PREVALENCE OF MICROFLORA AND POTENTIALLY TOXIGENIC FUNGI IN POULTRY FEED MIXTURES

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Abstract
Eight different commercial poultry feeds obtained from their trade outlets in Dhaka city, was studied to determine the degree of risk of the occurrence of selected bacteria and microscopic fungi. Recorded results indicated that the bacterial count was higher in Broiler (3.9 x 10^3) cfu/g and it was relatively lower in Mash (1.6 x 10^2) cfu/g. Added to this; fungal count was highest in Starter (7.5x10^5) cfu/g and least in Pellets (1.8x10^3) cfu/g. Elevated number of pathogenic bacteria was found in feed samples while the majority (66.7%) of the microbial contaminants were detected as Gram negative rods,. The most common fungal genera detected in tested samples included, Penicillin, Aspergillus, Fusarium, Alternaria, Rhizopus and Mucor. Microorganisms require nutrients as source of energy for the production of ATP. Results of Mineral analysis indicated that the feed samples contain essential nutrients including Na^+, K^+, Ca^{2+}, Mg^{2+} and P. Proximate composition revealed the presence of moisture, ash, fat, protein contents and crude fibre. These data provide a clear insight that a Hazard Analysis Critical Control Point (HACCP) program should be instituted for the animal feed industry to reduce the risk of microbial contamination as well as potential human health hazards.

Keywords: bioburden, toxigenic fungi, microbial contamination, poultry feed, minerals.

Submitted: 01.04.2015 Reviewed: 29.05.2015 Accepted: 08.06.2015

1.INTRODUCTION

Poultry Feeds are composed largely of grains including, corn, wheat, barley, cake meal, sunflower seeds, peanuts and protein products of animal origin like fish meal, meat or bone meal, slaughter house offal's etc (Arotupin et al., 2007). Many authors (Wilson, 1990; Dhand et al., 1998; Ceveger and Yalcin, 2003) have already summarized that these feeds are the source of nutrients which contain essential vitamins and essential inorganic compounds to generate the heat and to support chemical reactions that allow the expressions of animal potentials.

From epidemiological and safety point of view, proper management of animal feeds are necessary as they considered as the major infectious routes for human due to the possible presence of pathogenic microorganisms that could represent a potential health hazards. Poultry feed mixtures are reported to be contaminated with various types of microorganisms depending on the function of material, location of its origin, climatic conditions encountered, even at the time of harvesting, processing, storage condition, transportation, packaging materials etc. (Arotupin et al., 2007). The major contaminants of poultry feed include the bacterial genus- *Escherichia coli*, *Salmonella* spp., *Listeria* spp., *Enterococcus fecalis, Erwinia herbicula* etc. (Klinger and Lapidot, 1993; Dhand et al., 1898; Jeffrey et al., 1998; D'Mello, 2006). The main sources of fugas in animal food mixture originate primarily from cereals (the plant origin) (Creppy, 2002; Kwiatek and Kukier; 2008). Potential formation of mycotoxin can be occurred due to the development of Mould on the surface of curnels under storages condition which can results the incidence of diseases in poultry feed (Cegielska-Radziejewska et al., 2013). Due to their resistance to the action of high temperature as well as diversity of toxic effects, the presence of mycotoxins in poultry feeds constitutes a potential threat to human and fatal consequences in form of direct losses

due to animal mortality (Hussein and Brasel, 2001; Chełkowski, 2008).

In Bangladesh, the poultry sub-sector is particularly important for the people in the context of growth of agriculture, for the improvement of diets as it is the main source of protein where consumers purchase live poultry, processed poultry meat and eggs from poultry shop (Islam, 2003). According to the report of Bangladesh Food Security Investment Forum (May, 2010), poultry and poultry products are the most important source of export earnings; contributing 4.11% to the national GDP, 12% of the agricultural GDP. But the export market of Bangladesh is threatened for low quality processed products which are contaminated with different types of microorganisms. Therefore, the present study was conducted- (i) to identify the bacterial and fungal population associated with the poultry feeds. (ii) To analyze the proximate composition (iii) to determine the mineral composition of the poultry feed mixtures.

2. MATERIAL AND METHODS

Selection of the Study Area for Investigation*:* A total 50 samples of eight different commercial poultry feeds namely; mash, pellets, crumbles grower, layer, broiler, starter and scratch obtained from 14 different trade outlets of the Dhaka metropolis, was taken for microbiological analysis. Experiments were carried out within 1-8 hours after collecting the samples. All the samples were kept at 4°C until these were analyzed.

Isolation and Identification of the Bacterial Isolates-

Bacterial Examination: All the samples were serially diluted up to 10^{-3}. If discrete colonies were not detected in 10^{-3} dilution, further dilutions were prepared and the tests were then repeated. After incubation period of 24-48 hours at 37^0C, different types of colonies in various culture media were observed carefully. The number of organisms per ml of original culture was calculated by multiplying the number of colonies counted by the dilution factor. The equation is- Number of cells per ml=number of colonies x dilution factor/sample volume used.

Cultural Characterization and Biochemical studies: Morphological characteristics including shape, size, surface texture, edge, elevation, color, opacity etc. of the colonies on different media were studied. According to the Bargey's Manual of Determinative Bacteriology (1994), several biochemical tests were performed to identify the biochemical characteristics of the bacterial isolates. The tests were- Oxidase test, Catalase test, Indole production test, Methyl Red test, Voges-proskauer test, Urease test, Citrate utilization test, Triple Sugar Iron test and Carbohydrate (Lactose, Sucrose and Dextrose) fermentation tests.

Isolation and Identification of Fungi*:* Fungi grow on artificial media in variety and their gross colonies morphology alone can give a preliminary idea about identification of most fungal strains. A small amount of inoculums was placed on the Potato Dextrose Agar (PDA) plate using an inoculating loop and incubated at 25°C. The development of the colony over a period of three days was observed considering the texture, size, and color of the colony, sporulation, and reverse color. Lactophenol-cotton blue was used to observe the microscopic feature. The solution serves as a stain of fungal elements and also kills the poration of culture under examination. The suspected fungi were streaked on SDA (Saboraud Dextrose Agar) plates to confirm that they were fungi. This was followed by Standard biochemical test and microscopic observation.

Proximate and Mineral Analysis: Proximate composition including Moisture, Ash content, Fat content, Protein content, Crude fiber etc. analysis of the poultry feed sample was performed according to the procedure described by AOAC (1990) using nitrogen to protein conversion factor. On the other hand, minerals of commercially available poultry feed (Sodium, Phosphorus, Potassium, Calcium, and Magnesium) were detected by the

procedure of AOAC (1990) with Atomic Absorption Spectrophotometer.

3. RESULTS AND DISCUTION

Determination of Total Viable Bacterial and Fungal Count: For the determination of total bacterial count Nutrient Agar (NA) was used. Total bacterial count helps to determine the concentration of the aerobic or heterotrophic microorganisms present in the samples. Results showed that the mean TVC of bacterial isolates varied between (1.6×10^3) to (3.9×10^3) cfu/g. *Staphylococcus* spp. was the highest prevalent organism 32% (16 in 50) while *Micrococcus lutius* was the lowest prevalent 8% (4 in 50). On the other hand, for the determination of total fungal count, Potato Dextrose Agar (PDA) was used. The highest fungal count was recorded as (5.6×10^3) cfu/gm and the lowest count was (7.5×10^5) cfu/gm. The results are summarized in the following table 1. **Occurrence of Pathogenic Bacteria in Poultry Feed Samples:** Coliform, as the primary pathogens, was isolated on MacConkey agar media. Suspected lactose fermenting bacteria were then inoculated into EMB agar media. The isolated *E. coli* organisms showed green metallic sheen on the media. Biochemical tests also confirmed the presence of *E. coli.* Total Coliform Count (TCC) was ranged between (1.7×10^2) MPN/g to (2.3×10^3) MPN/g on average while the Total Fecal Coliform Count (TFCC) count was (1.7×10^2) MPN/g to (6.3×10^2) MPN/g among the samples analysed. Exposure of coliforms were distributed as; Total coliform (15 in 50, 30%), Fecal coliform (11 in 50, 22%) and *E. coli* (8 in 50, 16%) samples respectively.

Table 1: Mean profile of bacteria and fungi contaminating poultry feed mixture.

Poultry Feed	Number of sample	Mean of Bacterial Microflora (cfu/g)	Mean of Fungal Microflora (cfu/g)
Mash	8	1.6×10^2	9.0×10^2
Pellets	8	3.0×10^2	1.8×10^3
Crumbles	4	2.4×10^3	4.1×10^3
Grower	10	3.3×10^3	5.2×10^4
Layer	4	2.1×10^2	3.7×10^3
Broiler	8	3.9×10^3	3.2×10^4
Starter	3	3.1×10^3	7.5×10^5
Scratch	5	9.1×10^2	3.4×10^5

Table 2: Frequency of bacterial microflora in poultry in feed samples.

Food Categories	Frequency of Isolation n (%)					
	Pseudomonas spp.	*Escherichia coli*	*Micrococcus lutius*	*Alcaligenes*	*Staphylococcus spp.*	*Vibrio cholerae*
Mash (n=8)	3 (37.5%)	2 (25.0%)	1(12.5%)	0 (0.0%)	4 (50.0%)	0 (0.0%)
Pellets (n=8)	3 (37.5%)	1 (12.5%)	0(0.0%)	1 (12.5%)	3 (37.5%)	0 (0.0%)
Crumbles (n=4)	0 (0.0%)	1 (25%)	1 (25.0%)	0 (0.0%)	2 (50.0%)	0 (0.0%)
Grower (n=10)	2 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (20.0%)	0 (0.0%)
Layer (n=4)	0 (0.0%)	1 (25%)	0 (0.0%)	0 (0.0%)	2 (50.0%)	0 (0.0%)
Broiler (n=8)	3 (37.5%)	0 (0.0%)	1(12.5%)	1 (12.5%)	0 (0.0%)	1 (12.5%)
Starter (n=3)	0 (0.0%)	2 (66.6%)	0 (0.0%)	0 (0.0%)	1(33.3%)	0 (0.0%)
Scratch (n=5)	1 (20.0%)	1 (20.0%)	1 (20.0%)	0 (0.0%)	2 (20.0%)	0 (0.0%)
Total (n=50)	**12 (24%)**	**8 (16%)**	**4 (8%)**	**2 (4%)**	**16 (32%)**	**1 (2%)**

Table-3: Percentages of poultry feed samples within limit values according to Regulation

Fungal counts (cfu/gm)	Number of samples	Frequency (%)
>50.000 [x]	9/50	18%
>300.000 [y]	3/50	6%
Below limiting value [z]	37/50	74%

X-Value of cfu/ gm which is not in compliance with conditions determined in Regulation for young categories of animals; Y - Value of cfu/gm which is not in compliance with conditions determined in Regulation for older categories of animals; Z - Value of cfu/gm which is not in compliance with conditions determined in Regulation for both categories of animals.

After the primary identification by microscopic and biochemical characterization, the suspected *Pseudomonas* sp. was inoculated into cetrimide and blood ager. On cetrimide agar plate the bacteria showed bright yellowish green colony which also showed fluorescence under exposure to UV ray. On blood agar plate the bacteria showed β-hemolytic zone around the colony. To differentiate *Pseudomonas aeruginosa* from the other two species nitrate reduction test was performed. Only the *P. aeruginosa* gave positive nitrate reduction test and not the other species. These demonstrate that, there might be presence of *P. aeruginosa* in the poultry feed. Study results revealed that *Pseudomonas* spp. was the highest prevalent organism 28% (14 in 50). Pathogenic prevalence in poultry feed are presented in Table 2.

Micrococcus is generally thought of as harmless bacterium, but there have been rare cases of *Micrococcus* infections in people with compromised immune systems, as occurs with HIV patients. The species *M. luteus* was differentiated from the other species by the formation of pigment. Only the *M. luteus* species produce yellow pigment which can be falsely interpreted as staphylococci.

But oxidase test easily distinguish them. *M. luteus* is oxidase positive whereas staphylococci are oxidase negative. Results demonstrated the occurrence of *M. lutius* in only 8% (4 in 50) of the poultry feed samples while *Staphylococcus* spp. was detected in 24% (12 in 50) samples. Moreover, 2 isolates were detected as *Alcaligenes* and only1 isolate was confirmed as *Vibrio cholerae* from the biochemical tests.

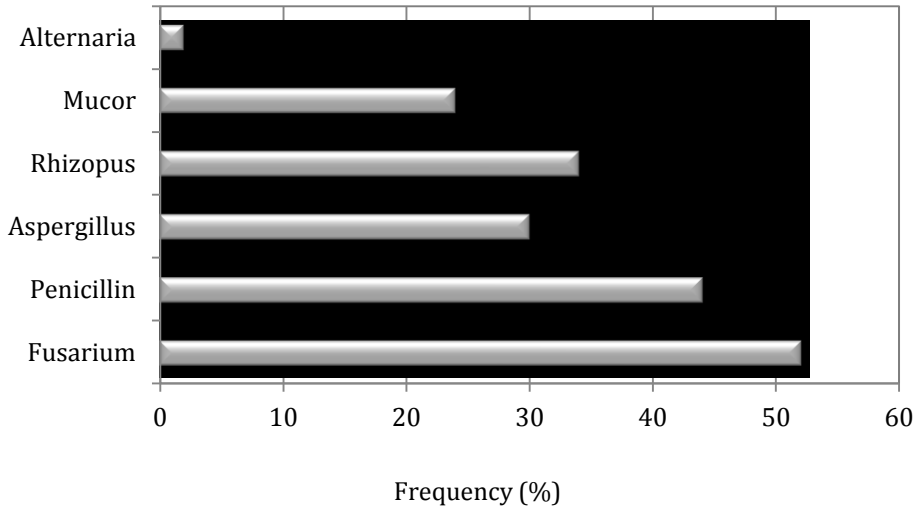

Figure 1: Frequency of fungal genera in poultry in feed samples.

Occurrence of Potentially Toxigenic Fungi

Total count of fungus is regarded as one of the criteria to evaluate the quality of animal feed. The Regulation on maximal quantities of harmful substances and components in livestock feed which is defined in Articles 8 and 9 (Official Journal of SRY 2/90), describes that the 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 of forage mixture for older animal categories or 50.000 cfu/g for younger animals. According to the defined criteria, around 18% of the feed samples for young category of poultry, 6% of the feed samples for old category and 74% of the feed samples for both categories did not satisfy the standard of microbiological adequacy (Table 3).

By microbiological analysis, 6 fungal genera have been isolated and identified. The green colored mold was confirmed as *Penicillin* from both microscopic and cultural characteristics. The presence of black mold of *Aspergillus* was also reported. Study results revealed that the highest prevalent fungal genera were *Fusarium* while *Alternaria* was the lowest prevalent. Frequency of Fungal genera in poultry feed samples are presented in Figure 1.

Proximate and Mineral composition in poultry feed samples

The proximate composition of the commercially available poultry feed was Moisture content, Ash content, Fat content, Protein content and Crude Fiber. Results showed that the Moisture content of the sample varied from 8.46% to 9.67%, Ash 6.42% to 13.51%, Fat 1.10% to 3.53%, Protein 12.03% to 14.56% and Crude fibre 2.49% to 9.35% (Table 4).

Poultry feeds are enriched with minerals which can help in the development of growth in poultry animals. Our study analysed the mineral composition including Sodium, Potassium, Magnesium, Phosphorus and Calcium of the poultry feed samples.

Results are presented in table 5.

Table-4: Proximate composition of the poultry feed mixtures.

Feed samples	Proximate composition (%)				
	Moistu conten	Ash content	Fat Content	Protein content	Crude Fiber
Mash	9.67	7.68	1.10	13.15	2.70
Pellets	9.51	7.55	1.29	12.03	2.49
Crumbles	8.87	6.42	1.56	14.02	3.89
Grower	8.10	10.85	2.75	14.25	9.35
Layer	9.87	6.85	2.23	13.56	3.60
Broiler	8.50	12.36	1.19	20.70	8.56
Starter	8.46	13.51	3.28	14.56	5.34
Scratch	8.88	7.85	3.53	13.04	5.87

Table-5: Mineral composition of the poultry feed samples.

Feed samples	Mineral composition (ppm)				
	Sodium	**Calcium**	**Potassium**	**Magnesium**	**Phosphorus**
Mash	86951.46	32456.25	2210.85	642.25	2651.56
Pellets	55914.53	38745.65	2589.25	5.98.69	2586.32
Crumbles	52625.78	35665.65	2987.53	632.65	2956.85
Grower	58923.53	42533.57	1654.41	3.65.10	3216.06
Layer	88781.52	53214.45	2579.54	656.36	2865.32
Broiler	71329.24	25896.24	836.47	235.95	2685.06
Starter	76824.32	45215.68	1972.15	565.92	3204.38
Scratch	55689.34	48566.85	1865.32	356.32	2069.36

*ppm-Part Per Million

A significant portion of the current world diet constitute from animal based food products derived from cattle, swine, sheep, poultry, and farmed fish (Sapkota et al., 2007). Animal food production researchers reported that the quality of these products is directly related to animal feeding practices (Capucille et al., 2004; Gatin et al., 2003; Zaghini et al., 2008). Pathogenic microorganisms and their secondary metabolities (especially mycotoxins) in general chain of nutrition represent the most important potential risk to poultry products and human health. Dhand *et al.,* (1998) and Hancock *et al.,* (1998) separately implicated the microbial infection outbreak occurred by *Bacillus cereus, Micrococcus lutius* and *Staphylococcus aureus* in poultry farming. The organisms are considerable high percentage indicates the alarming situation both for chicken farming and for public health as well. Reports of Aletor and Daramolla (1989) showed that the success of poultry production to rely largely on the quality of feeds, based on their nutrient formula. Bacterial and other living organisms require certain nutrients as energy source for their growth (Gottschalk, 1986). In poultry feeds various ingredients are mixed together which are enriched with animal proteins, vitamins and minerals like Na^{+}, P, K^{+}, CA^{2+}, Mg^{2+} etc. So, these raw feeding materials serve as important vehicles for bacterial contamination of poultry feed ingredients.

The detection of pathogenic and opportunistic bacteria in the poultry feed is of significant importance. Therefore, present study was carried out with a total 50 samples of poultry feed obtained from different trade outlets to assess the microbiological and nutritional qualities. Study results revealed that *Staphylococcus spp;* associated with food poisoning and capable to produce heat-stable protein enterotoxins, isolated most frequently from 32% (16 in 50) samples. On the other hand, *Vibrio cholerae* was the lowest prevalent organism isolated from 2% (1 in 50) of the samples. The results of this study were found to be in concordance to the study of Arotupin *et al*. (2007) and Sudershan *et al.* (2012) who reported high prevalence of *Staphylococcus spp in* poultry feeds.

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 (Krnjaja et al., 2008). In general the fungal propagules are a helpful indicator to determine feeds 'hygienic quality; these counts should not exceed the values of 1 x 10^5 CFU g-1 (Dalcero et al., 1998). Study results showed the presence of some potentially toxigenic fungal genera including, *Penicillin, Aspergillus, Fusarium, Alternaria, Mucor* and *Rhizopus* in poultry feed samples where *Fusarium* was the most prevalent fungus. The results of this study were found to be in concordance to the study of Cegielska-Radziejewska *et al*. (2013) who reported high prevalence of *Fusarium in* poultry feeds. *Aspergillus, Penicillium* were detected as the mold isolate. Requirement of low water activity for fungal growth allow them to grow in the dry poultry feed. Based on results, sole determination of total fungi count is not sufficient to estimate the quality of poultry feed.

4. CONCLUSIONS

From the above findings and discussion it could be concluded that, the poultry feeds those were tested for microbiological quality showed the presence of some pathogenic and opportunistic bacteria. The fungal isolates were also found in significant number and some of them like Fusarium and Aspergillus could produce mycotoxin. The findings of this study emphasize the need for constant quality assessment of the commercially available poultry feeds so that it can maintain the microbiologically stable poultry products for human consumption.

5. ACKNOWLEDGEMENT

We thank Microbiology Laboratory, North South University, Bangladesh for laboratory facilities, technical assistance and financial aid.

Conflict of Interests: The author (s) declared no conflict of interest.

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