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Research Article – Environmental Sciences

Isolation and identification of microbial contaminants associated with commercial poultry feeds

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Abstract

Poultry feeds are food materials used in raising poultry birds. Poultry is the second most widely eaten meat in the world, accounting for about 38% of the world meat. The diseases of poultry is like the disease of other animals. They may be caused by pathogenic organisms, nutritional deficiency and from wound. This study was designed and carried out to determine the load and species of fungi and bacteria contaminating poultry feeds. A total of 6 samples were collected from different feed types and source of feeds inside poultry farm and market feeds. The isolates were identified according to their cultural, microscopic and biochemical properties to the following gram negative bacteria include *Escherichia coli* and *Proteus* spp and gram positive bacteria include *Staphylococcus aureus* and *Streptococcus* spp. The fungi includes *Aspergillus niger*, *Penicillium* spp, *Fusarium* spp, *Rhizopus* spp, *Mucor* spp, and *Cladosporium* spp. It is concluded that poultry feeds, especially those inside farms are harbouring potential pathogenic bacteria and fungi loads that are far above the acceptable levels, thus constituting a public health hazard and necessitate the application of the standard measures for production of feeds by manufacturers and health authorities. Therefore, the study recommends that hygienic production of poultry feed is a public health issue, proper treatment of feed ingredients and application of hygienic measures such as HACCP, starting from harvesting of feed ingredients to the storage, processing of feeds, packaging, transporting and eventually marketing of the bagged feeds is need of the hour.

Keywords: Poultry Feeds, Gram negative Bacteria, Gram Positive Bacteria, Contaminants.

Introduction

The term "poultry" used in agriculture generally refers to all domesticated bird kept for egg laying or meat production. Poultry is the second most widely eaten meat in the world, accounting for about 38% of the world meat (Rafloff, 2003).The diseases of poultry is like the disease of other animals. They may be caused by pathogenic organisms, nutritional deficiency and from wound or cannibalism. Some of the diseases associated with fowls locally include; new castle disease, chronic respiratory disease, fowl typhoid and fowl pox diseases. The world consumption of poultry products namely meat and eggs is remarkably increasing with increase in number of people, and this is because of good quality and effective price. The wide- spread of human consumption of poultry meats and eggs necessitate the control of microbial contamination.

The safety of poultry products raises the importance of efforts that should be exerted towards evaluation and detection of microbial hazard, which represents a great risk to the consumer. Poultry feed is considered as one of the important sources of contamination of poultry products (Brown *et al.,* 2001). The safety and quality of poultry feeds are currently of major concern in developed countries, that safety of feed is a fundamental requirement for all birds. Unsafe feed may lead to great economic losses in case of destroying an infected flock of birds. The world feed manufacturer and stock industries have faced severe food safety issues throughout the last two decades such as the outbreak of bovine encephalopathy (BSE) and Belgium dioxin crisis, which occurred in 1999 due to contaminated fat supplied to stock feed manufacture (Brown *et al.,* 2001). These incidents showed the importance of feed safety in ensuring the safety of human food. One of the major areas of concern in the bacterial contamination of poultry feed come from the stock feed, raw materials and farms (Sazaki, 2000). There has been an increased focus on food as source of bacterial contamination of livestock production units, and there are standard measures that every feed factory or industry should follow and produce high quality, efficiency and pathogen-free feeds, industry must accept greater share of responsibility for the quality and safety of poultry feed production(Borland, 2002). Approaches have been made to reduce the contamination of poultry materials as well as the finished product, with some invasive *Salmonella*, the most important cause of infection in poultry (Borland, 2002). Non-typhi serotype of *Salmonella enterica* was reported in US poultry feed as early as 1948 (Edwards *et al.,* 1948). Studies from around the world have documented the presence of *Salmonella enterica* in a wide variety of animal feeds (Kidd *et al.,* 2002).

Several other microorganisms such as *Bacillus* spp, *E. coli*, *Campylobacter* and *Clostridium perfringens* can contaminate poultry feeds either from feed ingredients, through farms workers, equipment, air, handling, used bags or raw materials (Bryan and Doyl, 1995). There is a considerable evidence that poultry feed is frequently contaminated with food-borne bacterial pathogens (Bryan and Doyl, 1995). Risk assessment data for most poultry-borne hazards are lacking. However, these types of data are essential in developing food safety strategies. There is a need to evaluate poultry production, processing, handling and preparation procedures

to determine their impact on the risk of food-borne illness. Fully understanding the hazards associated with poultry consumption is the key to develop effective sampling, detection and identification methods, that in turn can be utilized to design control strategies (Mead *et al.,* 1993).

The presence of microscopic fungi affects the quality of feeds, their organoleptic attributes and nutritional quality (Shareef, 2010). Moulds like other microorganisms will assimilate and utilize the most readily available nutrients in the materials they grow upon and spoilage may result in the loss of 5 to 100% of nutrients in the feed (Okoli *et al.,* 2006). Regarding nutritional quality, lipids, protein and minerals are of essential importance for the proper development and growth of farm animals. The quantity and the nutritional requirements of feed depend on the weight and age of the poultry as well as the season. Healthy poultry require sufficient amounts of carbohydrates, lipids and proteins along with the necessary vitamins and dietary minerals (Dmello and Macdonald, 1998).

In addition to their negative impact on nutritional and organoleptic properties, mould can synthesize different mycotoxins (Stuper and Szablewski, 2013). It is well known that contamination of animal feed with mycotoxins may induce sanitary disturbances and mortality among animals and secondary contamination of human consumers via eggs, meat or milk (Nyamongo and Okioma, 2005). Consumption of a mycotoxin contaminated diet may induce acute and long-term chronic toxic effects (Binder, 2007). With respect to the humans and animals, in general terms, mycotoxins exhibit toxic actions and are characterized by carcinogenic, mutagenic, teratogenic and estrogenic properties (Shareef, 2010).

Most toxic species belong to the general *Aspergillus* spp, *Penicillium* spp, *Fusarium* spp and *Alternaria* spp (Pitt *et al.,* 1994). According to several authors, mycotoxins such as aflatoxins, Zealenone, deoxynivalenol, ochratoxin A, fumonisins, and patuli can be considered the most common mycotoxins found in feed and food (Gimeno and Martins, 2007; Hussein and Brasel,2004). Most mycotoxicoses of poultry are caused by an intake of low concentration of contaminants over a long with the typical chronic symptoms of poor growth, poor feed efficiency and suboptimal production. Ingestion of higher concentration however leads to acute clinical symptoms associated with specific vital organs, the immune system and other aspects of avian physiology as well as mortality (Mabbett, 2004; Shareef, 2010). For quality control the identification of the contaminating microbiota is essential because it provides data on the potential production of its mycotoxins and is a helpful indicator to determine feed hygienic quality (Khosravi *et al*., 2008).

Materials and Methods

Site of sample collection

The samples were collected from Rimi market in Kano Municipal Local government area, which is located along Emir Palace road opposite Murtala Muhammad General Hospital, Kano, and Sasakowa Farms in Kura local government area of Kano state.

Sample collection

The samples were collected from two different locations (farm and market), sample were mixed thoroughly and put in a sterilized polythene packets with a proper labelled named as chick mash, starter, broiler, grower, layer, and finisher respectively and then taken to the laboratory for analysis and processed as soon as possible.

Isolation of fungi

Poultry feeds dilution was prepared for the isolation of fungi from the poultry feeds. Serial dilutions of the sample were made in sterile test tube. 1ml of the serially diluted samples was pipette into each serially marked petri dish. Using pour plate method, the media were inoculated in an inverted manner at 37^{o}C for 3-5 days. The plates were observed and the single colonies pick for sub-culturing in other to obtain pure culture (Heuser, 1955).

Primary screening for fungi isolates

After the colonies were counted, 200ml of potato dextrose agar was prepared and the medium was sterilised and to cooled and anti-biotic reagents was added. Then the medium was aseptically poured in 10 plates and allowed to solidify. The fungal colonies were subcultured on the medium to obtain the pure culture. The plates were incubated at 37^{o}C for 3 days (Heuser, 1955).

Microscopic examination of fungi isolates

One or two drops of lacto phenol cotton blue was placed on a clean glass slide. Using a sterilized inoculating pin, a bit of the suspected fungal isolates were picked from the medium and placed in the stain on the slide. This was then teased out and covered with glass cover slip and pressed down slightly with the tip of the finger to expel any air bubble and further disintegrate the hypal growth to enhance observation. The slide was observed under ×10 magnification of a light microscope. Microscopic characteristic of fungi such as hyphae, conidial heads and arrangements of conidia were observed (Heuser, 1955; Barnnet and Hunter,1972).

Isolation of bacteria

Poultry feeds dilution was prepared for the isolation of bacteria from the poultry feeds. Five screw cap test tubes with distilled water was autoclave and arrange 1-5 into laminar flow for further processing. Serial dilution was carried using (Ogbulie, 1995) method.

Primary screening for bacterial isolate

After the colonies were count, 200ml of nutrient agar was prepared and the medium was sterilised. After sterilization the medium was cooled. Then the medium was aseptically poured in 10 plates and allowed to solidify. The bacterial colonies were streaked on the medium to obtain the pure culture. The plates were incubated at 37^{o}C for 24 hours (Ogbulie, 1995).

Gram staining for bacterial isolate

The Smear of each of the isolates were prepared by picking a small portion of microbial growth from the plates, then the slides were heated and fixed carefully. The heat fixed smears were stained with crystal violet for 60s, washed off with water and drained, then flood with Lugol's iodine for about 60s and wash off gently with water and drained. The slides were rinsed with 50-50 alcohol-acetone for 3s and were rinsed with water and drained. The slides were then counter stained with safranin for 1min after then, the stains were

washed off with water. The slides were air dried, Immersion oil was dropped on the smears and the smears were examined for cell morphology and arrangement, presence of capsule and staining reaction (Cruickshank *et al.,* 1973).

Biochemical test for identification of isolates

The selected bacterial isolates were subjected to Biochemical and Staining techniques as described and key provided in the Bergy's Manual of Determinative Bacteriology (Heuser, 1995; Isenberg, 2004). The following biochemical test were carried out catalase test, oxidase test, motility test), methyl red test, indole test, citrate test, and triple sugar iron agar test (Tsi) (Barrow and Felthman, 1993).

Results and Discussion

This study was conducted in order to determine and investigate the microbial contaminant associated with commercial poultry feeds. Six types of poultry feeds were examined using different media. Table 1 represents the colony count, shape and colour of fungi for all the samples from poultry feeds on a selective media.

Table 1: Fungal colony count, shape and colour from poultry feeds

Samples	Plate dilution factor	No. of colonies	Colour
Chick mash	10^{-6}	13	Green
	10^{-7}	00	-
	10^{-8}	09	Blue green, black
	10^{-9}	00	-
	10^{-10}	08	Brown, blue green
Total		30	
Starter mash	10^{-6}	13	Blue green, and dark brown
	10^{-7}	09	Blue green brown and dark brown
	10^{-8}	05	Black, green
	10^{-9}	00	-
	10^{-10}	00	-
Total		26	
Broiler mash	10^{-6}	26	Blue green, green
	10^{-7}	12	Blue green and dark brown
	10^{-8}	12	Blue green and brown
	10^{-9}	11	Blue green, dark brown
	10^{-10}	08	Yellowish, green, blue green
Total		69	
Layer mash	10^{-6}	18	Green, Blue green
	10^{-7}	14	Blue green, brown
	10^{-8}	09	Blackish
	10^{-9}	00	-
	10^{-10}	00	-
Total		41	
Grower mash	10^{-6}	13	Green, Blue green
	10^{-7}	12	Blackish, green, blue green
	10^{-8}	09	Blackish
	10^{-9}	08	Blackish, blue green
	10^{-10}	06	Greenish, brown
Total		48	
Finisher mash	10^{-6}	16	Blue green, green, brown
	10^{-7}	15	Blue green
	10^{-8}	00	-
	10^{-9}	00	-
	10_{-10}	00	-
Total		31	

Table 2: Occurrence of fungal isolated in different sample of poultry feeds

Fungal isolates	Chick mash	Starter	Broiler	Layer	Grower	Finisher
Aspergillus niger	2	3	2	1	1	2
Mucor spp	3	3	5	0	2	0
Rhizopus spp	1	0	1	0	0	0
Fusarium spp	0	0	1	0	0	0
Penicillium spp	1	1	2	2	1	3
Cladosporium spp	0	0	0	0	0	1
Total	7	7	11	3	3	6

The safety of poultry products for human consumption is a World Health Organization (W.H.O) requirement. Microbial contamination of poultry feed is a significant potential pathway for entry of pathogens into human food supply (Maciorowski *et al*., 2009). This study was designed and carried out to determine the loads and types of fungi and bacteria contaminating poultry feeds from different sources. These sources are the main sectors dealing with poultry feeds. Although feeds from farms and those from markets seem to have similar contamination, but they were considered separately due to the effect of storing time and conditions (humidity and temperature) in the markets.

This study revealed that four bacterial (Table 5) and six fungal genera (Table 2) were isolated in the feeds sample

analysed and time factor did not affect the bacterial and fungal isolates in the feeds. Higher fungal counts were obtained in the brands of poultry feed and this corroborates the report of studies carried out elsewhere (Andrews and Pitt, 1986; Pitt *et al*.,1994; Ogbulie, 1995). The high level of fungi obtained in this study can be associated with the low water activity of animal feed and the physiology of contaminating fungal genera. Animal feeds have been listed as one of the sources of microbes of farmed animals and poultry.

Table 3: Morphological Characteristics of Fungal Isolates from poultry Feeds

Fungal isolates	Morphological characteristics	Cultural Characteristics
AspergilSlus niger	Greenish-black, greenish, filamentous conidiosphore with rapid growing of black velvety spores.	Septate hyphae, branched conidiosphore with secondary branches. The conidiosphore is enlarge at the tip forming rounding vesicle like chains.
Rhizopus spp	Whitish and fluffy in appearance, sporangia turns blackish brown at maturity.	Profuse proliferation of filamentous conidiosphore.
Penicillium spp	Blue-green, the colonies are rapidly growing flat, filamentous and velvety, woolly or cottony in texture.	Chains of single-celled conidia are produce in basipetal succession from a specialized conidiogenous cell called a phialide.
Fusarium spp	White, cottony and change to yellow.	Septate hyphae with canoe-shape macroconidia, conidiospores bear conidia singly or in cluster.
Mucor spp	Grows quickly and cover agar surface with white fluff that later turns grey and reverse is white.	Hyphae practically non- septate, sporangiosphores are long, often branched and bear terminal spore filled sporangia.

Table 4: Bacteria colony count, shape and colour from poultry feeds

Samples	Plate dilution factors	No of colony	Colour
Chick mash	10^{-6}	12	Yellow, Whitish
	10^{-7}	04	Whitish
	10^{-8}	03	Milky
	10^{-9}	02	Whitish
	10^{-10}	00	-
Total		21	
Starter	10^{-6}	07	-
	10^{-7}	04	Whitish, milky
	10^{-8}	03	Yellowish
	10^{-9}	00	-
	10^{-10}	00	-
Total		17	
Broiler	10^{-6}	06	Whitish
	10^{-7}	05	Whitish, Milky
	10^{-8}	03	Milky
	10^{-9}	00	-
	10^{-10}	02	Yellowish
Total		16	
Layer	10^{-6}	05	Whitish
	10^{-7}	03	Whitish
	10^{-8}	00	-
	10^{-9}	00	-
	10^{-10}	02	Yellowish, Whitish
Total		10	
Grower	10^{-6}	06	Milky, Yellowish
	10^{-7}	03	Whitish
	10^{-8}	00	-
	10^{-9}	03	Whitish, Milky
	10^{-10}	01	Milky
Total		13	
Finisher	10^{-6}	08	Yellowish, Whitish, Milky
	10^{-7}	05	-
	10^{-8}	02	Milky
	10^{-9}	01	Milky
	10^{-10}	00	-
Total		16	

Thus, the high fungal and bacteria recovery may indicate a potential hazard to the animal. The high occurrence of fungal and bacteria species of public health concern may indicate obvious health hazard in terms of direct consumption of bacteriological or fungal contaminated feed or their toxins by farmed animal (Fraizier and Westhoff,1978). Also the *Staphylococcal* spp may suggest both bad manufacturing practice and contamination through handling, Studies elsewhere have indicated such high magnitude of *Staphylococcal* spp contamination to be hazardious (Bergoll, 1979; dos Santos *et al.*, 1981). The source of these organisms may vary extensively while bacterial genera may have

originated from nitrogenous waste products used in compounding animal feeds such as dung, chicken etc as reported by (Ogbulie, 1995). Fungal species may have resulted from carry-over of over seasoned fungal species from the field. Also handling and other post-harvest process may contribute amongst the primary sources of contamination. Most of the fungal species have been isolated from cereals (Pitt *et al*., 1994) and physiological adaptation of these fungal genera may have supported their survival. On the other hand, the presence of *E. coli* spp, *Proteus* spp, *Staphylococcus* spp and *Streptococcus* spp may suggest faecal as well as environmental contamination. Some of these organisms are well known pathogens of birds and farmed animals (Mallinson, 1984). For instance, *E. coli* is implicated in disease condition such as colibacillosis which occurs in forms whereas *Staphylococcus* spp, *Streptococcus* spp and *Proteus* spp are capable of producing acute and chronic infections in all or most types of birds and animals (Linnan *et al.*, 1988; Mallinson, 1984). 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).

Table 5: Occurrence of bacteria isolated in different sample of poultry feeds

Bacteria isolates	Chick mash	Starter	Broiler	Layer	Grower	Finisher
Stapylococcus aureus	3	3	2	1	1	2
Streptococcus spp	2	1	2	0	2	1
Proteus spp	0	1	1	0	0	0
E. coli spp	1	2	1	2	2	0
Total	6	7	6	3	5	3

Table 6: Identification of bacteria isolates from poultry feeds

Bacterial isolates	Gram stain reaction	Morphological characteristics
Stahylococcus aureus	+	Circular, pinhead colonies which are convex with entire margins. This is a gram positive coccus often produces colonies which have a golden brown colour.
Streptococcus spp	+	This is Gram positive cocci in cluster forms shiny, spherical and arranged in chains of varying length.
E. coli spp	-	This is a gram negative rod forms shiny, mucoid colonies which have entire margins and are slightly raised. Older colonies often have a darker center.
Proteus spp	-	This is a gram negative rod forms and facultatively anaerobic.

Table 7: Results of Biochemical Tests for Bacterial Isolates from Poultry Feeds

Test	***Staphylococcus aureus***	***Streptococcus* spp**	***E. coli* spp**	***Proteus* spp**
Indole test	-	-	+	-
Methyl red	-	-	+	+
Catalase test	+	-	+	+
Oxidase test	-	-	-	-
Citrate test	+	+	-	-
Motility test	-	-	+	+
Glucose	*	*	+	+
Sucrose	*	*	*	*
Lactose	-	+	+	-
Shape	Cocci	Cocci	Rod	Rod

(-negetive,+positive)

The socio-economic and health implication of these findings are enormous. Economically, the presence of these bacteria and fungi genera has been reported to overwhelmingly affect the viability of some animal husbandry undertaking and agriculture in general (Gimeno and Martins, 2007; Ogbulie, 1995). With the high colonization of bacteria and 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.

Conclusion

In conclusion, different species of fungi namely; *Aspergillus niger, Mucor* spp, *Rhizopus* spp, *Fusarium* spp, *Penicillium* spp, and *Cladosporium* spp were isolated and identified from poultry feeds and this is of concern because of the health hazards they present to the value chain actors. The bacteria counts obtained in this study were high and indicated the need for improved sanitary conditions in the markets and farms. Much of the contamination may occur after processing since the processing techniques utilized did not destroy many of the micro-organisms, especially the spore forming organisms. Potential pathogenic bacteria were isolated namely; *E. coli, Staphylococcus aureus, Streptococcus* spp, *Staphylococcus* spp, and *Proteus* spp. These organisms may contaminate poultry products and constitute a real hazard for public health. Feeds made inside farms are more contaminated and of higher bacterial loads compared to feeds from other sources.

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