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ISOLATION AND IDENTIFICATION OF BACTERIAL ISOLATES FROM POULTRY AND FISH FEEDS SOLD IN ABRAKA, DELTA STATE, NIGERIA.

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ABSTRACT
This study was done to determine isolates from four brand poultry feeds (Starter, Grower, Layer and Finisher) and fish feeds (Durante, Coppens, Aqua and Multi). Organisms isolated from both poultry and fish feeds were *Staphylococcus aureus, E.coli, Bacillus substilis, Salmonella spp, Proteus mirabilis, Shigella spp* and *Corynebacterium spp*. Results showed that Durante fish feed had the highest bacterial load of 3.32 X 10^5CFU/g while for poultry feed, starter feed had the highest bacterial load of 2.60 X 10^5CFU/g. *S. aureus* and *E.coli* had the highest prevalence 23.33% in fish feed while for poultry feed, *Shigella* had the highest prevalence of 21.62%. Results showed that the fish and poultry feeds sold in Abraka, Delta State may have bacteria present in them. Absolute quality control measures should be adhered to in the poultry and fish feed producing industries to ensure that raw materials used in preparing feeds are free from microbial contamination.

Keywords: Poultry feeds; Fish feeds; Bacterial isolates; Abraka; Nigeria.

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1. INTRODUCTION

Animal feed may serve as a substrate for a wide variety of microorganisms (Stotzky, 1997; Israel, Sunday, Mansong and Ubong, 2016). Some of the microflora are adapted to the desiccated conditions in soil and are transferred by insects, dust and wind to similar niche where they are capable of degrading organic matter and/or exist in a survival state until the moisture is high enough for bacterial action. While other microorganisms, primary moulds, actively grow within stored seeds including the low amount of available moisture as substrates (Crump *et al.,* 2002).

The chemical and nutritional constituents of animal feeds are important for livestock nutrition and growth, but are only part of the animal feed matrix. From an ecological standpoint, harvested grains are not only ingredients for livestock diet, but can act as substrate and transmission vector for simple unicellular prokaryotic and eukaryotic organisms. Feed may contain diverse microflora that is acquired from multiple environmental sources, including dust, soil, water and insects. Feed materials may be inoculated at any time during growing, harvesting, processing, storage and dispersal of the feed. According to Maciorowski et al (2004), microflora found in feed materials may come from a variety of ecological niches, which may include soil and gastrointestinal tracts, and have to adapt to the conditions found in animal feed and feed components in order to survive. The microbial diversity found in different feeds is dependent on the water activity, oxygen tension, pH and nutrient composition of the feed matrix. Microflora can decrease grain value through nutritional changes, physical damage or the production of toxins deleterious to animal health.

There are different types of feeds given to poultry and fish depending on the purpose. Poultry feeds such as breeders, starters, growers and layer feeds while for fish feeds includes Durante, Coppens, Aqua and Multi are used. The percentage of each ingredient in the feed varies depending on the type of feed. The ingredient include ground yellow corn, ground oats or barley, Alfata meat, fish meal, oyster shell, vitamin A, vitamin D and coccidiostat (which is given in form and at the level recommended by the manufacturer). The different percentage of each ingredient varies depending on the kind of feed. For example, ground yellow corn in starter and grower feed is 25% and 22% in breeder and layer feeds. There could be the same percentage of a particular ingredient in different feeds as in the case of alfafa meal which constitutes 10% in all the feed types. Some ingredients could be absent in a particular feed but present in the others as in the case of riboflavin supplement which is absent in starter feed and present at the level of 2% and 5% in layer and breeder feeds.

To maintain healthy fish and birds, the feeds are kept fresh as much as possible at all times. The amount of administered feeds are limited to the extent necessary to avoid wastage. Most poultry and fish feeds are prepared in dehydrated forms and because of this, there is the need to store them properly to avoid moisture uptake and damage by heat, in this way they can remain safe for a considerable period of time without losing their safeness and value. The feed should be stored in a suitable place where it will not be attacked by microorganisms, insects, rodents etc. Air tight storage of the various

feeds is not advisable because offensive odour might result when there is prevention of outflow and inflow of air (Leonard, 1981). The condition of feed storage and handling could be a source of contamination. When feeds are un-hygienically handled and stored, there could be a build-up of microbial contaminants.

2. MATERIALS AND METHODS

2.1 PROCUREMENT OF POULTRY AND FISH FEEDS

The poultry and fish feeds used in this study were purchased from the market retailers in Abraka and aseptically collected using sterile beakers and transferred into sterile universal bottles. The samples were labelled properly and transported immediately to the microbiology laboratory of Delta State University, Abraka where the samples were analysed. The samples collected include four types of poultry feeds namely Starter (S), Grower (G), Layers (L) and Finishers (F), also four brands of fish feeds namely Durante (D), Coppens (C), Aqua (A) and Multi (M).

2.2 ANALYSIS OF POULTRY AND FISH FEED SAMPLES

One gram (1g) of each feed sample were weighed out and homogenised into 9ml of distilled sterile deionized water using a sterile warring blender. Tenfold serial dilution of the homogenate were made using sterile pipette. Each feed sample was immediately inoculated (in duplicates) into nutrient agar plate, Salmonella-Shigella agar, Mannitol salt agar plate and MacConkey agar plate using per plate method. The plates were then incubated at 37oC for 18-24hrs. At the end of the incubation periods, colonies were counted. The characteristic bacteria isolates observed on the various media were isolated and subjected to microscopic and biochemical test after it has been sub cultured into agar plates for proper identification. Peptone water was used to grow the bacteria isolates from sugar utilization according to Buchanam and Gibbson (1974). Sub cultures of bacteria isolates were made to obtain pure cultures. Characterization and identification were carried out by methods as described by Mac Faddin (1980) and Buchanan and Gibbson (1974). Gram staining was carried out to distinguish between gram-positive and gram-negative bacteria.

3. RESULTS

Organisms from the feeds are shown in table 1. Durante fish feed had the highest bacterial load of 3.32 X 105CFU/g while for poultry feed, starter feed had the highest bacterial load of 2.60 X 105CFU/g as shown in table 2.

Table 1: Total Viable Count of Organisms
Samples

Fish feed	**Count (CFU/ml)**
Durante	3.32 X 10^5
Coppens	9.70 X 10^4
Aqua	1.75 X 10^5
Multi	1.82 X 10^5

Table 2: Total Viable Count of Organisms
Samples

Poultry feed	**Count (CFU/ml)**
Starter	2.60 X 10^5
Grower	2.30 X 10^5
Layer	2.22 X 10^5
Finisher	1.16 X 10^5

Table 3: Cultural and Biochemical Characteristics

Cultural characteristics	H_2S	Glucose	Lactose	Acid	Gas	Motility	Indole	Citrate	Coagulase	Catalase	Oxidase	Gram Staining	Tentative Organism
Colonies were creamy and convex with smooth edges	-	+	+	+	+	+	+	-	+	+	-	GPC	*Staph aureus*
Colonies were pink,convex with smooth edges	-	+	+	+	+	+	+	-	-	+	-	GNR	*E. coli*
Colonies were flat and white on agar plate	-	+	+	+	-	-	-	-	-	+	-	GPR	*B. Substilis*
Colonies were creamy, round,convex smooth edges	-	+	+	+	+	+	-	+	-	+	-	GNR	*Salmonella Spp*
Colonies were white and swampy	-	+	+	+	+	-	+	-	-	+	-	GNR	*Proteus mirabilis*
Black convex colonies with smooth edges	+	+	+	+	+	-	-	-	-	+	-	GNR	*Shigella spp*
Colonies were creamy convex with flat edges	-	+	+	+	+	-	-	-	-	+	-	GPR	*Corynebacterium spp*

Key + = Positive; - = Negative; GPC= Gram Positive cocci; GPR = Gram positive rod; GNR= Gram negative rods

A total number of seven organisms comprising of *Staphylococcus aureus, E.coli, Bacillus substilis, Salmonella spp, Proteus mirabilis, Shigella spp* and *Corynebacterium spp.* were isolated.

Table 4: Organisms isolated from fish feed

Durante	Coppens	Aqua	Multi
Staphylococcus aureus	*Salmonella spp.*	*corynebacterium*	*Salmonella sp.*
E.coli	*E.coli*	*S.aureus*	*Shigella sp.*
Salmonella sp.	*Shigella sp.*	*Bacillus sp.*	*S.aureus*
Proteus sp.	*Proteus sp.*	*Shigella sp.*	*Corynebacterium*

Table 5: Organisms isolated from Poultry feed

Starter	Grower	Layer	Finisher
Staphylococcus aureus	*Salmonella sp.*	*Corynebacterium*	*Salmonella sp.*
Bacillus sp.	*E.coli*	*Bacillus sp.*	*Shigella sp.*
Salmonella sp.	*Bacillus sp.*	*Proteus sp.*	*E.coli*
Shigella sp.	*Shigella sp.*	*Proteus sp.*	*Salmonella sp*

Table 6: Percentage of Occurrence of Bacterial Isolates contaminating poultry and fish feeds

Isolates	Fish feed	Poultry feed
Staphylococcus aureus	7(23.33)	7(18.91)
Salmonella sp.	6(20.00)	7(18.91)
Corynebacterium spp	1(3.33)	3(8.10)
Shigella sp.	4(13.33)	8(21.62)
Proteus mirabilis	1(3.33)	3(8.10)
E.coli	7(23.33)	3(8.10)
Bacillus substilis	4(13.33)	3(8.10)
Total	**30(100)**	**37(100)**

4 DISCUSSION

The microbiological analysis of poultry and fish feeds showed the presence of pathogenic bacteria like *Staphylococcus aureus, E. coli,Bacillus substilis, Salmonella spp, Proteus mirabilis, Shigella spp* and *Corynebacterium spp.* Results showed that Durante fish feed had the highest bacterial load of 3.32 X 10^5CFU/ml while for poultry feeds, starter feed had the highest bacterial load of 2.60 X 10^5CFU/ml.

In the study of Nwabueze and Nwabueze (2011) Coppens feeds had more bacterial load of 36 X 10^3cfug^{-1}. Dizengolf had 28 X 10^3cfug^{-1} while Durante feeds had 20 X 10^3cfug^{-1}. Coppens and Dizengolf feeds had more of *E. coli* than *S. aureus.* Durante had more of S. aureus than both Coppens and Dizengolf feeds.

From both poultry and fish feeds,the presence of E.coli suggest contamination most probably from the product retailers while the presence of *Staphylococcus aureus, Bacillus substilis* and *Proteus mirabilis* in the feeds suggests recent contamination most probably from the market sellers. This is because these three organisms are non-spore former and their presence in such samples like poultry feeds that are of very low water activity suggests recent contamination especially as the sellers and buyers have a feel of the feeds with bare hands thus exposing the feeds to microbial contaminants. Similar isolates were identified in the work of Obi and Ozugbo (2007) who found *Staphylococcus aureus, Bacillus substilis*, *Psuedomonas aeruginosa, Proteus vulgaris, Micrococcus sp.* And *E. coli* from poultry feeds in Umuahia. The presence of these organisms in poultry and fish feeds depicts a deplorable state of poor hygienic and sanitary practices employed in the manufacturing, processing and packaging of animal feeds.

From the total survey of this work it was discovered that the setting and operation of the market is not well organized structurally as the animal feeds retailers sections were close sanitary conveniences like public toilets and urinary and also heaps of refuse that constitute breeding grounds of disease vectors are constantly present around the market surroundings and this can lead to cross contamination by microbes via the activities of disease vectors like houseflies.

5. CONCLUSION AND RECOMMENDATION

The presence of these organisms in the feed samples is of great health concern and for attention in the storage strategies employed by the poultry and fish feeds manufacturers, the ware house condition, distributors and the sellers. The opened bag of feeds should be kept in clean cases and sealed in bags to prevent pathogens that can be transferred by houseflies and dust particles gaining access to the exposed feeds. Poultry farmers on the other hand, must ensure proper disposal of poultry droppings in other to prevent contamination of feeds via pathogens from the animal droppings to the feeds.

To rectify this situation from future occurrences, poultry and fish feeds should be stored in dry atmospheric environment to prevent the growth of organisms.

Furthermore, absolute quality control measures should be adhered strictly to in the poultry and fish producing industries to ensure that raw materials are free from microbial contamination during

production, packaging, storage and transporting to the various sites where it will be sold**.**

It is therefore strongly recommended that Government and public health organizations should raise public awareness on the diseases that could be possibly contracted from poultry birds and fishes that consume contaminated feeds. Also, producers of poultry and fish feeds should provide service points where subsistence poultry and fish farmers can purchase wholesome feeds without the risk of contamination.

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