COMPARATIVE ASSESSMENT OF BIOLOAD OF HEALTHY AND DISEASED *Oreochromis niloticus* AS MEANS OF FOOD SECURITY

Toochukwu Ekwutosi OGBULIE*, Harriet Chinyelu NWIGWE*, Sylvia Onyinyechi ANYADOH*

[a]*Department of Biotechnology, School of Science, Federal University of Technology Owerri (FUTO) Imo State Nigeria.*
Corresponding author: Mrs. T.E. Ogbulie, Department of Biotechnology, School of Science, Federal University of Technology Owerri (FUTO) Imo State, Nigeria; phone: +2348035472379; e-mail: ogbulie_toochi@yahoo.com

Abstract. Thirty-one (31) samples each of diseased and healthy Tilapia fish (*Oreochromis niloticus*) from Otamiri River, in Nekede, Owerri West; Imo State Nigeria was examined to detect the presence of bacterial and helminth fauna. The intestine, liver, gill, tissue and skin of the fish were examined. Bacteriological analysis revealed counts of healthy diseased organs to fall between $6.0 \times 10^4 - 3.5 \times 10^7$ cfu/g and $5.7 \times 10^6 - 1.9 \times 10^{11}$ cfu/g respectively. The result however indicated that the bacterial load of the diseased fish samples were higher than those of the apparently healthy fish. Identification tests of the probable bacterial isolates revealed the isolation of *Vibrio* sp, *Renibacterium* sp, *Aeromonas* sp, *Klebsiella* sp, *Yersinia* sp, *Pseudomonas* sp, *Nocardia* sp, *Lactobacillus* sp, *Sporocytophaga, Staphylococcus* sp, *Mycobacterium* sp, *Serratia* sp *Proteus* sp and *Edwardsiella* sp. Twenty-nine (29 ie 46.8%) of the 62 samples studied were found to be infected by helminth fauna identified as *Camallanus* sp, *Procamallanus beviconchus, Capillaria sp, Clinostonium tilapiae, Euclinostonium heterostoma, Cleidodiscus sp and Bothricephalus acheilognathi*. Percentage helminth infestation was found to be higher in males than females with sub adults recording the highest infection rate of 56.08%. Hence helminth infestation varies amongst age group. This study therefore reveals the bacterial & helminth load of cultured organs of *Oreochromis niloticus* with a view to provide information on the state of environmental and personal hygiene in the environment, the level of contamination of water and the security and/or insecurity nature of using fish as food.

Keywords: comparative assessment, bacterial load, helminth load, organs, diseased fish, healthy fish, Otamiri, Nigeria.

INTRODUCTION

Millions of people in the world today have been reported to lack adequate access to food [6]. This according to FAO [8] has been estimated to be about 840 million people, 25% of which are sub-Saharan Africa. Probably as the population grows and puts more pressure on national resources, more people will probably become food insecure, lacking access to sufficient amount of safe and nutritious food for normal growth, development and an active and healthy life [20].

Fish has been an important source of food for centuries and has been reported to contribute around 50% of total animal protein in the diets of many Africans especially in less developed or developing areas where other sources of animal protein are scarce and expensive [9]. But the lack of tradition of fish and water husbandry in sub Saharan Africa and the past socio-economic, environmental and political constraints have limited investment and slowed expansion of African aquaculture, leaving sub-Saharan countries in an extremely difficult final situation [19], although predictions suggest an improvement in the future economic state of sub Saharan Africa which may therefore reduce the prevalence of food insecurity and wide spread poverty [3].

Studies in various countries have also shown that about 80 – 100 percent of aquaculture products marketed is from rural area [6]. Tilapia (*Oreochromis niloticus*) is however, the most widely grown and marketed fish. This, according to Little [12] and Roderick [21], is due in part to their superior growth rate on either natural, grazing or formulated feeds and are acceptable to both rich and poor. As herbivores also, they are less sensitive to the “fish meal trap” facing much of the world’s commercial aquaculture and can be raised with a much smaller ecological foot print.

Despite technological improvements to increase global food energy per person, there are still regional differences in productivity and distribution; hence some areas have excess food, while others are lacking [23]. In other words, food secure house holds should not be at risk of loosing access to food, which should be acquired in socially acceptable ways without resorting to emergency food supplies, scavenging, stealing or other coping strategies [10]. Purchasing power is therefore essential to guarantee access to sufficient food at the house- hold level [5] because the more presence of food does not entitle a person to consume it. Therefore to achieve food security, components such as effective biological utilization of safe nutritious food amongst others should be feasible so that every person can lead a healthy and productive life [18]. Unfortunately, it is not practiced in developing countries like Nigeria. This, as stated by FIVIMS [10], Cunningham [6] and Ozor and Igbokwe [17], could be due to various factors like socio economic and political environment, the performance of the food economy, lack of good agricultural practices, hostile climate and the health and sanitation situation of the country. In other words, attempting to ensure food security is seen as an investment in human capital in less developed and developing countries, hence, there is prevalence of food insecure people. Poor people therefore resort to fishing in rivers directly as their means of sustainable access to animal protein irrespective of the health conditions and indirectly as cash generating activity and hence an important source of food.

Direct dependance on fish as means of sustainable access to protein can be detrimental, hence the insecurity nature of fish as food. This is because fish can serve as intermediate or definite hosts for some parasites or bacteria [13, 14] and this can have adverse effect on their fecundity, nutritional value, life span and growth rate, among other things. However, according to World Bank [24] an improperly fed, physically and mentally weakened population by an inadequate diet and hence poor health do not effectively contribute to the economic development of that country. This study was therefore designed to ascertain the incidence of bacteria and parasites in/on Tilapia in Otamiri River, to provide information on the environmental status and personal hygiene in the environment within some rural area (study area), level of water contamination, the danger facing aquatic life as well as the security or insecurity nature of fish as food.

MATERIALS AND METHODS

Sample Collection

Samples of *O. niloticus* used for this study were obtained from Otamiri River in Nekede, Owerri West, Imo State; Nigeria. The fishes were caught with surface sterilized cast net and taken to the laboratory using surface sterilized covered bucket. Surface sterilization of cast net and bucket was carried out by washing first with detergent and shaking in 75% ethanol for 30 seconds, rinsed three times with sterile water for 10 minutes each, after which they were washed with 5.25% sodium hypochlorite solution for 15 minutes and then rinsed three times with sterile water for 10 minutes per wash [25]. The samples were taken to the laboratory for examination within three hours of collection.

Bacteriological Analysis

Five grammes of each organ of the fish used for the analysis were aseptically collected from dissected samples. These were separately put in different stomacher bags into which 45ml of sterile 0.85% (w/v) NaCl solution was added as diluent and homogenized, using a stomacher blender (model 4000 Seward, London). 10 fold serial dilution as described by Ogbulie *et al* [14] was however carried out using the same diluent. Total heterotrophic bacterial counts were carried out by plating appropriate dilutions of the sample on sterile Triptone Soy agar (Oxoid) and incubated at 30^0C for 24 hours [4]. Representative isolates were characterized as described by Holt [11].

Helminth Isolation and Identification

The fish samples were examined for presence of parasites using the method reported by Ogbulie *et al* [14] while the identification of these helminths was as described by Anosike *et al* [1].

RESULTS

The probable genus of the bacteria isolates obtained from the different fish organs are as shown in Table 1. Table 2 on the other hand depicts the distribution of bacterial types isolated from different fish parts with the intestine habouring the highest number. Bacterial counts shown in Figure 1 indicate that the diseased parts contained higher bacterial load than healthy parts. Figure 2 however, shows the percentage occurrence of isolates per organ of diseased and healthy fish. Of the 62 samples of fish examined, 29 (46.8%) were found to be infected by helminthic parasites. These helminthes belonged to class Nematoda, Trematoda and Cestoda (Table 3). The percentage prevalence of these helminthes is however shown in Figure 3, while the relative abundance of helminthes in the different organ is as shown in Table 4. Statistical analysis carried out using X^2 at 95% confidence interval showed that there was significant difference between infection and age as well as sexes of fish samples; sub-adults were more infected than the others where as fewer males (41%; 16 out of 39) were infected than females (57%; 13 out of 23) (Fig. 4).

Table 1. Characteristics of probable bacterial isolates from fish samples.

Grams rxn/ Shape	Motility	Catala-se	Oxida-se	Citrate	Oxidation-fermentation	Coagu-lase	Indole	Methyl red	Urease	Probable Genus
-ve rods	-	+	-	+	F	-	-	+	-	*Klebsiella*
-ve rods	+	+	+	+	O	-	+	+	-	*Pseudomonas*
-ve rods	+	+	+	+	F	-	+	-	-	*Vibro*
-ve rods	+	+	+	-	F	-	+	-	-	*Aeromonas*
+ve rods	-	+	-	-	F	-	-	+	-	*Yersinia*
+ve rods	-	+	-	+	O	-	+	-	-	*Nocardia*
+ve rods	-	+	-	-	NR	-	-	-	-	*Renibacterium*
+ve rods	-	-	-	-	F	-	-	+	-	*Lactobacillus*
+ve rods	-	+	-	-	O	-	+	+	-	*Sporocytophaga*
+ve rods	-	+	-	-	F	+	-	+	+	*Staphylococcus*
+ve rods	+	+	-	-	O	-	-	+	+	*Mycobacterium*
-ve rods	-	+	-	-	O	-	+	+	-	*Serratia*
+ve rods	-	+	-	-	F	-	+	+	+	*Proteus*
+ve rods	+	+	-	-	F	-	-	-	+	*Edwardsiella*

Legend: -ve – Gram negative; +ve – Gram positive; F – fermentation test; O – Oxidation test ; rxn – reaction; + - positive test; - = negative test; NR – no reaction.

DISCUSSION

Study on assessment of bioload of *Oreochromis niloticus* as means of food security was undertaken. The study revealed variance in the occurrence of bacteria and parasites in diseased and apparently healthy fish. The parts of diseased fish studied had higher bacterial isolates than healthy ones; this collaborates with the findings of Okpokwasili and Alapiki [15] and lends more weight to the findings of Okpokwasili and Ogbulie [16].

Isolation of highest number of bacterial genera from the intestines supports the findings of Sakata *et al*, [22] which according to Yoshimizu *et al* [26] is an indication of the presence of favourable ecological niches in the intestine for these microorganisms. Of the fourteen (14) bacterial isolates obtained from the organs of diseased fish, only two organisms of genera *Rhenibacterium* and *Sporocytophaga* were not isolated. *Edwardsiella* sp was isolated from intestine, skin and fin of diseased fish. This agrees with the findings of Austin and Austin [2] and supports the findings of Ogbulie *et al*, [14] who associated some species of this genus, for example *Edwardsiella tarda,* with tissue and fin rot of fish. Furthermore, the presence of *Edwardsiella* has also been reported to be one of the characteristic pathogen of fish in the tropic [13].

Table 2. Relative abundance of bacterial isolates from anatomical site of both diseased and healthy Tilapia fish samples.

Bacterial isolates	DISEASED FISH PARTS						HEALTHY FISH PARTS					
	Intes-tine	Liver	Gill	Fin	Tissue	Skin	Intes-tine	Liver	Gill	Fin	Tissue	Skin
Klebsiella	+	-	+	-	+	+	-	-	-	-	-	-
Vibro	+	-	+	+	-	+	-	+	-	-	+	-
Pseudomonas	+	-	+	+	+	+	-	-	-	-	-	-
Aeromonas	+	+	+	+	-	+	-	-	+	+	-	+
Yersinia	+	+	+	+	-	-	-	-	-	-	-	-
Nocardia	+	-	-	-	-	+	-	-	-	-	-	-
Renibacterium	-	-	-	-	-	-	-	-	-	+	-	-
Lactobacillus	+	-	+	-	-	-	-	-	-	+	-	-
Sporocytophaga	-	-	-	-	-	-	+	-	-	+	-	-
Staphylococcus	+	-	+	+	-	+	+	-	-	-	-	+
Mycobacterium	+	-	+	-	-	-	+	-	-	-	-	+
Serratia	-	-	-	+	-	+	-	-	+	-	-	-
Proteus	+	-	-	-	-	+	-	-	+	-	-	+
Edwardsiella	+	-	-	+	-	+	-	-	-	-	-	-
Total	11	2	8	7	2	9	3	1	3	4	1	4

Table 3. Different types of helminth parasites identified in the fish samples.

Class	Genus	Number Identified
Nematoda	*Camallanus* specie	36
	Procamallanus bevichonchus	30
	Capillaria species	17
Trematoda	*Clinostomium tilapiae*	14
	Euclinostomum heterostoma	16
	Cleidodisus species	26
Cestoda	*Bothricocephalus acheilognathi*	9

Generally, the predomination of organisms in diseased fish has been reported [13, 14] to be associated with reduced immunity as a result of hydrologic problems that characterize fish culture in the tropics. Such high bacteria flora could also be contingent to microbial flora of the water where the fish lives [13].

Helminthological studies carried out in the research shows that parasitism in fish varies amongst different fish farms and contingent on the prevailing factors within the ecosystem. Such factors according to Ogbulie *et al*, [14] could be feeding differences amongst age groups as well as overcrowding. It could also be due to the type of feed used, hygienic practices and pattern of feeding [16]. This study however, revealed that helminthes infection is gender related with higher incidence in males than females. Furthermore, general occurrence revealed the preponderance of Nematodes followed by Trematodes while Cestodes recorded the least value.

Table 4. Relative abundance of Helminths in the location of recovery

Organ	Nematoda	Trematoda	Cestoda
Skin	10	4	-
Intestine	48	8	3
Gill	3	13	-
Liver	4	-	-
Fin	3	2	-
Tissue	15	29	6
Total	**83**	**56**	**9**

Bacteria and helminthes of public health concern have been isolated. Past studies revealed that locally processed fish feed is one of the major sources of this contamination [16]. Some other additives like poultry waste and dropping have not only increased the microbial and helminth population, but have incriminated incidence of fish diseases [16]. Rural populace, with lack of knowledge on the public health concern of these organisms and whose resort to obtain protein is by consumption of fish from locally made ponds and nearby rivers and streams, is however at risk. They are, according to DFID [7], FIVIMS [10] and Cunningham [6], referred to as food insecure people whose food intake falls below their minimum energetic requirements as a result of inadequate or unbalanced diet or from the body's inability to use food effectively due to infection, disease or poor sanitation.

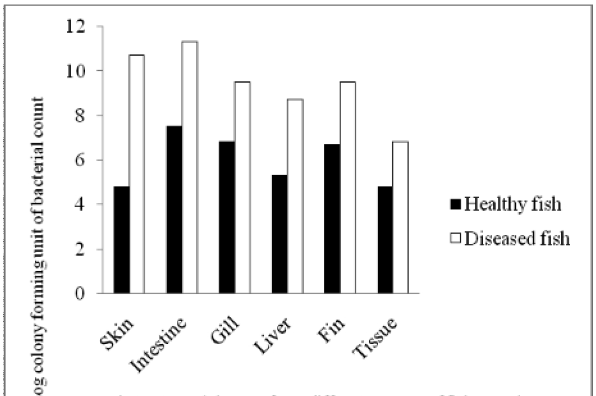

Fig 1: Bacterial count from different organs of fish samples

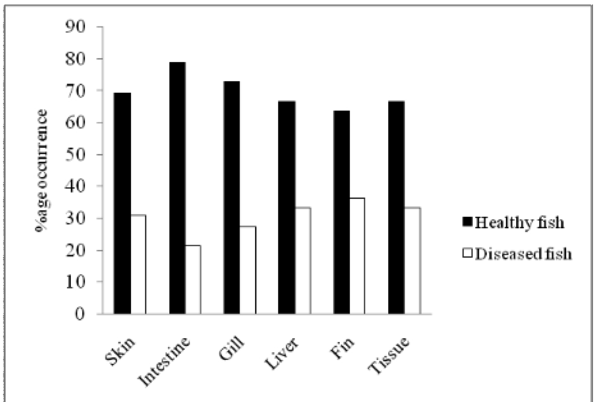

Fig2: Percentage occurrence of isolates per organs of diseased and healthy fish samples

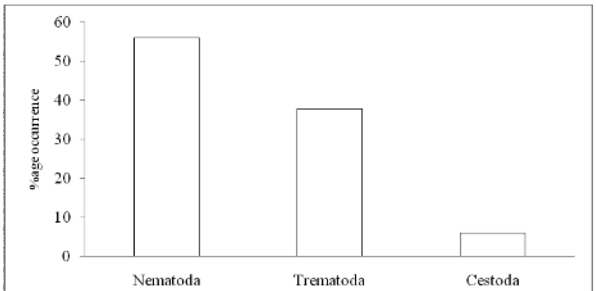

Fig 3: Percentage occurrence of different class of helminth isolated from fish samples

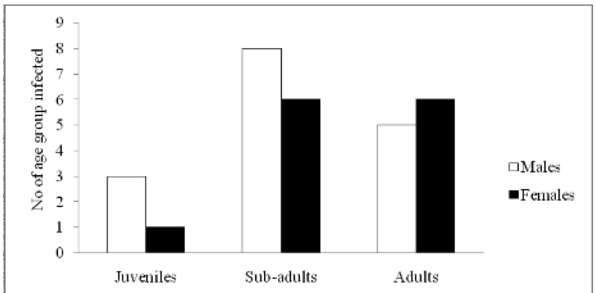

Fig 4: Age/sex related prevalence of Oreochromis niloticus infected with helminths

Adequate publicity and rural education have to be employed to educate rural consumers on the ideal of routine hygienic method of aqua cultural practices to avoid potential risks associated with these organisms. This will however enhance biological safety of aquacultural products and in this way food security is achieved.

REFERENCES

[1] Anosike, J.C., Omeriegie, E., Ofojekwu, P.C., Nweze, I.E., (1992): A survey of helminth Parasite of *Clarias geriepinus* in Plateau State. Nigeria Journal of Aquatice Science, 7: 39-43.

[2] Austin, B., Austin, D.A., (1987) ed. Disease in farmed and wild fish. Ellis Harwood Publishers, Chichester., 364 p.

[3] Bruinsma, J., (2003): World Agriculture: Towards 2015/2030. Earthscan Publications Ltd, Lodon. 432 p.

[4] Cheesbrough, M., (2004): District laboratory practice in Tropical countries. Part 2 Cambridge University press. 479 p.

[5] Clover, J., (2003): Food Security in sub-Sahara Africa. African Security Review, 12: 5-15.

[6] Cunningham, L., (2005): Assessing the Contribution of Aquaculture to Food Security: A Survey of Methodologies. FAO Fisheries Circular No 1010 FIPP/C1010(En), pp. 1-25.

[7] DFID, (2003): Policy Planning and Implementation: food security. Department for International Development, Overseas Development Institute, UK. Key sheet for sustainable livelihood. www.odi.org.uk>ODI>Resources 2003, 36 p.

[8] FAO, (2002): Food Insecurity: when people must live with hunger and fear starvation. The State of Food Insecurity in the World. Food and Agriculture Organization of the United Nations, Rome. www.fao.org

[9] FAO, (2003): The Role of Aquaculture in Improving Food Security and Nutrition. Committee on World Food Security, Food and Agriculture Organization of the United Nations, Rome. www.fao.org

[10] FIVIMS, (2003): Food Insecurity and Vulnerability Information and Mapping Systems. FAO, Rome. www.fao.org

[11] Holt, J.G., (1984): The Shorter Berger's Manual of Determinative Bacteriology. Williams Wilkins, Baltimore. 369 p.

[12] Little, D., (1998): Options in the Development of the "Aquatic Chicken". Fish farmers International file, England, 12(4): 35-37.

[13] Ogbulie, J.N., Okpokwasili, G.C., (1998): Haematological and histological response of *Clarias qariepious* and *Hetrobranchus bidorsalis* to tropical bacterial diseases. Journal of Natural Science Council of Sri Lanka, 26(3): 131-153.

[14] Ogbulie, J.N., Obiajuru, I.O.C., Ogbulie, T.E., (2003): Bacterial and heminth Bioload of cultural *Channa obscura* fish. Journal of Aquatic Science, 18(2): 93-99.

[15] Okpokwasili, G.C., Alapiki, A.M., (1990): Bacterial flora associated with a Nigerian Fresh water fish culture. Journal of Aquaculture in the Tropics 5: 87-90.
[16] Okpokwasili, G.C., Ogbulie, J.N., (1993): Bacterial and Metal quality of Tilapia (*Oreochromis niloticus*) aquaculture systems. International journal Environmental Health Research, 13: 190-202.
[17] Ozor, N, Igbokwe, E.M., (2007): Roles of agricultural Biotechnology in ensuring adequate food Security in developing societies. African Journal of Biotechnology, 6(14): 1597-1602.
[18] Pinstrup-Anderson, P., Pandya-Lorch, R., (1999): Achieving food security for all: Key policy issues for developing contries. pp. 13-19. In Ahmed, M., Delgado, C., Sverdrup-Jensen, S., Santos, R.A.V. (eds.): Fisheries Policy Research in Developing Countries: Issues, Priorities and Needs, ICLARM, Manila Philippines.
[19] Pinstrup-Anderson, P., Pandy-Lorch, R., Rosegrant, N., (1999): World Food Prospects: Critical Issues for the Early 21st Century. IFPRI, Washington DC., pp. 13-19
[20] Pretty, J.N., (1999): Can Sustainable agriculture feed Africa? New evidence on progress, process and Impacts. Environment, Development and Sustainability, 1: 253-274.
[21] Roderick, E., (1998): Latest research benefits versatile Tilapia. Fish Farmers International File, 12(4): 13-14.
[22] Sakata, T., Okabayash, J., Kakimoto, D., (1980): Variations in the intestinal microflora of Tilapia in fresh and sea water. Bulletin of the Japanese Society of Scientific Fisheries, 46: 313-317.
[23] Welch, R.M., Graham, R.D., (2000): A new paradigm for world agriculture: productive, sustainable, nutrition, healthful food systems. Food and Nutrition Bulletin, 21: 361-366.
[24] World Bank (1986): Poverty and Hunger: issues and options for food security in developing countries. International Bank for Reconstruction and Development, Washington DC. 379 p.
[25] Wu, C.H., Wood, T.K., Mulchandani, A., Chen, W., (2006): Engineering Plant microbe symbiosis for rhizoremediation of heavy metals. Applied and Environmental Microbiology, 72(2): 1129-1134.
[26] Yoshimizu, M., Kamiyama, K., Kimura, T., Sakar, M., (1976): Studies on the intestinal microflora of fresh water Salmon. Bulletin of the Japanese Society of Scienctific Fisheries, 42: 1281-1290.

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