Asian Journal of Medical and Biological Research ISSN 2411-4472 (Print) 2412-5571 (Online) www.ebupress.com/journal/ajmbr

Article

Microbiological quality of commercially available poultry feeds sold in Bangladesh

Nigar Sultana¹, Md. Atiqul Haque¹*, Md. Mostafizer Rahman¹, Mir Rowshan Akter¹, Mst. Deloara Begum¹, Md. Fakhruzzaman¹, Yeasmin Akter² and Md. Nurul Amin²

¹Department of Microbiology, Hajee Mohammad Danesh Science and Tecnology University, Dinajpur-5200, Bangladesh

²Department of General Animal Science and Nutrition, Hajee Mohammad Danesh Science and Tecnology University, Dinajpur-5200, Bangladesh

*Corresponding author: Dr. Md. Atiqul Haque, Lecturer, Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Tecnology University, Dinajpur-5200, Bangladesh. Phone: +8801717210631; E-mail: atique@hstu.ac.bd

Received: 15 February 2017/Accepted: 12 March 2017/ Published: 30 March 2017

Abstract: The study was conducted aiming at the isolation and identification of pathogens from poultry feed manufactured by four different brands namely A (broiler starter), B (broiler finisher), C (laver starter) and D (layer finisher) sold in retail stores of Rangpur city of Bangladesh. All these samples were collected from four randomly chosen outlets and analyzed by culturing in different culture media such as Nutrient broth (NB), Nutrient agar (NA), Salmonella-Shigella (SS) agar, Eosin methylene blue (EMB) agar, MacConkey agar, Triple sugar iron (TSI) agar slant, Motility, Indole, Urease (MIU) and Saboraud Dextrose agar (SDA) media. The bacterial agents were isolated and examined under light microscope for their gross morphological and conventional biochemical characteristics. The bacteriological analyses were done at the Microbiology Laboratory of Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period of January to June, 2014. Total bacterial colonies of all the samples were counted separately according to the American Public Health Association, using nutrient agar medium for total viable count (TVC), Eosine methylene blue (EMB) agar media for total E. coli count (TEC) and Salmonella-Shigella agar for TSC (total salmonella count). Saboraud Dextrose agar (SDA) media was used for detection of fungus. The virulence effect of the organism present in feed were observed by inoculating the organism in poultry. Recorded result showed that average TVC of feed sample A, B, C and D were 5.45 x 10^6 , 3.28 x 10^5 , 5.14 x 10^6 and 4.53 x 10^5 CFU/gm (colony forming unit per gram) respectively. TEC of feed sample A, B, C and D were recorded 6.25 x 10⁵, 8.26 x 10^3 , 5.52 x 10^5 and 5.65 x 10^4 CFU/gm respectively. TSC of feed sample A, B, C and D were recorded 3.15 x 10^4 , 2.68 x 10^3 , 4.46 x 10^3 and 1.19 x 10^4 CFU/gm respectively. The highest TVC, TEC and TSC were found in broiler starter (feed sample A) and lowest TVC, TEC and TSC were found in broiler finisher (feed sample B). Fungal count was 1.85×10^5 CFU/ gm in layer finisher (feed sample D) could be as a result of their high pathogenecity as reported by researchers elsewhere. These organisms can cause several poultry and farm animal infections specially mycotoxicosis having public health significance to both human and poultry. The presence of high numbers of E. coli and Salmonella spp. in poultry feed were indicative of poor hygienic practices during manufacture, post process contamination and unsatisfactory transportation and reservation. Therefore reinforce the need for preventive control measures, hygienic handling and processing of feeds to reduce the risk of potential human health hazards.

Keywords: poultry feed; total viable count (TVC); total *E. coli* count (TEC); total *Salmonella* count (TSC); colony forming unit (CFU)

1. Introduction

Poultry industry is one of the important industries in Bangladesh in terms of employment avenue and source of protein supply at cheaper price for the nation. Commercial poultry production has been growing rapidly in Bangladesh since early 1990 by using improved genetics, manufactured feeds and management (Raha, 2013). The poultry meat alone contributes a substantial 37% of the total meat production in Bangladesh (Begum *et al.*, 2011). It is estimated that there are about 137.2 million of poultry (Agricultural Census, 2008) and more than one lakhs poultry farms in Bangladesh (Saleque, 2007). Currently there are about 130 hatcheries (65 hatcheries with breeding farm) in Bangladesh, with 2 million broilers and 0.3 million layer parent stock which producing 4 to 5 million commercial day old chicks per week. The commercial broiler and layer farms supplying about 0.2 million metric ton of poultry meat and 5210 million table eggs per year in Bangladesh (Samad, 2005).

The advancement of poultry industry in Bangladesh is interrupted by a number of constraints of which major one is outbreak of disease causing about 30% mortality of chickens in every year (Ali, 2004). One of the major problems of the development of the poultry sub-sector in Bangladesh relates to the lack of sufficient and appropriate feeds (Mitchell 1997; Alam 1997). Relevant research suggests that a high priority is given to the improvement of the feed supply in this sector, which is expected to help in developing resistance to diseases, on one hand, and production of quality products, on the other (Islam, 2001).

Poultry feeds are food materials which referred as complete feeds as they are designed to contain all the nutritional materials needed for proper growth, meat and egg production in raising poultry birds (Obi and Ozugbo, 2007). Poultry feed 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). Various brands of poultry feeds are in existence depending on the functions they perform in the birds. Thus there are starter, growers, finishers, layers, among others. Poultry feeds can be contaminated directly and indirectly through contact with soil, rodent, birds, dust, human carrier, sewage or water during processing and storage (Ezekiel et al., 2011). Poultry feeds can potentially become contaminated with food borne pathogenic microorganisms during harvesting, processing, handling and marketing of the bagged feeds (Chowdhuri et al., 2011). Poultry feeds contaminated with bacteria pathogenic to humans can contribute to human food borne illness through the feed-poultry-food-human chain. Prominent bacterial species in the poultry feeds include Bacillus, Salmonella, E. coli, Enterococcus, Canpylobacter, Clostridium and Lactobacillus have been shown to be of critical importance in tropical region like Bangladesh and others (Onveze et al., 2013; Hossain et al., 2011; Nasrin et al., 2007). Reports by Gill and Best (1998) and Ruff (1992) have listed animal feed as one of the sources of microorganisms to animals. Specifically, some of the additives have been incriminated amongst the principle sources of bacteria of public health concern (Ogbulie and Okpokwasili, 1998). Various types of farm animal diseases such as diarrhoeal diseases like bacillary dysentery, amoebic dysentery, fowl cholera, Salmonellosis, Staphylococcosis, Colibacillosis, Erysipelus, Listeriosis etc. have been traced to the contamination of animal feed (Healing and Greenwood, 1991). A potential and more deadly hazard has been associated with the consumption of microbial toxin of bacterial and fungal origin in feed (Hesseltine, 1984; Betina, 1989; Gilbert, 1995). Studies elsewhere have associated some animal feeds with toxigenic strains of fungi and bacteria of public health concern (Bilgrami et al., 1995; White and Torman, 1995). Considering the health hazard posed to poultry and the unsuspecting consumers of such contaminated feed and its overwhelming socio economic impact, it is pertinent to undertake this study. This research is therefore designed to identify and characterize the microbial flora and fungus of commercially available poultry feeds sold in Bangladesh.

Bearing in mind the above facts the present study was undertaken with the following specific objectives:

- a) To assess the microbiological quality of commercially available poultry feeds.
- b) To determine the microbial loads associated with feed samples by using Total viable count (TVC), Total *E. coli* count (TEC) and Total *Salmonella* count (TSC).
- c) To isolate and identify the microorganisms from feed samples by using cultural and biochemical tests.
- d) To observe the effect of these feed in birds.

2. Materials and Methods

2.1. Study area

The present study was carried out at Microbiogy Laboratory of the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during the period from January to July, 2014.

2.2. Collection of feed sample

Four different brands of poultry feed samples were aseptically collected from poultry feed seller outlet at city corporation, Rangpur, Bangladesh. A total of 20 samples, each of five from different brand of commercial feed were collected. The collected samples of different brands were labeled as A (broiler starter), B (broiler finisher), C (layer starter) and D (layer finisher) and transported to the laboratory where bacteriological and mycological analysis were carried out within 2-6 hours of sample collection.

2.3. Laboratory preparation

All items of glass wares including test tubes, pipettes, cylinder, flasks, conical flasks, glass plate, slides, vials soaked in a household dishwashing detergent solution ('Trix' Recket and Colman Bangladesh Ltd.) for overnight, contaminated glassware were disinfected in 2% sodium hypochloride solution prior to cleaning. The glassware were then cleaned by brushing, washed thoroughly and finally sterilized either by dry heat at 160° C for 2 hours or by autoclaving for 15 minutes at 121° C under 15 lbs pressure per square inch. Autoclaved items were dried in a hot air oven over at 50° C. Disposable plastic were (micropipette tips) was sterilized by autoclaving. All the glassware was kept in oven at 50° C for future use.

2.4. Microbiological analyses

The bacteriological and mycological quality of four brands of feed sample was assayed with a view to establishing their autochthonous microorganisms. The different media such as nutrient broth (NB), Nutrient agar (NA), Salmonella-Shigella (SS) agar, Eosin methylene blue (EMB) agar, MacConkey agar, Triple sugar iron (TSI) agar slant, Motility, Indole, Urease (MIU) and Saboraud Dextrose agar (SDA) media were prepared separately. The last six media are called selective media. The above media were prepared separately by the following method:

2.4.1. Preparation of NB media

The nutrient broth media were prepared by suspending Thirteen grams of nutrient broth (Difco) was dissolved in 1000 ml of cold distilled water and heated up to boiling to dissolve it completely. The solution was then distributed in tubes, stoppered with cotton plugs and sterilized in the autoclave machine at 121°C and 15 pounds pressure per square inch for 15 minutes. The sterility of the medium was judged by incubating overnight at 37°C and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter and Cole, 1991).

2.4.2. Preparation of NA media

Twenty eight grams of nutrient agar powder (Hi-media, India) was suspended in 1000 ml of cold distilled water in a flask and heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. After autoclaving, the medium was poured into each sterile petridish and allowed to solidify. After solidification of the medium in the petridishes, these were incubated at 37^oC for overnight to check their sterility and used for culture characterization (Carter and Cole 1991).

2.4.3. Preparation of SS agar media

6 gms of dehydrated Salmonella-Shigella agar (difco) was suspended in 100 mi of cold completely. After sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass petridishes (large sized) to form a thick layer therein. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged by keeping the petridishes in the incubator at 37°C for overnight and then used for cultural characterization or stored at 4°C in refrigerator for future use (Carter and Cole, 1991)

2.4.4. Preparation of eosin methylene blue agar media

36 gm of EMB agar base (Hi-media, India) was added to 1000 ml of distilled water in a conical flask and heated until boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass petridishes (large sized) to form a thick layer therein. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged and used or stored at 4°C in refrigerator for future use (Carter and Cole, 1991).

2.4.5. Preparation of MacConkey agar

51.50 grams of dehydrated MacConkey agar (Difco) was suspended in 1000 ml of cold distilled water taken in a conical flask and was heated up to boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass petridishes (large sized) to form a thick layer therein. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged and used for cultural characterization or stored at 4° C in refrigerator for future use (Carter and Cole, 1991).

2.4.6. Preparation of MIU medium

Eighteen grams of MIU agar (Difco) was suspended in 950 ml of cold distilled water taken in a conical flask and heated up to boiling to dissolve the medium completely. Ninety five ml was dispensed into flasks and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Then was Cooled to about 50-55°C and aseptically add 5ml was added of sterile 40% basal medium. After mixing were dispensed into sterile test tubes. Allow to cool in an upright position. The sterility of the medium was judged and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter and Cole, 1991).

2.4.7. Preparation of sugar media

The medium consists of peptone water of which fermentable sugar added to the proportion of 100%. One gram of peptone (Hi-media, India) and 0.5 gram of sodium chloride were added in 100 ml of distilled water. The medium was boiled for 5 min, adjusted to pH 7.0, cooled and then filtered through filter paper. Phenol red, indicator at the strength of 0.2% solution was added to peptone water and then in 5 ml into cotton plugged test tubes containing Durham's fermentation and placed inverted. These were then sterilized by autoclaving at 15 lb/sq.inch 121°C for 15 minutes. The sugar used for fermentation was prepared separately 10% solution in distilled water (10 gram sugar dissolved in 100 ml of distilled water). A mild heat was necessary to dissolve the sugar. The sugar solutions were sterilized in Arnold steam sterilizer at 100°C for 30 minutes for 3 consecutive days. An amount of 0.5 ml of sterile sugar solution was added aseptically in each culture containing 4.5 ml sterile peptone water, indicator and Durham's fermentation tube. Before use, the sterility of the sugar media was examined by incubating it at for 24 hours (Carterand Cole, 1991).

2.4.8. Preparation of Saboraud Dextrose agar media

Sixty five grams of SDA medium (Difco) was suspended in 1000 ml of cold distilled water taken in a conical flask and heated up to boiling for one minute to dissolve the medium completely. After that it was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Then it was cooled to about 45-50°C and dispensed into sterile Petri dishes or test tubes. The sterility of the medium was judged and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter and Cole, 1991).

2.5. Isolation and identification of bacterial isolates

2.5.1. Bacteriological examination

The samples were first cultured into the nonselective media such as NA and NB media for total bacterial count. The feed samples were processed as described by Chowdhuri *et al.* (2011) by homogenizing 10 gms of each sample in 90 ml of peptone water in the beaker and mixed thoroughly. Then 900 μ l PBS was taken to each of the small bottles accordingly. Then 100 μ l mixed sample from beaker was taken to the small bottles for serial dilution. Thus the sterile dilution was made up to 10⁻⁴. These appropriate dilutions were cultured by spread plate technique using sterile bent glass rod on the NA media and incubated overnight at 37^oC in the incubator. Following incubation, plates exhibiting 30-300 colonies were counted and total viable count was calculated according to ISO recommendation. The results of the total bacterial count were expressed as the number of organism or colony forming units per gram (CFU/gm) of feed sample.

2.5.2. Cultural characterization and biochemical studies

The feed samples were subcultured into the selective media for identification of bacteria by their morphological characteristics including size, shape, surface texture, edge, elevation, color, opacity etc. of the colonies on different media were studied. Several biochemical tests were performed according to Holt *et al.* (1994) to identify the biochemical characteristics of the bacterial isolates. The tests were - Catalase test, Indole production, Urease test, Methyl red test, Voges-proskauer test, Triple Sugar Iron test and Carbohydrate fermentation test.

2.5.3. Determination of morphology of bacteria by Gram's staining method

The Gram's staining method was performed as described by Merchant and Packer (1976). Briefly, a small colony was picked up with a bacteriological loop, smeared on a glass slide and fixed by gentle heating. Crystal violet solution was then applied on the smear to stain for two minutes and then washed with running water. Gram's iodine was added to act as mordent for one minutes and then again washed with running water. Acetone alcohol was then added, which act as a decolorizer. After 10 second washing with water and safranine was added as counter stain and allowed to stain for 1 to 2 minutes. Then the slide was washed with water, blotted and dried in air and then examined under microscope with $100 \times$ objectives.

2.6. Isolation and identification of fungi

Fungus grows on artificial media in variety and its gross colony morphology alone can give a preliminary idea about identification of most fugal strains. For isolation of fungus in pure culture, the suspected mixed culture was inoculated into Saboraud dextrose agar media by streak plate technique to confirm that they were fungi.

2.7. Statistical analysis

Data were analyzed using SPSS for Windows (version 20.0). Prevalence of bacterial and fungal isolates was expressed in simple descriptive statistics such as means and standard deviation. For cfu/gm values, one-sample test was used to test if there is any statistical association between different feed samples and isolated pathogens.

3. Results and Discussion

3.1. Determination of TVC, TEC, TSC and TFC of feed sample

The results of TVC, TEC, TSC and TFC of 20 feed samples of four different brands were not uniform and varied quite considerably. The average total viable count (TVC) of feed sample A, B, C and D were 5.45×10^6 , 3.28×10^5 , 5.14×10^6 and 4.53×10^5 CFU/gram respectively (Table 1). The average Total *E. coli* count (TEC) of feed sample A, B, C and D were 6.25×10^5 , 8.26×10^3 , 5.52×10^5 and 5.65×10^4 CFU/gm respectively (Table 2). The average Total *Salmonella* count (TSC) of feed sample A, B, C and D were 3.15×10^4 , 2.68×10^3 , 4.46×10^3 and 1.19×10^4 CFU/gm respectively (Table 3). The highest TVC, TEC and TSC were found in broiler starter (feed sample A) and lowest TVC, TEC and TSC were found in broiler finisher (feed sample B). On the other hand Table 4 showed that the average Total fungal count (TFC) was 1.85×10^5 CFU/ gm in layer finisher (feed sample D).

| Feed sample | No. of sample tested | No. of positive sample | Mean of TVC (cfu/g) ±SD |
|-------------|----------------------|------------------------|---------------------------|
| А | 5 | 4 | $5.45 \ge 10^6 \pm 0.004$ |
| В | 5 | 2 | $3.28 \ge 10^5 \pm 0.003$ |
| С | 5 | 3 | $5.14 \ge 10^6 \pm 0.002$ |
| D | 5 | 3 | $4.53 \ge 10^5 \pm 0.006$ |

Table 1. Total viable count (TVC) of different feed sample.

TVC= Total viable count, SD = Standard deviation

Table 2. Total E. coli count (TEC) of different feed sample.

| Feed sample | No. of sample tested | No. of positive sample | Mean of TEC (cfu/g) ±SD |
|-------------|----------------------|------------------------|---------------------------|
| А | 5 | 4 | $6.25 \ge 10^5 \pm 0.003$ |
| В | 5 | 2 | $8.26 \ge 10^3 \pm 0.002$ |
| С | 5 | 4 | $5.52 \ge 10^5 \pm 0.004$ |
| D | 5 | 3 | $5.65 \ge 10^4 \pm 0.003$ |

TEC= Total E. coli count, SD = Standard deviation

Table 3. Total Salmonella count (TSC) of different feed sample.

| Feed sample | No. of sample tested | No. of positive sample | Mean of TSC (cfu/g) ±SD |
|-------------|----------------------|------------------------|---------------------------|
| A | 5 | 4 | $3.15 \ge 10^4 \pm 0.004$ |
| В | 5 | 2 | $2.68 \ge 10^3 \pm 0.006$ |
| С | 5 | 3 | $1.19 \ge 10^4 \pm 0.011$ |
| D | 5 | 2 | $4.46 \ge 10^3 \pm 0.003$ |

TSC= Total Salmonella count, SD = Standard deviation

Table 4. Total fungal count of different feed sample.

| Feed sample | No. of sample tested | No. of positive sample | Mean of Total fungal count (cfu/g) |
|-------------|----------------------|------------------------|------------------------------------|
| А | 5 | 0 | 0 |
| В | 5 | 0 | 0 |
| С | 5 | 0 | 0 |
| D | 5 | 2 | $1.85 \ge 10^3$ |

Table 5. Percentage and range of *E. coli* and *Salmonella sp* from different feed sample.

| Bacterial species | No. of sample tested | No. of positive sample | Range | Percentage |
|--------------------------|----------------------|------------------------|----------------------|------------|
| E. coli | 20 | 13 | 0 to $6.25 \ge 10^5$ | 65% |
| Salmonella spp. | 20 | 11 | 0 to $3.15 \ge 10^4$ | 55% |

Table 6. Percentage and range of TVC and TFC from different feed sample.

| Parameter employed | No. of sample tested | No. of positive sample | Range | Percentage |
|--------------------|----------------------|------------------------|----------------------|------------|
| TVC | 20 | 12 | 0 to $5.45 \ge 10^6$ | 60% |
| TFC | 20 | 02 | 0 to $1.8 \ge 10^3$ | 10% |

Table 7. Cultural and Biochemical properties of isolated bacteria and fungus.

| Test parameter | E. coli | Salmonella spp. | Fungal species |
|---------------------------------|-------------------------------------|----------------------------------|----------------|
| Grams reaction | | | |
| | GN, pink color, large rod shaped | GN, pink color, small rod shaped | ND |
| Culture media | | | |
| SS (Salmonella-Shigella) agar | - | + | ND |
| EMB (Eosin methylene blue) agar | + | + | ND |
| MacConkey agar | + | + | ND |
| SA (Saboraud Dextrose) agar | ND | ND | + |
| Biochemical tests | | | |
| Motility | + | + | ND |
| Catalase | + | + | ND |
| Indole production | + | - | ND |
| Urease | - | - | ND |
| Methyl red | + | + | ND |
| Voges-proskauer | - | - | ND |
| H ₂ S production | - | + | ND |
| Glucose | A/G | A/G | ND |
| Sucrose | - | - | ND |
| Lactose | А | - | ND |
| Maltose | А | А | ND |
| Mannitol | А | А | ND |

GN = Gram Negative, ND = Not done, A = Acid production, AG = Acid and gas production, AG = Aci

+ = Positive, - = Negative

3.2. Occurrence of pathogenic bacteria and fungus in poultry feed sample

E. coli was isolated on selective MacConkey agar and EMB agar media where it showed bright pink and metallic green sheen colonies respectively. The microscopic examination of Gram's stained smears from MacConkey and EMB agar revealed Gram-negative, pink colored, small rod shaped organisms arranged in single, pairs or short chain (Buxton and Fraser, 1977). Biochemical test also confirmed the presence of *E. coli*. Similarly *Salmonella* spp. was isolated on selective Salmonella-Shigella (SS) agar, MacConkey agar and EMB agar media producing black centered, pale smooth and colorless transparent colonies respectively. In Gram's stained smears the organisms revealed gram-negative, pink color, large rod shape with opaque and translucent appearance (Buxton and Fraser, 1977). Biochemical test was also done for the presence of *Salmonella* spp. On the other hand fungal species was isolate on Saboraud dextrose agar (SDA) media where it produced colonies with white cottony growth. The overall identification criteria employing cultural and biochemical tests for the

isolation of bacterial and fungal species were shown in Table 7. The present study was conducted to assess the microbiological quality of commercially available poultry feed using different cultural, biochemical tests, and staining techniques for identification and isolation of pathogenic microorganisms harmful for both human and animal health that contracted the infection from contaminated poultry feed. This study revealed that the percentage occurrence of pathogenic bacteria and fungus was 60% and 10% respectively (Table 6). The high occurrence of total viable count of bacterial and fungal species of public health concern may indicate obvious health hazard in terms of direct consumption of contaminated feed or their toxins by farm animals and poultry (Frazier and Westhoff, 1978). Total fungal count is one of the criteria in evaluation of hygienic quality of feed (Krnjaja *et al.*, 2008) and these counts should not exceed the values of 1×10^5 CFU/g (Dalcero *et al.*, 1998). Possible reason for the absence of fungal count in feed sample A, B and C might be inclusion of antifungal agents, use of high temperature during preparation and manufacturing. The study showed that the percentage occurrence of E. coli was 65% (Table 5) which is in line with the findings of Akond et al. (2009), Chowdhuri et al. (2011) and Da Costa et al. (2007) where it was 58%, 57.14% and 50% respectively. On the other hand the percentage of Salmonella spp. was 55% (Table 5) which is lower than the finding of Chowdhuri et al. (2011) and higher than the finding of Onyeze et al (2013), Alshawabkeh (2006), Refai et al. (1992) in that it was 71.43%, 24.4%, 2.33% and 4.4% respectively. A potential and more deadly hazard has been associated with the consumption of microbial toxins of bacterial and fungal origin in feed (Gilbert, 1995). On the other hand presence of *E.coli* and *Salmonella* spp. may suggest fecal as well as environmental contamination (Uwaezuoke and Ogbulie, 2008). For instance E. coli known as coliform bacteria are normal inhabitants of the digestive tract and are abundant in the poultry environment, some of them is implicated in disease conditions such as collibacillosis occurring various forms such as enteric and septicemic collibacillosis that cause increased mortality and performance of birds whereas *Salmonella* spp. is a serious threat to consumer health due to its ability to adapt to many different environments and broad range of transmission routes producing acute and chronic infections in all or most types of birds and animals (Bains 1979, Mallinson 1984, Barnes et al., 2003, Maciorowski et al., 2004). According to Shirota et al. (2001) broiler and layer feed is one of the important sources of chicken farm contaminated with Salmonella. The result of poultry inoculation in this study revealed that lower dose (2.68×10^3) of Salmonella organism was not produced any clinical sign due to resistant power but higher dose (3.15×10^4) of the same organism was able to produce clinical signs including depression, reluctant to move and diarrhea (Wray et al., 1996). These organisms can survive and multiply at refrigerator temperatures and in a wide range of pH, hence even a small amount of contamination may be significant (Linnan et al. 1988).

4. Conclusions

The role of poultry farmers in ensuring food safety when handling animal droppings and contaminated feed need to be emphasized. Poultry feed having pathogenic bacterial and fungal contamination is of public health concern posing threat to both human and animal. The presence of above bacteria and fungus in all commercial feed samples in the study area therefore calls for attention in both production and storage strategies employed by the poultry feed manufacturers, whole sellers, retailers and consumers. Good husbandry and management practice, avoid overcrowding and stress condition, sanitation, adequate ventilation and maintain bio-security measures of the poultry shed, several improvement in the feed processing industry including improve hygiene, feed treatment, transportation, packing materials and good manufacturing practices in the feed mill may be effective for prevention and control of pathogenic microorganisms at the pre-harvest and post-harvest phase of poultry production as well as improvement of the microbiological quality of the feed sample.

Conflict of interest

None to declare.

References

- Alam J, 1997. Non-Crop Sector in Rehman Sobhan (ed.). Growth or Stagnation? A Review of Bangladesh's Development, 1996, University Press Limited, Dhaka.
- Agriculture census, 2008. Structure of agricultural holdings and livestock population. Volume 1. Bangladesh Bureau of Statistics, Statistic division, Ministry of Planning, Government of People's Republic of Bangladesh.
- Ali MI, 2004. Current status of veterinary biologics production in Bangladesh and their quality control. Proceeding of the BSVER symposium held on July, 28, 2004 at NIPSOM auditorium, Mohakhali, Dhaka, Bangladesh.

Alshawabkeh KM, 2006. Occurrence of Salmonellas in poultry feeds in Jordan. J. Agril. Sci., 2: 46-51.

- Akond MA, SMR Hassan, S Alam and M Shirin, 2009. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Am. J. Environ. Sci., 5: 47-52.
- Arotupin DJ, RMO Kayode and Awojobi, 2007. Microbiological and physiochemical qualities of selected commercial poultry feeds in Akure, Nigeria. Journal of Biological Sciences, 7: 981-984.
- Bains BS, 1979. A Manual of Poultry Diseases. F. Hoffmann-La Roche & Co. Limited Company, Basle, Switzerland. p. 81.
- Barnes HJ, JP Vaillancourt and WB Gross, 2003. Colibacillosis, In: Diseases of Poultry, 11th edition, Iowa State University Press, Ames, IA, USA.
- Begum IA, MJ Alam, J Buysse, A Frija and G Van Huylenbroeck, 2011. A comparative efficiency analysis of poultry farming systems in Bangladesh: A Data Envelopment Analysis approach. Applied Economics, 44: 3737-3747.
- Betina V (Ed), 1989. Mycotoxins Chemical, Biological and Environmental Aspects. Amsterdam.
- Bilgrami KS, KK Sinha, AA Ansari and M Rahman, 1995. Widespread occurrence of toxigenic Alternaria in cereals and oil seeds. Indian Phytopathology, 48: 150-153.
- Buxton A and G Fraser, 1997. Animal Microbiology. Vol.1. Blackwell scientific publications, Oxford, London, Edinburg. Melbourne. pp. 103-115.
- Carter GR and JR Cole, 1991. Diagnostic Procedure in Veterinary Bacteriology and Mycology. 5th Ed. Academic Press Inc. pp. 372-373.
- Chowdhuri A, A Iqbal, M Giasuddin and AA Bhuiyan, 2011. Study on Isolation and identification of *Salmonella* and *Escherichia coli* from different poultry feeds of Savar Region of Dhaka, Bangladesh. J. Sci. Res., 3: 403-41.
- Da Costa PM, M Oliveira, A Bica, P Vaz-Pires and F Benardo, 2007. Antimicrobial resistance in *Enterococcus* spp. and *Escherichia coli* isolated from poultry feed and feed ingredients. Vet. Microbiol., 120: 122-131.
- Dalcero A, C Magnoli, M Luna, G Ancasi, MM Reynoso, S Ciacchiera, R Miazzo and G Palacio, 1998. Mycofiora and naturally occurring mycotoxins in poultry feeds in Argentina. Mycopathologia, 141: 37-43.
- Ezekiel CN, AO Olarinmoye, JMA Oyinloye, OB Olaoye and AO Edun, 2011. Distribution, antibiogram and multidrug resistance in Enterobacteriaceae from commercial poultry feeds in Nigeria. Afr. J. Microbiol Res., 5: 294-301.
- Fraizier WC and DC Westhoff, 1978. Food Microbiology, 3rd edition. Tata McGraw Hill publishing company Limited, New Delhi.
- Gilbert J, 1995. Analysis of mycotoxin in food and feed. Certification of DIN in wheat and Maize. American Pathology, 3: 263-268.
- Gill C and R Best, 1998. Antibiotic resistance in USA: Scientist to look more closely. Feed International. 19: 16-17.
- Hesseltine CW, 1984. Toxigenic fungi their toxins and health hazard. Koclarisha Press.
- Holt JG, NR Krieg, HA Sneath, JT Staley and ST William, 1994. Bergey's Mannual of Determinative Bacteriology. 9th edn. Baltimore: Williams and Wilkins.
- Hossain MA, MM Islam, AF Islam and PA Iji, 2011. Constraints to use all-vegetable feed ingredients and strategies to improve such diets for poultry birds: A review. Bangladesh. Res. Pub. J. 6: 120-125.
- Healing TO and MH Greenwood, 1991. Frequency of Isolation of *Campylobacter* spp., *Yersinia* spp. and *Salmonella* spp. from small mammals. Southern Britain International Journal of Environmental Health Research, 1: 54-62.
- Islam N, 2001. Demand for poultry feeds: implications for wheat and maize production in Bangladesh. Food Management & Research Support Project, Ministry of Food, Government of the People's Republic of Bangladesh. FMRSP Working Paper No. 31.
- ISO (International Standardization Organization), 1995. Recommendation of meeting of the subcommittee, International Organization for Standardization on meat and meat products. ISO/TC. 36, section 6, pp. 10-18, The Netherlands.
- Krnjaja V, L Stojanovic, R Cmiljanic, S Trenkovski and S Tomasevic1, 2008. The presence of toxigenic fungi in poultry feed. Biotechnology in Animal Husbandry, 24: 87-93.
- Linnan MJ, L Mascola and DE Xiao, 1988. Epidemic listeriosis associated with Mexicanstyle cheese. New England Journal of Medicine, 319:823.
- Maciorowski KG, FT Jones, SD Pillai and SC Ricke, 2004. Inidence, sources and control of food-borne *Salmonella* spp. in poultry feeds. World's Poultry Science Journal, 60: 446-457.
- Mallinson ET, 1984. Infectious Diseases. In: Animal Health. Jack Hayes (ed). Bureau of Animal Industry (Publisher), Maryland, U.S.A.

- Merchant IA and RA Packer, 1976. Veterinary Bacteriology and Virology 7th edn, the Iowa State University Press. Ames, Iowa, USA. pp. 286-306.
- Mitchell D, 1997. The Livestock and Poultry Sub-Sector in Bangladesh, Mission Report, World Bank.
- Nasrin MS, MJ Islam, KHMNH Nazir, KA Choudhury and MT Rahman, 2007. Identification of bacteria and determination of their load in adult layer and its environment. J. Bangladesh. Soc. Agri. Sci. Technol., 4: 69-72.
- Obi CN and Ozugbo IJ, 2007. Microbiological analyses of poultry feeds sold in Umuaia Main Market, Abia state, Nigeria. Res. J. Appl. Sci., 2: 22-25.
- Ogbulie JN and GC Okpokwasili, 1998. Efficacy of chemotherapeutic agents in controlling bacterial diseases of cultured fish. Journal of Aquaculture in the Tropics, 13: 61-72.
- Onyeze RC, GT Onah and OC Eluke, 2013. Bacterial contaminants associated with commercial poultry feeds in Enugu Nigeria. Int. J. Life. Sc. Bt & Pharm. Res., 2: 432-437.
- Refai M, G Abdel-Gaber, H Hammam, N Aly, N El-Danaf, E Safawt and H Ibrahim, 1992. Detection of salmonellae in poultry feeds by ELISA and their control by BioAdd. J. Egypt. Vet. Med. Ass., 52: 371-381.
- Raha SK, 2013. Poultry industry in Bangladesh: ample opportunities for improvement. In: proceedings of the seminar, 8th international Poultry Show and Seminar. World's Poultry Science Association, Bangladesh Branch, pp.13-19.
- Ruff MD, 1992. New Methods of Disease control. Feed Mix., 1: 15-18.
- Saleque MA, 2007. Poultry industry in Bangladesh: current status and its challenges and opportunity in the emerging market environment. Poultry Business Directory. Khamar Bichitra, Dhaka.
- Samad MA, 2005. Poultry Science and Medicine. 1st edn. Published by M. Bulbul, BAU Campus, Mymensingh, Bangladesh.
- Shirota k, H Katoh, T Ito and K Otsuki, 2001. Salmonella contamination in commercial layer feed in Japan. J. Vet. Med. Sci., 62: 789–791.
- Tavechio AT, Ghilardi AC, Peresi JT, Fuzihara TO, Yonamine EK, Jakabi M and Fernandes SA, 2002. Salmonella serotypes isolated from nonhuman sources in São Paulo, Brazil, from 1996 through 2000. J Food Prot., 65:1041-4.
- Uwaezuoke JC and JN Ogbulie, 2008. Microbiological quality of commercially available poultry feeds sold in parts of Eastern Nigeria. J. Appl. Sci. Environ. Manage, 12: 113-117.
- Wray C, RH Davies and JD Corkish, 1996. Enterobacteriaceae. In Poultry Diseases, 4th ed.; Jordan, T. W., Pattison, M., Eds.; W. B. Saunders: Philadelphia, PA, USA, pp. 9–43.