Microflora associated with commercial fish feeds sold in Abeokuta, South West, Nigeria

AMREVUAWHO, M.O.[1], EZERI, G.N.O.[1], AKINDUTI, A.P.[2], and D.D.[1]OLOWOOKERE

[1]*Department of Aquaculture and Fisheries Management, College of Environmental Resources Management, Federal University of Agriculture, Abeokuta, Ogun State*

[2]*Department of Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State*

Abstract

Microbial load in feed has become a major source of disease outbreak in fish culture hence, the study aimed to determine the microbial load of four commercial fish feeds. Four new commercial fish feeds of different particle size [Ranaan feed (6mm), Optimum feed (4mm), Aller Aqua (8mm), Multi feed (2mm)] were purchased in the months of March and July, 2016. Feeds were analyzed for proximate nutrient composition and total microbial load using spread plate method. Proximate analysis showed moisture content and crude protein was less than and within the specified range for fish culture (12% and 32 %) respectively. Bacteria isolated include *Klebsiella* species, *Pseudomonas aeruginosa*, *Eschericia coli*, *Pseudomonas fluorescens*, *Micrococus* species, *Bacillus subtilis*, and *Staphylococus saprophyticus* (March) and *Bacillus mycoides* was included in the month of July. Fungi species include *Penicillium notatum* and *Fusarium oxysporum* (March) while *Fusarium solani*, *Altenaria alternaria* ,*Aspergilus flavus* and *Acremonium* species were included in the month of July. Highest bacteria count was obtained in the month of July in Optimum feed (5.3×10^6cfu/ml) and fungi count also in the month of July in Ranaa feed (3.4×10^6cfu/ml). Conclusively, utmost attention should be paid to fish feed ingredients, fish feeds, packaging, storage among others in a bid to prevent contamination and transfer of pathogenic organisms to the fish and human consumers.

Introduction

Globally, aquaculture is the fastest growing food-producing sector. Fish make up about 60 % of total world protein supply. However, like other animals, the place of nutrition (fish feed) cannot be over emphasized. The advantage of fish as food is that it is easily digestible and has high nutritional value. As human food, fish protein contains most of the essential amino acids especially lysine, methionine and tryptophan. Due to its low cholesterol level coupled with high quality nutrient profile, it is most desirable (World Fish Center, 2013).

Feed accounts for over 50 % of total cost of production in fish culture at a satisfactory level. However, if fish feed are not properly packaged and stored, microbes and environmental alteration will take place. Farmers buy commercial fish feeds that have been in the warehouse for a period of time without knowing the actual nutritive value as at the time of purchase. Fish require a specific ratio of digestible protein to grow, however, protein are among the most challenging raw material in feed production and the human food industry. Feeds may contain organic and in-organic materials that have beneficial, negligible or deleterious effects on the growth or health of the fish or the sensory quality of the processed fish. Hence, it is essential to have adequate knowledge of the nutritional requirement of the cultured fish and also the nutrient composition of the purchased feed.

The singular most important input in increasing aquaculture production and profits is feed (Martin, 2002). The success or failure in augmenting yield with feeding, rest largely on the quality of the diet as ease of digestibility is a factor of feed quality. Although, the quality of feed is generally perceived as the responsibility of the feed manufacturer, it is affected by other factors such as handling, storage and use, which onus lies on the farmer. High rate of disease outbreak and eventual mortality of cultured fish is a major problem facing the aquaculture industries. Studies have shown that microorganisms especially bacteria and fungi are the major cause of disease outbreak in aquaculture (Amrevuawho *et al.*, 2014). it is important that fish farmers familiarise themselves with the nature and occurrence of major feed quality problems and able to prevent and control them (SEAFDEC, 2015). Also, previous research has indicated the possibility of transfer of infection from feed to fish (Maciorowski *et al.*, 2006). There is therefore the need to determine the load of microbes in stored commercial fish feeds.

This study was therefore aimed to determine the proximate composition of these commercial fish feeds, examine their microbial load and compare the microbial load of the feeds in two months (March and July) of 2016.

Materials and Methods

Procurement of fish feed samples

500g of new commercial fish feed samples of different sizes (Multi-feed (2mm), Optimum feed (4mm), Ranaa feed (6mm) and Aller-aqua feed (8mm)) were purchased from four selected outlets in Abeokuta metropolis in the months of March and July 2016 each month representing dry and wet months of the year. Expiration dates were noted in all the feed samples. Feed were transported in sterile polyethylene bags to the laboratory for further

studies on proximate composition of the feed and microbial load.

Laboratory procedure for proximate analysis of the different feed samples

Proximate analysis of each of the commercial feed samples was carried out according to the procedures of AOAC (2000) for ash, moisture, crude fiber, ether extract and protein content using nitrogen to protein conversion factor of 6.25.

Microbiological Analysis of the different feed samples

Preparation of materials

The materials needed for this experiment which included: glass wares (Conical flasks, Bijou bottles, Pipettes, MacCartney bottles, Petri dishes) were washed with detergents, rinsed thoroughly with distilled water and left to air dry before sterilizing them in hot-air oven at 160^{0}C for 1 hour. The wire loop to be used was sterilized by flaming it red-hot using a spirit lamp. Also, the laboratory cabinets on which the work was carried out was swabbed with cotton wool soaked in ethanol to sterilize it before any microbiological analysis was carried out, to avoid the growth and isolation of other organisms not present in the samples.

Preparation of media

Media for microbiological analysis (Nutrient Agar, Potato Dextrose Agar and MacConkey Agar) were weighed and prepared according to the manufacturer's specifications before being used.

Isolation and enumeration of associated microorganisms

Serial dilution was determined according to Hedges method as described by Ben-David (2014). One gram of each of the sample was first measured and dissolved in 10 mL of sterile distilled water prior to serial dilution. One milliliter aliquot was diluted with 9 mL of sterile water in different test tubes to give 1:9 dilutions. From this, ten-fold serial dilutions were made up to 10^{-6}. One milliliter of the sample was plated on nutrient agar for bacteria, *Eosin methylene blue* (EMB) agar for coliform organisms and *Mannitol Salt agar* (MSA) for ***Staphylococcus aureus*** . Dilution of 10^{-6} was plated on Potato Dextrose Agar (PDA) for fungi count. All the plates in triplicates were incubated at 37°C for 24 h for bacteria, while the plates for fungi were incubated at 25°C for 24-72 h.

Enumeration of associated microorganisms

Colonies of microorganisms that developed on the plates after incubation were counted, recorded and expressed as standard numbers of colony forming unit per milliliter (cfu mL^{-1}). The discrete colonies that grew were sub-cultured on fresh media to obtain pure cultures. The pure cultures were maintained at 4°C as stock culture for further tests

Identification of fungi

This was carried out according to James and Natalie (2008) using cotton blue in lactophenol stain. The identification was done by placing a drop of the stain on a clean slide with the aid of a mounting needle; a small portion of the mycelium from the cultures (from an area 4 mm from the edge) was removed and placed in a drop of lactophenol on the slide. The cover slip was gently lowered onto the preparation, while allowing the heat from the microscope lamp to spread the medium evenly and to eliminate air bubbles. Each of the slides was then mounted and viewed under x10 and x40 objective lenses respectively.

Identification of bacterial isolates

The isolated bacteria were identified using cultural characteristics (i.e. the color, shape, elevation, capacity, consistency, edge), morphological and biochemical test was by the hanging drop technique and according to the method of Cowan and Steel (1993).

Results

The proximate composition of the different feed samples

The proximate composition of the different feed samples is shown in Table 1.

Biochemical identification of feed samples in both months

Microbial analysis of commercial fish feeds for the months under study showed the presence of bacteria and fungi cells. Organisms isolated, characterized and identified are as presented in Table 2

Total microbial count of the different feed samples in both months

Tables 3a - e shows the bacteria and fungi count of the different commercial fish feed sampled in the months of March and July. The result of the bacteria and fungi load in feed samples showed that the highest bacteria count was observed in Optimum feed in the month of July ($5.3.3\times10^6$cfu/ml) and fungi in Ranaa feed also in the month of July (3.4×10^6 cfu/ml).

Discussion

Microbial count of the different fish feeds was observed to be high in both months (Table 3) especially in the wet month. Arotupin *et al.*, (2007) in their study of microbial load in stored commercial poultry feed reported similar high microbial load of the feeds and attributed reasons to reflect the contaminants in the feed ingredients. However, packaging, packaging materials, environment and handling circumstances, and storage condition including the nature and extent of the quality control measures (Hancock *et al.*, 1998) adopted could still be responsible for such high load of microbes which also holds true for the findings of this study as there were variations in the microbial load of the feeds examined in the different months.

The presence of these microorganisms which include *E. coli*, *S. saprophyticus*, *B. subtilis*, *Micrococcus* sp, *P. fluorescens*, *Klebsisella* sp, *P. notatum* and *F. oxysporum*, *F. solani*, *A. alternaria*, *Aspergilus* and *Acremonium* spp (Table 2) in the feed samples shows that there was sufficient nutrient in the feeds to promote the growth of these organisms. Most of these isolates are highly pathogenic in aquaculture and even to man thus being a serious course of public health concern.

For farm animal's growth and good performance, the nutritional requirements of their feeds are essential. The required percentage ash, fat, crude fiber and protein content are a factor of the different stage of their growth. However, these nutrients also play vital role in the development and build-up of contaminating microorganisms. Highest crude protein was found in Multifeed (31.17%) and the least in Aller Aqua (25.56%). Although, crude protein percentage for multifeed (2mm) were within the specified range for cultured fish and even less for the size of fish (fingerlings), it could however, be responsible for the diversity of microbes observed in the feed. Wilson (2000) posited that commercial fish feeds for Catfish production and grow out should contain 32% crude protein for adult catfish which correspond with observed study. Similarly, Eyo (1996) and Robinson *et al.* (2006) estimated that the crude protein requirement for tropical Catfish to be 35-40, 25-35 and 28-32% for fry, grow-out and broodstock.

Moisture content observed in this study although, were within the specified limit (12%) for farm animals, including fish could justify the increased microbial load observed in the feed especially in the month of March which is a dry month with hot temperature. Activities of these different factors: increased moisture, high temperature could be said to be responsible for the increased microbial load in the feeds. This agrees with the findings of previous researchers on the effect of these factors on microbial growth (Ray, 1996; Jay, 2000). Also, fungi count in the month of July was observed to be highest in all the feed samples and reasons could be due to high water activity during the wet period of the year and likely storage condition. Jubeen *et al.* (2012) reported significant increase in fungi load during storage period of feed at high moisture levels in grounds and tree nuts.

Microbial load was higher in the month of July than March which corroborates other studies on the effect of storage condition in the wet season and dry season on microbial growth. Nwabueze and Nwabueze (2011) in their study posited that occurrence of microbial strains may depend on the storage condition of the feed especially as a result of the temperature of the store house.

Conclusion

In conclusion, nutrient composition of all feed samples analyzed was within the acceptable range required for the different stages of fish growth which they are meant to feed. Also, other factors such as moisture content, period of the year, handling and nutrients levels can influence the growth of microorganisms in stored feed and feed ingredients. Constant quality assessment of commercial fish feeds on sale should be emphasized.

References

Amrevuawho, M.A., Akinyemi, A.A., Ezeri, O.G.N., Bankole, O.M. and Takeet, O.V.A. (2014). Pathological study of *Clarias gariepinus* (Burchell, 1822) sub-adult artificially infected with *Pseudomonas aeruginosa*. *Braz. J. Aquat. Sci. Technol.* 18(2): 65-70. eISSN 1983-9057. DOI: 10.14210/bjast.

Arotupin, D.J., Kayode R.M.O. and Awojobi, K.O. (2007). Microbiological and Physicochemical Qualities of Selected Commercial Poultry Feeds in Akure, Nigeria. *Journal of Biological Sciences*, 7: 981-984 **DOI:** 10.3923/jbs.2007.981.984.

Association of official Analytical Chemists (AOAC) (2000). Official methods of Analysis. Association of official Analytical Chemists, Inc, Washington DC, USA, 35p.

Ben-David, A. and Davidson, E.C. (2014). Estimation method for serial dilution experiments. *Journal of Microbiological Methods*, 107: 214–221. http://dx.doi.org/10.1016/j.mimet.2014.08.023.

Cowan, S.T. and Steel, K.J. (1993). *Enterobacteriacea*. In G.I Barrow, R. K. A. Felthan, (Eds). *Manual for the Identification of Bacteria* (3rd edition), Cambridge University press, United Kingdom, 213-218.

Eyo, A.A. (1996). Fish feed formulation. NIFFR Extension Guide series number 7. In collaboration with GTZ and NAERLS.

Hancock, D.D., Besser, T.E., Rice, D.H., Ebel, E.D., Herriott, D.E. and Carpenter, L.V. (1998). Multiple sources of *Escherichia coli* 0157 in feed lots and dairy farms in the Northern USA. *Preventive Vet. Med.*, 35: 11-19.

Hedges, A.J. (2002). Estimating the precision of serial dilution and viable bacteria count. *International Journal of Food Microbiology* 76(3): 207-214.

James, G.C. and Natalie, S. (2008). Microbiology. A Laboratory Manual (8th edition). Pearson Education, Incorporation, USA, page 569.

Jay, J.M. (2000). Modern food Microbiology. 6th ed. Gaithersburg (MD): Aspen. page 679.

Jubeen, F., Bhatti, I.A., Maqboo, U. and Manhboob, S. (2012). Fungal incidence, aflatoxin B1, Tocopherols and fatty acids dynamics in ground and tree nuts during storage at two moisture levels. *International Journal of Agricultural Biology* 14: 521-527.

Maciorowski, K.G., Herrera, P., Jones, F.T., Pilai, S.D. and Ricke, S.C. (2006). Effects on poultry and livestock of feed contamination with bacteria and fungi. Elsevier B.V. pages 109-136.

Martins, J.D. (2002). Proper feed handling, storage can help minimize aquaculture feed expenses. Feed stuffs. In: FISON conference proceedings page 2011.

bueze, A.A. and Nwabueze, E.O. (2011). Microbial ra of Fish Feeds sold in Asaba, Southern Nigeria. *merican journal of Experimental Agriculture (2): 27-31.*

Ray, B. (1996). Fundamental food microbiology. Boca Raton (FL): CRC Press. 516 p.

Robinson, E., Menghe, H.L. and Hogue D.C. (2006). Catfish Nutrition Nutrient Requirements. Mississi State University, Extension Service Publication 2412.

SouthEast Asian Fisheries Development Center (SEAFDEC) (2015). Feed quality problems and management strategies. In: Santiago, C.B., Coloso, R.M., Millamena, O.M. and Borlongan, I.G. (Eds.). Feeds for Small-Scale Aquaculture. Proceedings of the National Seminar-Workshop on Fish Nutrition and Feeds, 64-73.

Wilson, R.P. (2000). Channel Catfish, *Ictalurus Punctatus* In: Handbook of Nutrient Requirement of Finfish, Wilson, R.P. (Ed.). CRC Press, Boca Raton, USA, 35-53 pp..

World Fish Centre (WFC) (2013). An issue for everyone. A concept paper for 'Fish for All' produced by World Fish Centre, Penang, Malaysia, 2-4 pp..

Table 1. Proximate composition of commercial feed samples

Parameter	A	B	C	D
Crude Protein (%)	25.56	30.12	31.17	29.94
Ether Extract (%)	16.00	15.00	14.00	15.00
Ash (%)	6.00	9.50	7.00	8.50
Moisture (%)	12.60	10.00	10.20	11.00
Crude Fiber (%)	5.50	7.00	4.00	6.00

Key: A - Aller Aqua, B – Optimum, C – Multifeed, D - Ranaa

Table 2: Microbial isolates from fish feeds in both dry and wet months

s/n	Bacterial isolates		Fungi isolates	
	Dry	Wet	Dry	Wet
1	*Klebsiella* sp	*Klebsiella* sp	*F. oxysporum*	*F. oxysporum*
2	*P. aeruginosa*	*P. aeruginosa*	*P. notatum*	*P. notatum*
3	*E. coli*	*E. coli*		*F. solani*
4	*P. fluorescens*	*P. fluorescens*		*A. alternaria*
5	*Micrococcus* sp	*Micrococcus* sp		*A. flavus*
6	*B. subtilis*	*B. subtilis*		*Acremonium* sp
7	*S. saprophyticus*	*S. saprophyticus*		
8		*B. mycoides*		

Table 3a: Total bacteria count of the feed sample during the dry month

SAMPLE	TBC ($<10^6$cfu/ml)	A	B	C	D	E	F	G
S1	1.4	+	+	+	+	-	-	-
S2	1.3	+	-	+	+	+	+	-
S3	1.7	+	+	+	+	-	+	+
S4	2.1	+	+	-	+	+	+	+

TBC=Total bacteria count, cfu/ml=colony forming unit per ml; - (Negative); Positive); S1- Ranaa feed (6 mm), S2- Optimum feed (4 mm), S3 – Aller Aqua feed (8 mm), S4 – Multifeed (2 mm), A = *Escherichia coli*; B = *Pseudomonas aeruginosa*, C= *Staphylococcus saprophyticus*; D= *Bacillus subtilis*; E= *Micrococcus* sp; F= *Pseudomonas fluorescens*; G= *Klebsisella* sp

Table 3b: Total Fungi count of the different feed samples during the dry month

SAMPLE	TFC (*10^6cfu/ml)	A	B
SAMPLE 1	0.0	-	-
SAMPLE 2	0.0	-	-
SAMPLE 3	0.1	+	-
SAMPLE 4	0.3	+	+

TFC= Total fungal count; - (Negative); + (Positive); CFU/MI= Colony forming unit; A= *Penicillium notatum*; B= *Fusarium oxysporum*

Table 3c: Total bacteria count of the feed sample during the rainy month

SAMPLE	TBC (×10^6cfu/ml)	A	B	C	D	E	F	G	H
S1	1.5	-	+	+	+	-	+	-	+
S2	5.3	+	+	-	+	+	+	-	+
S3	1.3	-	+	+	+	-	+	+	-
S4	1.2	+	+	+	+	+	+	-	+

TBC=Total bacteria count, cfu/ml=colony forming unit per ml; - (Negative); Positive); S1- Ranaa feed (6 mm), S2- Optimum feed (4 mm), S3 – Aller Aqua feed (8 mm), S4 – Multifeed (2 mm), A = *Escherichia coli*; B = *Pseudomonas aeruginosa*, C = *Staphylococcus saprophyticus*; D = *Bacillus subtilis*; E = *Micrococcus* sp; F = *Pseudomonas fluorescens*; G = *Klebsisella* sp, H = *Bacillus mycoides*

Table 3d: Total fungi count of the feed samples during the rainy month

SAMPLE	TFC (×10^6cfu/ml)	A	B	C	D	E	F
S1	3.4	+	-	+	+	+	+
S2	1.0	+	+	+	+	-	-
S3	2.3	+	-	+	-	+	+
S4	1.5	+	-	+	-	+	+

TFC=Total fungi count, cfu/ml=colony forming unit per ml; - (Negative); Positive); S1- Ranaa feed (6 mm), S2- Optimum feed (4 mm), S3 – Aller Aqua feed (8 mm), S4 – Multifeed (2 mm), A = *Penicillium notatum* B = *Fusarium oxysporum* C = *Fusarium solani*, D = *Altenaria alternaria*, E = *Aspergilus flavusm*, F = *Acremonium* sp

Table 3e: Microbial count of fish feed samples

Feed Samples	Bacterial count (×10^6cfu mL^{-1})		Fungi count (×10^6cfu mL^{-1})	
	Dry	Wet	Dry	Wet
S1	1.4	1.5	0.0	3.4
S2	1.3	5.3	0.0	1.0
S3	1.7	1.3	0.1	2.3
S4	2.1	1.2	0.3	1.5

cfu/ml=colony forming unit per ml; S1- Ranaa feed (6 mm), S2- Optimum feed (4 mm), S3 – Aller Aqua feed (8 mm), S4 – Multifeed (2 mm),