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An investigation into the microbial infestation of poultry feeds

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Abstract

The Experiments were carried out during the period of July 2001 to December 2002. Infestation of poultry feeds by fungi and aflatoxins content in feeds were determined. Ten feed samples were collected from ten different farms located in three different areas of Bangladesh. Five different fungal species belonging four genera were detected which were *Aspergillus flavus, Aspergillus niger, Fusarium spp, Rhizopus stolonifer and Penicillium spp.* Among them *Aspergillus flavus* and *Aspergillus niger* were found in all the feed samples and the rest were occasionally found. Effect of storage and moisture content of feeds were studied for determining the influence on occurrence of fungal flora in feeds. Higher the storing period of feed along with higher moisture content resulted superior incidences of fungal population in stored feeds. Studied on storage practice with 4 duration of F_1 sample revealed that the presence of aflatoxins were 30.52ppb, 33.36ppb, 37.32ppb and 48.35ppb of 5, 10, 15 and 20 days after production of feeds, respectively.

Keywords: Investigation, Poultry Feed, Storage, Fungi and Alfatoxin

Introduction

The people of Bangladesh like other developing countries are experiencing the lack of nutrition. There is a great deficiency of protein, especially animal protein, we have in our daily food menu. In order to build a nation of people with good mental and physical health, dietary supply should include adequate animal protein. The proportion of animal protein to vegetable protein in the daily diet is more than 66% in the advanced societies, whereas the availability of animal protein in the diet of the people of Bangladesh is only 9% (Ahmed *et al*., 1977). Poultry production is an important part of livestock production. Poultry meat contributes around 37% of the total animal protein supply in Bangladesh (Ahmed and Islam, 1990). By increasing the productivity of poultry meat and eggs, the existing gap between supply and demand of animal protein can be bridged. Poultry meat and eggs are quality food in respect amino acid ingredients and therefore, can improve considerably an otherwise imbalanced diet. It also provides cash income and creates employment opportunity. Though presently only 15% of the total poultry products are coming from commercial farms (Ahmed and Islam, 1990). Poultry industry has established its position as the fastest growing segment in the agricultural sector.

Profitable poultry farming is a highly specialized job in which a lot of factors may be responsible to offset the profit amount. Among these, the availability of quality feeds is the most important as nutrition is one of the most important factors for successful poultry farming, Feed alone accounts for approximately 65-70% of the total cost of production of poultry meat and eggs. A booming poultry feed business thus is appearing all over the country. A number of feed mills are producing poultry feed, which is purchased by farm owners according to their need. Poultry farmers do not have enough facility to analyze and monitor the quality of feed. In view of availability and source of different feed ingredients, the level of nutrients in the prepared ration may vary from what is actually desired. As the ingredients used to prepare poultry feed are mostly from animal origin and cereal grains, these are easily contaminated by storage fungi that affect the quality of poultry feed. Bangladesh is a warm and humid country. In rainy season, the humidity persists at around 90-95%, which is very favorable for the growth of fungus (Sert, 1991). Feed ingredients containing moisture contents safe level are often stored for several months in the godown. Many of the storage fungi *viz: Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger, Fusarium spp.* produce mycotoxins. Among them *Aspergillus flavus* and *Aspergillus parasiticus* produce a mycotoxin known as aflatoxin (Skau, 1964). Besides, the persistent humid environment of the country facilitates the production of aflatoxin in the poultry feed. Aflatoxin once in the food chain is one of the most important mycotoxin that causes great damage to poultry and other domestic animals as well as human being (Humphreys, 1999). Available evidence indicates that aflatoxin B_1 causes dramatic alterations in nucleic acid and

protein synthesis in liver (Rao, 1981). Aflatoxin causes aflatoxicosis in poultry when feed efficiency and growth rate of poultry are seriously decreased. It reduces the number of egg production as well as egg weight. Pathological alterations associated with aflatoxicosis are enlarged fragile liver with yellow discoloration, atrophy of bursa of frabricus and thymus (Sapcota, 2001). In extreme situation, it may cause death of the birds (Rao, 1981). It increases susceptibility of chickens to salmonellosis, aspergillosis, coccidiosis and Merek's disease (Pier, 1981). Aflatoxin is reported to be a potent carcinogen. Considering the deleterious effect of aflatoxin on poultry farming, its presence in the feed needs to be monitored and certified before marketing. Expiry date should also be determined and printed on the feed package marketed. Before implementing such regulatory measures, enough research data supporting the regulations must be made available. The objectives of the present studies were determination of occurrence of fungal flora with the poultry feeds. Quantification of phycotoxins produced by the fungal flora present in the stored feeds. Evaluation of storing period and moisture content on the quality of poultry feeds.

Materials and Methods

***In-vitro* Experiments:** The *in-vitro* experiments were conducted at Department of Plant Pathology, Bangladesh Agriculture University (BAU), Mymensingh. Toxins were determined in Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka.

Locations: Feed samples were collected from ten different poultry farms located in different areas of Bangladesh. Four of them are situated at Mymendingh, four at Gazipur and two at Dhaka.

Table 1. Precise information about the poultry farms under investigation were sample collection in July 2002 to December 2002

No. of farms	Location	Types of farm	No. of birds	Sources of feed	Types of feed
Farm-1	Mymensingh	Broiler production	500	Feed-mill	Mash
Farm-2	Mymensingh	Broiler production	1000	Feed-mill	Mash
Farm-3	Mymensingh	Layer	500	Self-production	Mash
Farm-4	Mymensingh	Breeder	10,000	Own feed-mill	Pellet
Farm-5	Dhaka	Broiler production	800	Poultry feed shop	Mash
Farm-6	Dhaka	Broiler production	1000	Poultry feed shop	Mash
Farm-7	Gazipur	Big hatchery	10,00,000	Own feed-mill	Layer-Mash
Farm-8	Gazipur	Broiler	5,000	Owned feed-mill	Layer-Mash
Farm-9	Gazipur	Broiler layer	10,000	Owned feed-mill	Pellet
Farm-10	Gazipur	Breeder	10,00,000	Own feed-mill	Layer-Mash

Composite sampling: In case of big farms, ten bags were selected randomly. From each bag about 0.5 kg of feed sample was pooled together so that the total amount became 5 kg. They were mixed thoroughly and about 1 kg of composite sample was collected from the mixed feed. In case of small farms, composite sampling was made from only 2-3 bags of feed. The deviation was accepted because the small farms generally procure or prepare less amount of feed at a time. The composite sample was then divided into two. One part was kept in the laboratory, Department of plant pathology, BAU, Mymensingh for isolation and identification and other part was taken to the BLRI, Savar, Dhaka for toxin identification and quantification. A code number was given to each collected sample following the rules mentioned below-The numerical digit attached to F represents the serial number and sample number of the farm. So the code number of the feeds sample of farm were- F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8, F_9, and F_{10}.

Determination of Dry matter (DM): Ten grams of fresh ground sample were weighed by an electrical balance. The sample was kept in an oven at 200^0C for 5 hours. After every 1- hour, the samples were transferred into desiccators for the same period. Afterwards each sample was weighed in an electric balance and data was recorded. Percent DM was determined by the following formula:

$$\%DM = \left[\frac{\text{Wt. of dry samples (b)} \times 100}{\text{Wt. of fresh sample (a)}} \right], \text{ Percent moisture was calculated as } M = \frac{(a-b) \times 100}{a}$$

Preparation of dilution series: One g sample of feed (on oven dry weight basis) was placed in 9 ml water blank and mixed thoroughly thus making a dilution of 1: 10. The test tube was shaken constantly for five minutes to make uniform suspension. One ml aliquote from this suspension was ovansferred to a fresh 9 ml blank with a sterile pipette. The second dilution (1: 100) was also shaken thoroughly. One aliquote from the second dilution was withdrawn and transferred to a fresh 9 ml water blank with a fresh pipette and shaken thoroughly making a dilution of 1: 1000. 2 ml aliquote were transferred to a 8 ml water blank making a dilution of 1: 5000. 1 ml aliquote were transferred to a 9 ml water blank making a dilution of 1: 10,000.

Plating: One ml aliquots from the final diluents were withdrawn with a sterile pipette, put in a sterile pipette, and put in a sterile petridish. Thus, sample @1 ml from each of the dilution series were plated to make 5 plates having 5 dilutions. Cooled (45^0C) acidified PDA was poured into five plate. For each composite sample, there were 3 replications. After pouring culture medium to the plate, the plates were given gentle swirling motion for some time to mix the feed diluents and the medium in order to disperse fungal spores and mycelia fragments more or less uniformly in the culture plates. After solidification, the plates were kept in inverted position, and incubated to allow fungal growth.

Recording of microorganism: The inoculated petridishes were incubated at room temperature for 4 days, and since after 48 hours of incubation, the appearing fungal colonies were daily counted until the colonies start to coalesce due to growth. From the colonies appearing per ml diluents the population of the fungus per g of feed was estimated by multiplying the average number of colonies/ml in 5 petridishes by the dilution factor. Population of fungi = Average number of total colonies/ml in 3 (replicates) petridishes $\times$ dilution factor.

Purification

The fungal pathogens were purified by hyphal tip and single spore culture method, the Fungus was grown in the petridishes containing 2% water agar(WA) medium. The medium supported a sparse growth of fungi and hypha remained well apart from each other. The plates were observe under stereoscopic microscope and Tips of the hyphae, along with a small block of medium, were cut by a thin and sharp sterilized needle. The blocks were immediately transferred into sterile PDA containing plates and incubated at room temperature for seven days. The cultures were stored in a refrigerator.

In single spore culture method, a small block of solid agar medium was taken on the tip of a sterile inoculating needle and touched to the surface of the fungal culture gently to adhere spore on the agar block. The block was glided over a solid PDA plate gently. The spores were dispersed on the medium along the sliding line. The plates were observed under stereoscopic microscope and well separated spores were collected by means of a tiny piece of sterile filter paper previously fixed on the tip of a sterilized needle. The filter paper was transferred immediately to a PDA plates and incubated at room temperature for 7 days. The purified culture thus obtained was kept in a refrigerator for later use.

Identification: Slides were prepared from the pure cultures and different structures of the organisms were observed under microscope. The fungus was identified upto genus and species with the help of manuals (Barnett, 1965; Hanlin, 1990).

Determination of aflatoxin

Reagents

- Methanol
- 70% methanol solution: 70% methanol solution was prepared by mixing 70 ml pure methanol with 30 ml distilled water.
- Distilled water.

Preparation of samples: The samples were stored in a cool place, protected from light. A representative drawn sample (according to accepted sampling techniques) was ground and thoroughly mixed prior to proceeding with the extraction procedure.

- 5 g of ground sample were weighed and added it to a suitable container with 25 ml of 70% methanol)
- It was shaken vigorously for three minutes (with shaker)
- The extracts were filtered through what man no. 1 filter
- 1 ml of the obtained filtrate were diluted with 1 ml of distilled water
- 50 ul per microwell were used in the test

Test procedure

- A sufficient number of wells were inserted into the microwell holder for all standards and samples to be run. It was recorded standard and sample positions.
- 50 ul of standard or prepared sample was pipette to separate well; a new pipette tip was used for each standard or sample.
- 50 ul of enzyme conjugate were added to the bottom of each well (red cap).
- 50 ul of the aflatoxin antibody were added to each well (black cap). It was mixed thoroughly and incubated for 10 min (+/-1) at room temperature (18-300C).
- The liquid was dumped out of the wells into a sink. The microwell holder was tapped upside down onto a clean filter towel to remove all remaining liquid from the wells. Using a wash bottle or multichannel pipette, the wells were filled with distilled water. The wells were emptied again and removed all remaining liquid. The washing steps were repeated two times.
- 2 drops (alternatively 100 ul) of substrate/chromogen (white dropper) were added to each well. It was mixed thoroughly and incubated for 5 min (+/-0.5) at room temperature (18-300C; 65-860F) in the dark.
- 2 drops (alternatively 100 ul) of stop solution were added to each well (yellow dropper). It was mixed well and measured the absorbance at 450 nm against an air blank. It was read within 10 minutes on mixing.
- Calculation

The absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standards) and multiplied by 100. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentage.

$$\% \text{ absorbance} = \frac{\text{absorbance satndard (or sample)}}{\text{absorbance zero standard}} \times 100$$

Experimental design: The experiments were laid out in completely Randomized Design (CRD) with 3 replications for each.

Analysis of data: Treatment means were compared with Duncan's Multiple Range test (DMRT). Regression analysis was performed on the relationship of microbial population as against moisture content in the feed samples. Analysis of all the data were performed on treatment values calculated by is sine transformation method (Zaman *et al.,* 1982).

Results and Discussion

Dry matter (DM) content of the feed

It was observed that % DM in the feed varied from sample to sample. Which ranged from 84.5% to 89.5%. Highest %DM was found in F_8 and F_{10} samples and lowest in F_3 samples.

Detection and identification of fungi associated with poultry feeds

Comprising of 10 feed samples obtain from ten different farms were tested to observe the prevalence of fungi associated with poultry feeds. Five different fungi were identified through dilution plate method. The fungi were as follows *Aspergillus flavus, Aspergillus niger*, *Fusarium spp, Rhizopus stolonifer* and *Penicillium spp.* The fungi associated which individual feed samples are presented in Table 2.

Fungi associated with F_1 feed sample

Four fungi genera were associate with F_1 feed sample. These were *Aspergillus flavus, Aspergillus niger, Fusarium spp, Rhizopus stolonifer and Penicillium spp.* The recorded average number of colonies per gram of feed was 2.07×10^4, 0.67×10^4, 0.27×10^4 and 0.13×10^4 of *Aspergillus flavus, Aspergillus niger, Fusarium spp, Rhizopus stolonifer and Penicillium spp,* respectively (Table 2).

Fungi associated with F_2 feed sample

A total of three fungi genera were recorded from **F_2** feed sample. The associated fungi
Were *Aspergillus flavus, Aspergillus niger and Fusarium spp,* Average no of colonies per gram of feed were 1.47×10^4, 1.13×10^4 and 0.33×10^4 of *Aspergillus flavus, Aspergillus niger and Fusarium spp,* respectively as shown in Table 2.

Fungi associated with F_3 feed sample

Three fungal genera were recorded from F_3 feed sample. These were *Aspergillus flavus, Aspergillus niger and Fusarium spp.* The average colonies recorded per gram of feed were 3.67×10^4, 2.20×10^4 and 0.40×10^4 due to *Aspergillus flavus, Aspergillus niger* and *Fusarium spp,* respectively as are presented in Table 2.

Fungi associated with F_4 feed sample

A total of four fungal genera/species were recorded from F_4 feed sample. These were *Aspergillus flavus, Aspergillus niger, Fusarium spp* and *Rhizopus stolonifer.* The recorded average colonies per gram of feed were 0.53×10^4, 0.67×10^4, 0.33×10^4 and 0.13×10^4 of *Aspergillus flavus, Aspergillus niger, Fusarium spp* and *Rhizopus stolonifer* respectively as shown in Table 2.

Fungi associated with F_5 feed sample

Four fungal genera/species namely *Aspergillus flavus, Aspergillus niger, Fusarium spp, Rhizopus stolonifer and Penicillium spp.* associated with F_5 feed sample. The average colonies recorded per gram of feed were 1.67×10^4, 1.47×10^4, 0.33×10^4 and 0.20×10^4 due to *Aspergillus flavus, Aspergillus niger, Fusarium spp,* and *Rhizopus stolonifer* respectively as shown in Table 2.

Fungi associated with F_6 feed sample

Three fungal genera/species were recorded from F_6 feed sample. These were *Aspergillus flavus, Aspergillu sniger* and *Fusarium spp.* The average colonies recorded pergram of feed were 0.63×10^4, 1.87×10^4 and 0.13×10^4 of *Aspergillus flavus, Aspergillus niger* and *Fusarium spp,* respectively (Table 2).

Fungi associated with F_7 feed sample

Total of three fungal genera/species were recorded from F_7 feed sample. These weregenera/species were *Aspergillus flavus, Aspergillus niger and Fusarium spp.* The average colonies recorded per gram of feed were 1.53×10^4, 0.93×10^4 and 0.20×10^4 due to *Aspergillus flavus, Aspergillus niger* and *Fusarium spp,* respectively presented in Table 2.

Fungi associated with F_8 feed sample

Four fungal genera/species namely *Aspergillus flavus and Aspergillus niger were* associated with F_8 feed sample. The average colonies of *Aspergillus flavus and Aspergillus niger were* recorded per gram of feed were 0.73×10^4 and 0.67×10^4, respectively (Table 2).

Fungi associated with F_9 feed sample

A total of two fungal genera/species namely were recorded from F_9 feed sample. These were *Aspergillus flavus and Aspergillus niger.* The recorded average colonies of *spergillus flavus* and *Aspergillus niger* recorded per gram of feed were 1.47×10^4 and 0.80×10^4 of *Aspergillus flavus and Aspergillus niger,* respectively as shown in Table 2.

Fungi associated with F_{10} feed sample

Two fungal species associated with F_{10} feed sample. These were *Aspergillus flavus and Aspergillus niger.* The recorded average colonies were 0.80×10^4 and 0.13×10^4 gram of feed due to *Aspergillus flavus and Aspergillus niger,* respectively (Table 2).

Table 2. Fungal infestation of feeds of different sources

Sample	Fungal population/gram of feed × 10^4				
	Aspergillusflavus	***Aspergillusniger***	***Fusarium spp.***	***Rhizopusstolonifer***	***Penicilliumspp***
F_1	2.07 b	0.67 c	0.27 bc	0.00 b	0.13 b
F_2	1.47 c	1.13 d	0.33 ab	0.00 b	0.00 c
F_3	3.67 a	2.20 a	0.40 a	0.00 b	0.00 c
F_4	0.53 d	0.67 e	0.33 ab	0.13 a	0.00 c
F_5	1.67 e	1.47 c	0.33 ab	0.00 b	0.20 a
F_6	0.73 d	1.87 b	0.13 d	0.00 b	0.00 c
F_7	1.53 c	0.93 de	0.20 cd	0.00 b	0.00 c
F_8	0.73 d	0.67 e	0.00 e	0.00 b	0.00 c
F_9	1.47 c	0.80 e	0.00 e	0.00 b	0.00 c
F_{10}	0.80 d	0.13 f	0.00 e	0.00 b	0.00 c
LSD(0.01)	0.352	0.275	0.104	0.023	0.023
CV (%)	10.37	11.14	20.41	72.98	55.88

Figures in column having the similar letter (s) do not differ significantly as per DMRT

Occurrence of Fungal population in feeds upto 20 days after production (DAP)

It appeared from the Table 2 that feed sample after 5 days of production comprises average fungal colonies of 1.67×10^4, 1.054×10^4, 0.199×10^4, 1.013×10^4 and 0.033×10^4 of *Aspergillus flavus, Aspergillus niger, Fusarium spp, Rhizopus stolonifer and Penicillium spp,* respectively. Infestation of *Aspergillus flavus* was higher than other recorded fungi presented in Table 3.

Table 3. Occurrence of fungal population in feeds up to 20 days after production

No of sample	Fungal population/gram of feed x 10					Fungal population/gram of feed x 10				
	5 DAP					10 DAP				
	A. flavus	***A. niger***	***F. spp***	***R. stolonifer***	***P. spp***	***A. flavus***	***A. niger***	***F. spp***	***R. stolonifer***	***P. spp***
F1	2.07	0.67	0.27	0.00	0.13	4.53	2.07	0.40	0.00	0.27
F2	1.47	1.13	0.33	0.00	0.00	3.47	2.60	0.53	0.20	0.00
F3	3.67	2.20	0.40	0.00	0.00	6.33	4.47	0.67	0.27	0.00
F4	0.53	0.67	0.33	0.13	0.00	1.07	1.00	0.40	0.33	0.00
F5	1.67	1.47	0.33	0.00	0.20	3.40	2.47	0.53	0.00	0.60
F6	0.73	1.87	0.13	0.00	0.00	1.00	2.87	0.27	0.00	0.00
F7	1.53	0.93	0.20	0.00	0.00	2.60	1.67	0.27	0.00	0.13
F8	0.73	0.67	0.00	0.00	0.00	1.13	1.47	0.00	0.00	0.00
F9	1.47	0.80	0.00	0.00	0.00	2.87	1.47	0.27	0.00	0.00
F10	0.80	0.13	0.00	0.00	0.00	1.67	0.33	0.07	0.00	0.00
LSD(.01)	0.352	0.275	0.104	0.023	0.023	1.092	0.854	0.367	0.194	0.194
CV (%)	10.37	11.14	20.41	72.98	55.88	16.76	17.99	46.81	102.06	81.65

Table 3. Contd.

No of sample	Fungal population/gram of feed x 10					Fungal population/gram of feed x 10				
	15 DAP					20 DAP				
	A. flavus	*A. niger*	*F. spp*	*R. stolonifer*	*P. spp*	*A. flavus*	*A. niger*	*F. spp*	*R. stolonifer*	*P. spp*
F1	6.80	4.13	0.73	0.00	0.53	8.93	6.67	0.93	0.20	0.83
F2	6.67	5.13	0.73	0.20	0.00	10.13	7.13	1.43	0.73	0.13
F3	11.87	7.20	0.73	0.33	0.00	16.47	9.20	0.73	0.63	0.00
F4	3.60	2.80	0.67	0.40	0.00	5.13	4.20	0.80	1.13	0.00
F5	7.40	5.53	0.53	0.00	0.87	12.20	8.67	0.80	0.00	1.17
F6	2.20	4.53	0.33	0.00	0.00	3.60	7.00	0.53	0.00	0.00
F7	4.67	2.47	0.33	0.00	0.33	7.00	3.60	0.80	0.00	0.40
F8	2.27	2.67	0.00	0.00	0.00	3.80	4.00	0.00	0.00	0.27
F9	4.93	2.80	0.47	0.00	0.00	7.20	4.47	0.73	0.33	0.33
F10	2.13	0.93	0.20	0.00	0.00	3.60	1.87	0.93	0.00	0.00
LSD(.01)	1.255	0.999	0.525	0.265	0.255	3.222	1.997	0.232	0.320	0.232
CV (%)	10.29	11.27	47.55	123.72	63.20	17.76	15.13	12.92	45.32	3.245

It was found that feed produced 10 days before testing yielded an comparatively higher fungal colonies that feed produced 5 days before testing feed 10 days stored (old) yielded 2.807×10^4, 2.065×10^4, 0.334×10^4, 0.08×10^4 and 0.1×10^4 of *Aspergillus flavus, Aspergillus niger, Fusarium spp, Rhizopus stolonifer and Penicillium spp,* respectively. Infestation of feed with different fungi differed significantly from one feed to another.

The feed infestation by the fungi were significantly higher in 15 days stored (old) feed than the 5 days and 10 days old feed. The average colonies were 5.245×10^4, 3.819×10^4, 0.472×10^4, 0.09×10^4 and 0.173×10^4 of *Aspergillus flavus, Aspergillus niger, Fusarium spp, Rhizopus stolonifer and Penicillium spp,* respectively. Maximum infestation was due to *Aspergillus flavus* and minimum for *Rhizopus stolonifer*.

The fungal genera/species odtained in do days stored (old) samples namely *Aspergillus flavus, Aspergillus niger, Fusarium spp, Rhizopus stolonifer and Penicillium spp.* were 7.806×10^4, 5.28×10^4, 0768×10^4, 0.302×10^4 and 0.313×10^4, respectively which were higher than that's of other group of 5, 10, and 15 days old feed sample. The samples included this group comprising more fungal infestation in general than the other sample, except *Rhizopus stolonifer.*

Aflatoxins in the feed samples

Considering the storage duration, varying concentration of aflatoxins was detected in feed samples from each group. The levels of aflatoxins were 30.52 ppb, 33.36 ppb, 37.32 ppb and 48.35 ppb in the sample of 5, 10, 15 and 20 DAP respectively. The corresponding association of total fungal genera were 2.97×10^4, 5.39×10^4, 9.81×10^4 and 14.47×10^4 at 5 DAP, 10 DAP, 15 DAP and 20DAP respectively.

Correlation and regression study

Correlation study was done to examine the relationship between % feed moisture content and fungal population variation at different days after production (DAP) of feeds. Relationship between % content and fungal population revealed a significant and positive correlation followed by the regression equation $y = 0.6562x - 5.1867$ ($r = 0.7769$), $y = 1.1743x - 8.8658$ ($r = 0.7004$), $y = 2.2031x - 16.908$ ($r = 0.7708$) and $y = 2.9952x - 21.442$ ($r = 0.7932$) at 5 DAP, 10 DAP, 15 DAP and 20 DAP respectively. This indicated that with the increasing moisture content in the feed, the fungal growth correspondingly increased.

The fungal flora present in feeds were isolated, detected and indentified by using dilution plate method after 5, 10, 15 and 20 days after production of feeds. All the feeds were infested in common by *Aspergillus flavus* and *Aspergillus niger*. Though *Rhizopus stolonifer* and *Penicillium spp*. were occasionally found. A positive correlation was found to exist between the moisture content and fungal infestation in feeds. Feed at 20 DAP was found to contain significantly higher amount of fungal flora as well as aflatoxin than that of feeds stored 5, 10 and 15 days after storing.

Conclusion

The information obtained through this research work is basic in nature in Bangladesh that make the foundation for initiation of taking a well-planned research program for developing the quality of poultry feed in the country. However, the finding of this study will be of immense use by the personnel's in this discipline.

The Bangladesh poultry diet is deficient in balance in terms of poultry nutrition. In poultry industry, quality and cost of feed determine the profitability of the business. So, it deemed necessary to investigate the quality of poultry feed. Considering the prevailing humid condition of Bangladesh, the role of storage fungi and the occurrence of fungus produces toxins, such as aflatoxin, which is deteriorating the quality of poultry feed. Besides presence mycotoxins are introduced in the food chain it persistently affects every member in the chain. Therefore, presence mycotoxin in the poultry feed and the factors infusing their concentration need to be investigated for that matter. Ten feed samples were collected from ten different farms located in different areas of Bangladesh. Four of them are situated at Mymensingh, four at Gazipur and two at Dhaka. The fungal flora present in feeds were detected, isolated and indentified by using dilution plate method after 5, 10, 15 and 20 days after production of feeds. Five different fungal species belongings to four genera were identified, *viz. Aspergillus flavus, Aspergillus niger, Fusarium spp., Rhizopus stolonifer* and *Penicillium spp*.

All the feeds were infested in common by *Aspergillus flavus* and *Aspergillus niger,* where *Rhizopus stolonifer* and *Penicillium spp*. were occasionally found. A positive correlation was found to exist between the feed moisture content as well as duration of storage after production influenced fungal infestation in feeds. Feed at 20 DAP was found to contain significantly higher amount of fungal flora as well as aflatoxin that feeds stored 5, 10 and 15 days after storing.

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