated the moisture content employing the following formula,

$$\text{Percentage of moisture content} = \frac{W_1 - W_2}{W_2} \times 100$$

Here, W_1= weight before dry and W_2= Weight after dry.

Detection of fungal load in poultry feed

Enumeration of fungal flora in the feed samples was perfumed by serial dilution technique according to Reiner (1982). Briefly 100 ml sterilized distilled water was taken in a McCarty tube. Then 1gm of feed sample was taken in the tube and shacked vigorously by vortex to make homogenous suspension. This gave a dilution of $1:10^2$. Then 1ml of first dilution was transferred by a micropipette to another test tube, which containing 9 ml of sterilized distillled water to make the dilution of $1:10^3$. From this solution a small amount (0.1ml/plate) of samples spread on PDA, Czapek's and Richard's media in Petri dish and the plates were sealed by paraflim and incubated at 30±2°C for 5-7 days. The developments of fungal colony of the plates were observed and cfu was counted by visual observation. Total cfu were calculated according to the following formula:

$$\text{cfu} = \frac{\text{Colony count on agar plate}}{\text{Total dilution of tube} \times \text{Amount plated}}$$

Identification of fungal flora

The fungi were isolated from different types of poultry feed on PDA, Czapek's and Richard's media and bacterial contamination was avoided by incorporating the 50% lactic acid in the media. A small amount of collected poultry feed ingredients sprinkled on the medium in Petri dish and incubated at 30±2°C for 7-10 days and examined periodically for the developments of fungal colony. Identification of the fungi was made grossly by their cultural and microscopical characteristics. Identification up to genus of each colony was confirmed and identification up to species level was tried, wherever possible with the help of standard mycological books and manuals (Gilman, 1957; Anisworth, 1971; Raper and Fennel, 1966; Barnett, 1962; Booth, 1971; Subramanian, 1971; Ellis, 1971 and Alexopoulos and Mims, 1979).

Determination of fungal abundance

The abundance of genera calculated on the basis of the number of colonies of a genus against the total number of colonies of all recorded genera during the entire sampling period.

Statistical analysis of the data

The experiment was conducted by using completely randomized design with six replications. Analysis of the variance (ANOVA) was done accordingly and significant differences among the treatments mean were identified by least significant differences (LSD) test at 5% level.

RESULTS AND DISCUSSION

The fungal load in different poultry feed samples were assessed on PDA, Czapek's and Richard's media (Plate 1). A high degree of variability was present to total fungal plate counts performed in this study (Table **1**). The total viable fungi were counted and ranged as 1.01×10^4 to 4.01×10^8, 1.03×10^5 to 6.01×10^9 and 0.81×10^4 to 7.01×10^7 cfu/g and approximately 25-75%, 25-100% and 25-50% of the samples showed $>10^6$ cfu/g on PDA, Czapek's and Richard's media, respectively. Total mould enumerations in poultry feed in a study by Tabib *et al.* (1984) ranged from 5 to 1.2×10^6 cfu/g. Begum *et al.* (1995) counted total fungal colony ranges $10\text{-}26.3 \times 10^3$ cfu/g. Uwaezuoke and Ogbulie (2008) recorded total fungal count 1.4×10^7 to 2.4×10^8 cfu/g in different commercial poultry feeds. This variability can be explained as mold spores are not produced in similar amounts by the various molds that can contaminate poultry feed or feed ingredients. Furthermore, once they are produced, they are not uniformly dispersed throughout a feed sample. Therefore, the type of mold

present on a feed particle, its degree of sporulation, and its tendency to become dissociated from the mycelial mass can account for a high degree of variability in the fungal plate count.

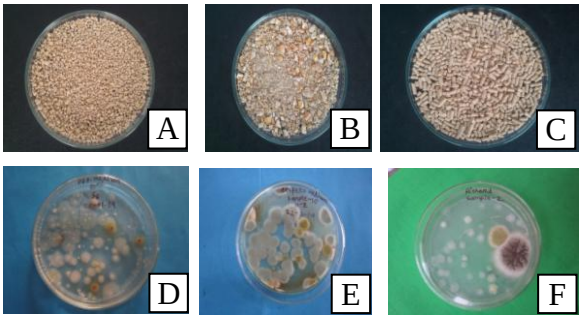

Plate 1: Poultry feeds (A, B & C) and fungal colonies on PDA (D), Czapek's (E) and Richard's (F) media.

The p^H along with moisture is the key factors for the growth of microbes. In this study, the p^H of the samples was recorded as 5.3 - 6.3 and moisture was 8.42 - 11.67% (Fig 1). Among the samples the highest number of fungal colonies in respected medium was recorded as 215 (PDA), 316 (Czapeck's) and 228 (Richard's) in the feed of Juwel Poultry Farm while maximum moisture and minimum pH was recorded. In general the number of fungi increase with the increases of moisture content and below pH is favorable for fungal growth. Thus the pH and moister of the tested feed was favourable for the growth of fungal flora. In another study, Alam *et al.* (2001) measured the moisture content of poultry feed ingredients as maize, rice bran, soybean oil cake and wheat husk as 8-11.2%, 7.9-10.5%, 10.2-15.2% and 5.9-9.2% and no. of fungal colonies were114-159, 93-115, 18-29 and 90-189, respectively. Mould growth has been associated primarily with the moisture content of feed ingredients (Thompson and Henke, 2000), but may be also positively associated with zinc concentrations in feed and the surface area available for mycofloral attack (Jones and Hamilton, 1987).

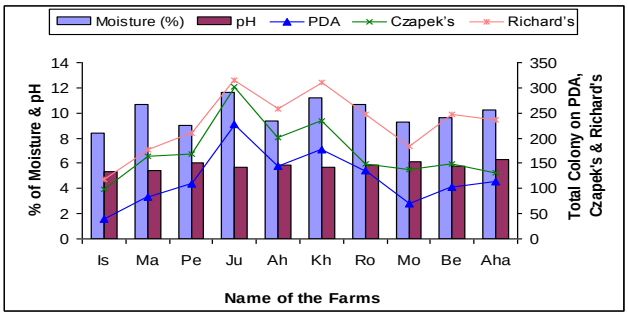

Fig. 1 The moisture (%), pH and total no. of fungal colonies occurred in the feeds of different poultry farms in Rajshahi Metropolis.

[IS= Ismail, Ma= Mahbub, Pe= Pearul, Ju= Juwel, Ah= Ahadur, Kh= Khokon, Ro= Rosid, Mo= Mostak, Be= Belal, Aha= Ahab]

Table 1. Total fungal colony count of different poultry feed samples on PDA , Czapek's and Richard's media.

Sampling area	Main Component of PF	PDA N=6		Czapek's N=6		Richard's N=6	
		Ranges of cfu/g	% of sample showing >10^6 cfu/g	Ranges of cfu/g	% of sample showing >10^6 cfu/g	Ranges of cfu/g	% of sample showing >10^6 cfu/g
Ismail PF Court	Maize	1.01×10^4 to 3.01×10^5	–	1.03×10^4 to 1.75×10^5	–	0.81×10^4 to 1.07×10^5	–
Mahabub PF, Meherchondi	Maize+ric bran	1.06×10^4 to 1.09×10^5	–	1.08×10^4 to 2.02×10^5	–	1.03×10^4 to 1.02×10^5	–
Peyarul PF, khorkhory	Maize	7.05×10^5 to 8.49×10^7	50%	1.15×10^5 to 4.19×10^7	50%	2.05×10^5 to 1.09×10^7	25%
Juwel PF, Chockpara	Maize+rice bran	3.02×10^5 to 4.01×10^8	75%	5.07×10^7 to 6.01×10^9	100%	3.02×10^6 to 7.01×10^8	50%
Ahadur PF, Narikelbaria	Maize+rice bran	1.01×10^4 to 2.05×10^5	–	1.01×10^5 to 4.08×10^5	–	1.07×10^3 to 4.09×10^4	–
Khokon PF, Chormaajardia	Maize+rice bran	1.03×10^4 to 1.5×10^8	75%	1.01×10^7 to 4.1×10^9	100%	2.08×10^5 to 5.06×10^7	50%
Roshid PF, Budpara	Maize	2.06×10^4 to 4.09×10^7	50%	3.37×10^6 to 4.4×10^7	50%	5.01×10^5 to 1.07×10^7	25%
Mostak PF, Dasmari	maize	1.07×10^4 to 3.06×10^7	25%	6.04×10^5 to 5.5×10^7	25%	1.03×10^5 to 1.1×10^7	25%
Belal PF, Noudapara	Maize+rice bran	5.05×10^5 to 6.12×10^7	25%	1.06×10^6 to 1.65×10^8	50%	3.02×10^5 to 1.04×10^7	50%
Ahab, Baya	Maize+rice bran	1.02×10^5 to 1.03×10^7	50%	1.04×10^5 to 1.01×10^7	25%	1.01×10^5 to 1.06×10^7	25%

Key PF= Poultry feed, N= Number of the samples

All the feed samples of different poultry farms found to be contaminated with the fungal flora and significantly (P= 0.05) varied with the number of fungal colonies (Table 3-5). Total 1299, 1195 and 1749 fungal colonies were counted on PDA, Czapek's and Richard's media, respectively. Among them total 4184 fungal colonies were identified and 59 were not identified. The identified fungi assigned to six genera belonging to *Aspergillus, Rhizopus, Penicillium, Alternaria, Fusarium* and *Paecilomyces* (Table 2). *A. flavus, A. niger, Rhizopus* sp, and *A. nidulans* were dominating fungi recovered on PDA. In Czapek's dominating fungi were *A. niger, A. flavus, A. fumigatus* and *Penicellium* but in case of Richard's it were *A. flavus, A. niger, Fusarium* sp. and *A. fumigatus.* All the identified genera were found in all the farms only *Fusarium* was not found in Ismail farm and *Paecilomyces* sp. was not found in Ismail, Mahbub and Roshid farm in PDA; *Pacilomyces* and *Alterneria* were not found in Ismail farm in Czapek's; and for Richard's *Alternaria* was not detected in Ismail farm and *Paecelomyces* not found in Ismail, Peyarul, Roshid, Mustak and Belal Farm. Alam *et al.* (2001) identified eight fungal genera i.e. *Aspergillus Penicillium, Rhizopus, Fusarium, Paecilomyces, Alternaria, Scopulariopsis* and *Candida* from the poultry feed. Begum *et al.* (1995) identified seventeen fungal species and among them *A. niger, A. flavus* and *Rhizopus* sp. were present in all the samples. Cook *et al.* (1991) found that *Fusarium moniliformis* was the predominant fungus in maize samples.

Table 2. Identification of fungal isolates

Serial No	Macroscopic characteristics and texture	Microscopic characteristics	Identified as
1	Velvety, wooly, whitish but later turned green fungal colony with yellowish reverse side.	The mycelium branched, colorless.Conidial head globose, light yellow green color. Vesicles globose, shaped Sterigmata uniseriate or biseriate. Conidia globose, echinulate and 3.5 to 5 µ in diameter.	*Aspergillus flavus*
2	Colony velvety, wooly, whitish but later turned black with yellowish reverse side.	Conidial head globose, large, black, Vesicles globose shaped, sterigmata two series. Conidia globose and echinulate	*A. niger*
3	Velvety, whitish but later turned bluish fungal colony.	Conidial head columner, compact, dark green in color .Conidiophores short, vesicles flask shaped, Sterigmata single series. Conidia globose and echinulate	*A. fumigatus*
4	Colony velvety, whitish but later turned yellowish green fungal colony	Conidial head short columnar, dark yellow green in. Vesicles hemispherical. Sterigmata two series. Conidia globose and echinulate	*A. nidulans*
5	Colony velvety, whitish but later turned salphur yellow fungal colony	Conidial head sulphur yellow, globose and slight splitting. Vesicles globose, hyalineSterigmata two series. Conidia globose and smooth.	*A. ochraceus*
6	Colony hairy, creamy powdery growth that later turned black	Aseptate hyphae, unbranched sporangiosphores are from the foot of rhizoids that enlarged in a cup-shaped form with the mycellial region.	*Rhizopus* sp.
7	Powdery whitish surface but later turned bluish-green with whitish reverse side and edges	Branched septate hyphae with flask shaped sterigmata. The conidiophore are unbranched with a penicillate or bluish appearance.	*Penicillium* sp.
8	Cottony, tufted and wooly, whitish to graywish but turned almost black	Branched septate hyphae... conidiadark brown in color, Septet tapering beak sometimes branched. 5-10 transverse septa and with longitudinal septa generally obclavate in shape. Each of them had borne singly on the conidiophore	*Alternaria* sp.
9	Fluffy creamy growth that later turned pinkish with a yellowish reverse side	Septate with branched conidiophore and oblong conidia	*Fusarium* sp.
10	Colony floccos, grayish brown with pale yellow shading reverse brownish.	Hyphae branched and septet. Phialides single, flask-shaped colorless.Conidia produce basipetally in very long chains at the tips of the phialides very pale brown.	*Paceilomyces* sp.

Among the samples the abundance of the fungal flora were recorded 2.08- 29.71, 2.10-21.11 and 1.49-23.46% on PDA, Czapeck's and Richard's medium, respectively (Table 3-5). *Aspergillus flavus* was dominating fungi and contributed 29.71% in PDA, 21.72 % in Czapek's and 23.465% in Richard's medium over the total fungal

flora.The high level of fungi obtained in this study can be associated with the low pH and water activity of animal feed and the physiology of the contaminating fungal genera. Animal feeds have been listed as one of the sources of microbes of farmed animals and poultry. Thus the high fungal recovery may indicate a potential hazard to the animal. Most of the fungal species have been isolated from cereals (Pitt, *et al.*, 1994) and the physiological adaptation of these fungal genera may have supported their survival.

Table 3. Number of fungal colonies and their abundance in the feed of different poultry farms on PDA medium.

Name of fungi	Ismail PF N=6	Maha-bub PF N=6	Peyarul PF N=6	Juwel PF N=6	Ahadur PF N=6	Khokon PF N=6	Roshid PF N=6	Mostak PF N=6	Belal PF N=6	Ahab PF N=6	Total no. of colony	Grand total of all colonies	Abund-ance (%)	F value	LSD ($_{p\leq0.05}$)
A. flavus	24	46	52	56	48	54	42	26	30	28	386		29.71	75.022	3.666
A. niger	18	30	38	42	32	40	36	20	28	24	272		20.94	32.082	3.816
A. fumigatus	4	12	8	18	6	16	10	8	4	6	92		7.08	11.644	3.666
A. nidulans	4	10	5	16	10	12	13	6	8	15	99		7.62	7.944	3.816
A. ochraceus	2	6	2	9	4	14	8	2	4	10	65		5.41	10.790	3.210
Rhizopus sp.	8	12	9	28	15	24	18	11	14	10	149	1299	11.47	18.805	3.960
Penicillium sp.	6	10	6	12	8	10	6	2	3	4	67		5.15	5.251	3.696
Alternaria sp.	2	2	6	8	6	10	4	2	2	1	43		3.31	8.245	2.760
Fusarium sp.	-	4	8	12	8	12	6	4	8	10	72		5.54	7.714	3.542
Paecilomyces sp.	-	-	4	8	2	6	4	-	1	2	27		2.08	9.870	2.270
Non identified	1	1	2	6	2	8	4	-	3	1	27		2.08	7.680	2.366
F value	48.95	111.11	153.403	115.71	108.410	113.318	76.603	58.978	74.477	56.575	-		-	-	-
LSD$_{(p\leq0.05)}$	2.856	3.397	3.397	3.837	3.541	3.633	3.889	2.713	3.058	3.091	-		-	-	-

Key PF= Poultry farm N= Number of the samples - = Not detected

Table 4. Number of fungal colonies and their abundance in the feed of different poultry farms on Czapek's medium .

Name of fungi	Ismail PF N=6	Maha-bub PF N=6	Peyarul PF N=6	Juwel PF N=6	Ahadur PF N=6	Khokon PF N=6	Roshid PF N=6	Mostak PF N=6	Belal PF N=6	Ahab PF N=6	Total no. of colony	Grand total of all colonies	Abund-ance (%)	F value	LSD ($_{p\leq0.05}$)
A. flavus	22	30	38	48	40	50	32	22	28	33	343		21.72	33.259	4.363
A. niger	30	38	46	62	51	39	22	38	26	30	382		21.11	55.378	4.099
A. fumigatus	26	30	22	48	28	42	22	26	20	21	285		15.75	37.643	3.960
A. nidulans	2	5	8	16	12	18	7	6	6	5	85		4.69	11.965	3.874
A. ochraceus	2	6	10	22	12	15	16	10	15	11	119		6.57	14.995	3.712
Rhizopus sp.	3	11	12	19	8	16	8	8	12	5	102	1195	5.63	12.824	3.510
Penicillium sp.	12	28	16	42	30	38	23	16	18	12	235		12.99	42.604	4.233
Alternaria sp.	-	8	6	10	5	7	8	4	6	4	58		3.20	4.833	3.279
Fusarium sp.	2	6	5	19	7	13	8	6	12	10	88		4.86	11.484	3.727
Paecilomyces sp.	-	2	6	10	4	8	2	1	4	1	38		2.10	11.218	2.549
Non identified	-	-	-	6	4	2	1	-	1	-	14		4.09	9.143	1.771
F value	115.84	108.20	107.049	161.554	124.107	118.484	58.168	75.025	40.348	82.694	-		-	-	-
LSD$_{(p\leq0.05)}$	2.750	3.368	3.596	3.837	3.759	3.889	3.368	3.494	3.596	3.186	-		-	-	-

Key PF= Poultry farm N= Number of the samples - = Not detect

Table 5. Number of fungal colonies and their abundance in the feed of different poultry farms on Richard'smedium

Name of fungi	Ismail PF N=6	Maha-bub PF N=6	Peyarul PF N=6	Juwel PF N=6	Ahadur PF N=6	Khokon PF N=6	Roshid PF N=6	Mostak PF N=6	Belal PF N=6	Ahab PF N=6	Total no. of colony	Grand total of all colonies	Abund-ance (%)	F value	LSD ($_{p\leq0.05}$)
A. flavus	8	8	24	40	30	36	38	26	32	30	282		23.46	46.224	4.294
A. niger	4	5	20	32	21	25	29	12	22	18	188		15.64	52.027	3.347
A. fumigatus	3	6	12	22	12	26	18	8	9	16	132		10.98	22.019	4.016
A. nidulans	6	8	10	20	16	18	11	3	8	6	106		8.82	16.749	3.573
A. ochraceus	2	12	10	22	12	14	8	2	4	2	88		7.32	33.825	2.917
Rhizopus sp.	4	6	8	20	9	12	6	3	5	8	81	1749	6.74	11.769	3.727
Penicillium sp.	2	4	7	18	6	8	4	4	4	7	61		5.07	12.272	3.313
Alternaria sp.	-	4	6	18	12	10	6	2	4	2	64		5.32	22.533	2.993
Fusarium sp.	10	16	12	22	18	19	14	10	13	19	153		12.72	6.671	4.153
Paecilomyces sp.	-	3	-	6	4	4	1	-	2	2	22		1.83	5.449	2.270
Non identified	-	2	-	8	4	2	-	-	-	3	18		1.49	18.455	1.570
F value	13.050	11.141	25.914	37.519	29.504	52.682	92.024	22.030	52.271	66.800	-		-	-	-
LSD$_{(p\leq0.05)}$	2.360	3.186	3.541	3.965	3.705	3.624	3.186	5.359	3.426	2.891	-		-	-	-

Key PF= Poultry farm N= Number of the samples - = Not detect

The occurrence of *Aspergillus*, *Fusarium* and *Penicillium* could be as a result of their high pathogenicity as reported by researchers elsewhere (Pitt *et al.*, 1994). These organisms although on their own can cause several poultry and farmed animal infections, they also produce mycotoxins that are also of public health importance to both humans and their farmed animals. The socio-economic and health implication of these findings are enormous. Economically, the presence of these fungal genera has been reported to overwhelmingly affect the viability of some animal husbandry undertaking and agriculture in general (Misra *et al*., 1995; Ogbulie, 1995). With the high colonization of fungi of public health concern in poultry feeds, good manufacturing practice, handling and retailing methods need to be improved to enhance the microbiological quality of these products. Such information is also required to take necessary actions for the prevention and control of disease.

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