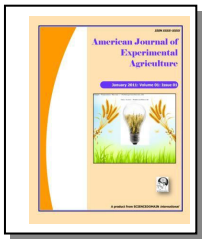

American Journal of Experimental Agriculture
1(2): 27-32, 2011

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Microbial Flora of Fish Feeds Sold in Asaba, Southern Nigeria

A. A. Nwabueze[1]* and E. O. Nwabueze[2]

[1]*Department of Fisheries, Delta State University, Asaba Campus, Asaba, Nigeria.*
[2]*Department of Agricultural Technology, Delta State Polytechnic, Ozoro, Nigeria.*

Research Article

Received 30th January 2011
Accepted 21st February 2011
Online Ready 28th February 2011

ABSTRACT

The microbial flora of fish feeds was investigated in this study. Commercially available fish feed samples were procured from sales outlets in Asaba, southern Nigeria. Three common fish feeds of three different particle sizes each, Coppens (6mm, 4.5 and 3mm), Dizengolf (10mm, 4.5mm and 2mm) and Durante (6mm, 3mm and 2mm) were cultured under laboratory conditions for bacteria and fungi growth. Serial dilutions of the fish feeds were made. The sub-cultured and pure cultures of samples were obtained. Bacteria isolates were characterized and identified. Bacterial count was determined using spread plate count. Isolated and identified bacteria were *Escherichia coli* and *Staphylococcus aureus.* Out of the nine sizes of cultured fish feed samples examined, *E. coli* was found in eight while one had *S. aureus.* Four of the samples had both *E. coli* and *S. aureus.* Bacterial counts from Coppens feed was significantly higher ($P<0.05$) than bacterial counts from Dizengolf and Durante fish feeds which had counts not significantly different ($P>0.05$) from each other. No fungus was found. Results obtained show that fish feeds sold in Asaba may have microbial flora with bacteria as the dominant microorganism. Storage conditions and unhygienic handling during storage, probably introduced bacterial flora in the fish feeds. Long duration of storage and scooping of feeds in small quantities during sales should be discouraged. Also, improved storage conditions to prevent fish feed contamination by microorganisms are recommended.

Keywords: Fish feeds, microbial flora, Asaba, Nigeria;

**Corresponding author: Tel: +234 806 413 8580; Email: arimiche@yahoo.com*

1. INTRODUCTION

Fish like other animals have a requirement for essential nutrients in order to grow properly. In the wild, natural feeds are available and as the fish forage for these, they are able to meet their body needs. When fish is removed from its natural environment to an artificial one, enough food must be supplied in order to enable them grow. Artificial diets may be either complete or supplemental. Complete diet supply with all the ingredients (protein, carbohydrates, fats, vitamins and minerals) is necessary for the optimal growth and health of the fish. Supplemental diet do not contain a full complement of nutrients needed but are used to help fortify the naturally available diets. Riche and Garling (2003) reported that fish reared in intensive tank systems requires all nutrients in a complete pelleted diet since natural food is limited and fish cannot forage freely for natural foods. This has the advantage of high quality and consistency of diet. Good nutrition in fish production system is essential to economically produce healthy, high quality fish products.

Fish feeds are constantly in contact with environmental organisms and become readily colonized by various microbial species. According to FAO (1987), environmental factors during storage predispose the fish feeds to microbial spoilage. The presence of bacteria in feeds causes their decomposition and subsequently, fish diseases. Bacteria such as *Salmonella, E. coli* and other bacteria strains have been reported to contaminate fish feeds (Zmyslowska and Lewandowska, 1999, Ciceron *et al*., 2008, Kaarine, 2010). Fungal contamination of fish feed has been reported to result in aflatoxicosis (Ashley, 1970). Aflatoxins are chemical produced by fungi like *Aspergillus flavus* and *A. parasiticus* commonly known as mold (Russso and Yanong, 2006). Mold-infested fish feeds have been reported to impact negatively on the growth of *Heterobranchus bidorsalis* fish (Effiong and Alatise, 2009). Aflatoxins in fish have been known to be capable of having carcinogenic effects on human consumers of contaminated fish (Brown, 2009). The occurrence of these microbial strains in fish feeds has been reported to depend on the storage conditions of the feeds, particularly temperature.

The quality of fish feeds and the hygienic levels of technological process employed during feed formulation determine the level of risk of microbial contamination aided by temperature. According to Zmyslowska (2000), storage conditions especially temperature and humidity are important factors affecting microbial quality of fish feeds. Improper storage temperature may prolong survival of the microorganism in fish feeds by enhancing their multiplication and production of toxic substances which may be injurious to fish.

Rearing of fish in concrete tanks using artificial diet has become a common practice in Asaba. Several outlets for sale of artificial fish feeds now exist. There is a need to investigate the microbial flora of fish feeds sold in Asaba and to ascertain their storage conditions in order to ensure their safety as food for fish. This is to forestall contamination of fish feed and by implication prevent transmission of harmful toxic effects on human consumers of fish.

2. MATERIALS NAD METHODS

2.1 PROCUREMENT OF FISH FEED SAMPLES

Commercially available fish feed samples were procured from ten randomly selected sale outlets in Asaba in 2008. One kg of each different sizes of three common fish feeds namely: coppens (6mm, 4.5mm and 3mm), Dizengolf (10mm, 4.5mm and 2mm) and Durante (6mm, 3mm and 2mm) were purchased and transported to the laboratory in sterile polyethylene bags to be cultured under laboratory conditions for bacterial and fungal growth.

2.2 ANALYSIS OF FISH FEED SAMPLES

One gram of the different sizes of fish feeds were dissolved in one liter distilled water (1g/l). Serial dilution to get clear solutions were made for the nine samples of different sizes of fish feeds. Mac Conkey agar was used to select and differentiate between the enteric bacteria based on their ability to ferment lactose (ICMSF, 1980). 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 described by Mac Faddin (1980) and Buchanan and Gibbson (1974). Gram staining was carried out to distinguish between gram-positive and gram-negative bacteria. Biochemical characterization was also determined. Bacterial count was determined using spread plate count according to APHA (1998).

2.3 STORAGE CONDITION OF FEEDS

The storage conditions of these sale outlets were considered as well as the duration of storage of feeds before sales in these outlets. Environmental factors prevailing in the stores considered were: temperature, relatively humidity, insect and rodent invasion and ventilation.

2.4 ANALYSIS OF DATA

Data collected were analyzed using analysis of variance at 95% confidence interval. Significant means were separated with Duncan Multiple Range test (DMRT).

3. RESULTS AND DISCUSSION

3.1 BACTERIAL ISOLATES

Microbial analysis of fish feeds showed the presence of only bacteria cells. Bacteria isolated and identified were *Escherichia coli* and *Staphylococcus aureus.* Out of the nine sizes of cultured fish feed samples examined, eight had *E. coil* and one had *S. aureus.* Four of the samples had both *E. coli* and *S. aureus.* Fungi were not found in the fish feeds examined. Gram staining and biochemical characterization of *E. coli* and *S. aureus* isolated are presented in Table 1.

Table 1: Character of bacterial isolates obtained from different fish feed samples

Fish Feeds	Growth	G. Stain	Indole RXN	Urease RXN	Catalyse	Confirmation
Coppens (6mm)	+	GNB	+	–	–	*E. coli*
Coppens (4.5mm)	+	GNB	+	–	–	*E. coli*
Coppens (3mm)	+	GNB, GPC	+ –	– –	– –	*E. coli* & *S. aureus*
Dizengolf (10mm)	+	GNB	+	–	–	*E. coli*
Dizengolf (4.5mm)	+	GNB, GPC	+ –	– –	– –	*E. coli* & *S. aureus*
Dizengolf (2mm)	+	GNB	+ –	–	–	*E. coli*
Durante (6mm)	+	GNB, GPC	+ –	– –	– –	*E. coli* & *S. aureus*
Durante (3mm)	+	GNB, GPC	+ –	– –	– –	*E. coli* & *S. aureus*
Durante (2mm)	+	GPC	–	–	–	*S. aureus*

Key: + = Positive; GNB = Gram Negative Bacilli; – = Negative; GPC = Gram Positive cocci

3.2 BACTERIAL COUNTS

Coppens feeds had more bacterial load of 36 x 10^3 cfug^{-1} of *E. coli* and 12 x 10^3cfug^{-1} of *S. aureus.* Dizengolf had 28 x 10^3 cfug^{-1} of *E. coli* and 12 x 10^3 cfug^{-1} of *S. aureus* while Durante feeds had 20 x 10^3 cfug^{-1} of *E. coli* and 30 x 10^3 cfug^{-1} of *S. aureus.* Coppens and Dizengolf feeds had more of *E. coli* than *S. aureus*. Durante had more of *S. aureus* than both Coppens and Dizengolf feeds. Bacterial isolates and counts from different sizes of fish feed samples are shown in Table 2.

Table 2: Bacterial counts of isolates from Fish Feed Samples (10^3 cfug^{-1}).

Fish Feeds	*E. coli*	*S. aureus*
Coppens 3mm	10	8
Coppens 4.5mm	14	4
Coppens 6mm	12	-
Dizengolf 2mm	8	-
Dizengolf 4.5mm	12	12
Dizengolf 10mm	8	-
Durante 2mm	-	6
Durante 3mm	6	12
Durante 6mm	14	12

This study has shown that *S. aureus* counts were lower compared with *E. coli* count. Bacterial counts from Coppen feeds were significantly ($P<0.05$) higher than counts from Dizengolf and Durante fish feeds. However, bacterial counts from Dizengolf and Durante fish feeds were not significantly ($P> 0.05$) different. Bacterial counts of *E. coli* and *S. aureus* were not significantly ($P>0.05$) different in the three types of feeds examined. Table 3 shows the mean bacterial counts of isolates from the three fish feeds. Bacteria counts reported in this study were lower than the standard plate count of 1.0 to 5.0 x 10^4 ml^{-1} of bacteria concentration in water (Buras, 1990). This bacterial concentration according to Buras (1990) is the critical concentration amounting to 1.0 to 2.0 x 10^4 ml^{-1} standard plate count for total bacteria for common carp under culture conditions.

Table 3: Mean bacterial counts of isolates in fish feeds (10^3 cfug^{-1}).

Types of Feed	*E. coli*	*S. aureus*
Coppens	14.33^b	5.33^b
Dizengolf	7.00^a	1.33^a
Durante	2.33^a	3.33^a

** Values with different superscript are significantly different (P< 0.05).*

3.3 STORAGE CONDITIONS

The storage conditions of the feeds in most of the sale outlets were observed to vary from one store to the other. The temperature readings obtained in the stores ranged from 26-32^{0}C. Relative humidity was similar in all the stores visited. Feeds, coppens (6mm, 4.5mm and 3mm), Dizengolf (10mm, 4.5mm and 2mm) and Durante (6mm, 3mm and 2mm) were found to be stored in 15kg bags with expiry dates of 6 months from the manufacturing date. The bags of feeds were stacked together one on top of the other on platforms with bags resting on walls in most of the stores. Feeds were observed to be sold in bags and scooped in smaller quantities measured with

kitchen scale to buyers. Some of the stores visited had expired feeds and some feeds almost due to expire. All the stores visited had adequate ventilation and clean floors. The storage conditions observed compared with standard storage conditions of fish feeds is presented in Table 4. According to FAO (1987), environmental factors like moisture, relative humidity, temperature, light and oxygen cause deteriorative changes and losses in stored feedstuff. High levels of moisture content and relative humidity in feeds during storage result in direct losses due to growth of micro-organisms, making it difficult to use the material in its original form. Scooping of fish feeds in small quantities for sale and sometimes using damp sacks may also predispose the fish feeds to bacterial contamination. *E. coli* and *S. aureus* were the bacteria isolated in this study. *E. coli* was observed to occur more than *S. aureus* in the fish feeds. *E. coli* contamination of fish feeds has been documented (Malicki *et al.*, 2004). The elimination of bacteria either by treatment of feeds with antibiotics or increasing the storage temperature to eliminate fungi growth is possible. The absence of fungi in fish feeds in this study shows that the storage temperature available in stores may not be suitable for the growth of fungi.

Table 4: Storage conditions of fish feeds in sale outlets compared with standards.

Storage Conditions	Observed Storage Conditions	Standard storage Conditions
Temperature (^{0}C)	26-32	5 and 20 (Zmyslowska and Lewandowska, 1999)
Relative humidity (%)	82	75 (Michael, 1987)
Insect invasion	Nil	Nil
Rodent invasion	Nil	Nil
Ventilation	Adequate	Well ventilated (FAO, 1987)

4. CONCLUSION

Results obtained show the presence of bacteria in most fish feeds sold in Asaba. Bacterial counts were however below the threshold of contamination. Coppens feeds had more *E. coli* than Dizengolf and Durante feeds. *S. aureus* was low in Coppen feed. Dizengolf and Durante also had low counts of *E. coli* and *S. aureus.* *E. coli* is the dominant micro-organism of fish feeds sold in Asaba. Unhygienic handling of feeds during sales and storage conditions may have influenced the type and bacterial counts in feeds observed. Expired feeds should not be sold to unsuspecting customers. Also, long storage periods in stores should be avoided. Improved hygienic conditions of fish feeds during storage and sales are essential for microbial-free fish feeds. Also, scooping of fish feeds in small quantities from bags during sales should be properly and hygienically handled to prevent occurrence of micro-organisms in fish feeds.

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