Bacterial flora of Cultured Catfish Fed with Poultry Hatchery Waste from selected Farms in Ibadan Southwestern Nigeria

Monday Onwenefah and Olufemi Bolarinwa Adedeji

Faculty of Veterinary Medicine, University of Ibadan, Nigeria.
Oluadedeji2001@yahoo.com

Abstract: Hatchery waste comprises empty shells, infertile eggs, dead embryos, late hatchings, dead chickens, a viscows liquid from eggs and decaying tissue which is used as a cheap fish feed by some farmers. From these animal based feedstuffs, there is a possibility of hazard arising from the presence of viable and contaminating microorganisms. Microbial quality of poultry hatchery wastes from three selected commercial poultry hatchery units and catfish (*Clarias gariepinus*) fed hatchery waste obtained from five purposively selected aquaculture farms in three local government areas in Ibadan Southwest Nigeria were studied using standard microbiological methods. The result obtained were subjected to statistical analysis. The total bacterial count obtained ranged from 1.2 x 10^5 to 4.6 x 10^6 cfu/g and 1.2 x 10^5 to 5.6 x 10^5 cfu/g for the hatchery waste and catfish respectively. The total enterobacterial count ranged from 6.0 x 1.4 to 3.0 x 10^5 cfu/g and 4.0 x 10^4 to 3.0 x 10^5 cfu/g for hatchery waste and catfish respectively. The Bacteria isolated from hatchery waste were *Staplylococcus epidermidis, Esherichia coli, Bacillos spp, Klebsiella pneumonia and Pseudomonas aeruginosa*, while those isolated from catfish organs (skin, stomach and intestines) are *salmonella sub sppl, Leclercia adecarboxylata, Bacillus spp, klebsiella pneumonia, Eschericia coli* and *Staphylococcus aureus, Citrobacter spp, Pseudomonas aeruginosa. Salmonella arizonal sub spp3A*. The types of bacterial organisms that are associated with the hatchery waste and catfish fed hatchery waste found in this study call for concern.
[Onwenefah M. and Adedeji O.B. **Bacterial flora of Cultured Catfish Fed with Poultry Hatchery Waste from selected Farms in Ibadan Southwestern Nigeria.** *N Y Sci J* 2013;6(7):106-110]. (ISSN: 1554-0200).
http://www.sciencepub.net/newyork. 16

Key Words: Bacterial Floral, Hatchery waste, Cultured Catfish

Introduction

Aquaculture shares the same challenges with agriculture in increasing food supply and brings about competition in the use of feeds for livestock and fish. Shortages of major feedstuff have been on the increase in recent times in Nigeria with the poultry and livestock industry expanding. Sourcing for critical feed ingredients is increasingly becoming difficult in aquaculture (Adejinmi, 2000; Adikwu,2003; Fagbenro et al.,2003**).** Poultry litter has been considered to have some nutritional values containing about 25.75% crude protein (Ndifon, 1987). Concept of utilizing poultry litter is highly desirable since it will not only eliminate problem of waste disposal but also provide cheap fish feed (Ayoola, 2011).

Poultry hatchery waste is an unconventional feed now increasingly used in freshwater aquaculture for economic reasons (Ayoola, 2010). Poultry industry produces large amounts of hatchery waste which includes solid waste and wastewater. Solid hatchery waste comprises empty shells, infertile eggs, dead embryos, late hatchings and dead chickens and a viscous liquid from eggs and decaying tissue. Wastewater comes from water used to wash down incubators, hatchers and chick handling areas (Phil et al., 2011).

Use of livestock wastes in fish production may however, pose health risks to humans and aquatic organisms through possible introduction of pathogens (FAO, 2003; FAO/OIE/WHO, 2005). Edwards (2008) stated that fish and plants passively accumulate microbial contaminants on their surfaces, but these rarely penetrate into edible fish flesh or muscle except for trematodes (parasitic tissue flukes). Aquaculture products like other foods have hazards that may adversely affect the consumers' health. The production system also presents risks to public health. Major health risks of aquaculture products are biological especially for the organisms produced in waste water or water receiving animal and human wastes. Safety of consuming fish products from such environments becomes questionable (Erondu and Anyanwu, 2005).

The use of poultry hatchery waste as fish feeds poses serious biological hazards to growing aquaculture industry due to poor information on the bacterial organisms of the hatchery waste fed to cultured fish by the fish farmers. Production system also presents risks to public health and again, consumers' health may be adversely affected. There is need to ensure that these catfish are safe for human consumption. Hence, the need to carry out a study to investigate the bacterial flora of cultured catfish

(*Clarias gariepinus*) that are fed with hatchery waste in Ibadan south western Nigeria.

Materials and Methods

Study Location

This study was conducted in four target fish farms and selected poultry hatcheries in Ibadan. The choice of the study sites was based on their accessibility. Ibadan is the capital city of Oyo state, Southwest Nigeria. The city is located in the geographical grid of reference longitude $3^0$5E, latitude $7^0$2N (Filani, 1994).

Collection and Processing of Samples

Hatchery wastes samples (15) were collected in a sterile polythene bags immediately after hatching the chicken eggs. The samples were collected for a period of two weeks. Live catfish (20) were randomly selected from the 5 selected farms that fed poultry hatchery waste to fish on their farms. The fish were put in a sterile plastic container containing the culture pond water. All the samples were transported in a cooler box containing ice packs to the Food and Meat Hygiene Laboratory of the Department of Veterinary Pubic Health and Preventive Medicine, University of Ibadan

Tissues samples (skin, stomach and intestine) of the fish (1 cm^3) were harvested aseptically and weighed from each of the 20 catfish, and 1g from each of the hatchery waste samples was aseptically weighed and taken for bacteriological examinations. Each of the samples was homogenized in 9ml peptone water that had been prepared aseptically according to manufacturer's instruction. Two folds serial dilution of each homogenized sample of the fish tissue and three folds serial dilution of the hatchery waste samples was further carried out.

Bacteriological Examination of the Samples

Using surface spread technique, 0.1ml of the each sample was taken and inoculated aseptically unto already prepared cultured media namely; Nutrient agar, Eosin-methylene blue (EMB) agar, MacConkey agar and Salmonella-shigellar agar (SSA). The inoculated media plates were then incubated at 37^0C for 24 hours. After incubation, the distinct bacterial colonies were counted to determine the colony forming unit (CFU) per gram of the sample (Horsely, 1977, APHA, 1995). Distinct colonies were further sub cultured on freshly prepared culture media; Nutrient agar, MacConkey, EMB agar and SS agar to obtain pure isolates of the organism.

Identification of Pure Isolates

Following the isolation of the pure colonies, the isolates were further identified morphologically and biochemically (Baron and Murray, 1999), using gram staining technique and Microbact Identification Kits (Microbact ^{TM} GNB 12A/B/E,24E, Oxoid). Organisms identification was based on pH change and substrate utilization (Farmer, et al, 1985). Octal coding system; an octal coding system was adopted for Microbact ^{TM}

Each of three reactions produced a single digit of the code. Using the results obtained, the indices of the positive reactions were noted and the sum of these indices in each group of the three reactions formed the code number. Computer aided identification package; Microbact ^{TM} aided computer package was then used for final identification of the organisms.

Statistical Analysis

Data entry and analysis was done using SPSS version 15. Both descriptive and inferential statistics were used. The mean total bacterial and enterobacterial counts were calculated. One-way analysis of variance was used to compare means among the tissues. The level of significance was set as $p \leq 0.05$.

Results.

Table 1.0 shows the bacterial isolates obtained from the different catfish organs and the hatchery waste. The species of the bacterial isolated are those in the genera *Citrobacter, Esherichia, Staphylococcus, Pseudomonas, Salmonella, Bacillus, Leclercia* and *Klebsiella*. The mean total bacterial load count and the mean enterobacterial load count in poultry waste is as shown in tables 2 and 3 respectively. All the samples of the hatchery waste were contaminated with the microbial load ($\log_{10}$ CFU/g) in the range of 5.28-5.66. The enterobacterial load ($\log_{10}$ CFU/g) of the hatchery waste samples was in the range of 4.30-5.48. Tables 4 and 5 show the respective mean total bacterial count and the mean enterobacterial count of the selected organs of the catfish fed hatchery waste. The organs were contaminated with bacteria load ($\log_{10}$ CFU/g or cm^2) in the range of 5.08-5.75. The enterobacterial load ($\log_{10}$ CFU/g or cm^2) was in the range of4.06-5.66 with skin having the lowest. The result of total bacterial count of the fish are presented in table 4 while that of enterobateriaceae count in the skin, stomach and small intestine are presented in table 5.

Table 1. Bacterial isolates obtained from different catfish organs and hatchery waste

SAMPLES	BACTERIA ISOLATES
Skin	*Citrobacter spp, Eschericia coli, Staphylococcus aureus, Pseudomonas aeruginosa*
Stomach	*Salmonella subsppI, Leclercia adecarboxylata, Bacillus spp, Klebsiella pneumonia, Eschericia coli, Staphylococcus aureus*
Intestine	*Eschericia coli, Salmonella arizonae subspp3A*

Hatchery waste	*Pseudomonas aeruginosa, Eschericia coli, Staphylococcus epidermidis, Bacillus spp, Klebsiella pneumonia,*

Table 2. Meantotal bacteria load count in poultry hatchery waste

	DESCRIPTIVE	ANOVA		
Sample	Mean± SD(n=5)	F-value	P-value	Remarks
Hatchery A	31.4±8.2	2.07	0.17	Not Significant
Hatchery B	37.6±7,9			
Hatchery C	40.2±4.3			

Table 3. Mean Enterobacterial load count in poultry hatchery waste

	DESCRIPTIVE	ANOVA		
Sample	Mean± SD(n=5)	F-value	P-value	Remarks
Hatchery A	19.6±8.6	0.48	0.63	Not Significant
Hatchery B	17.4±6.3			
Hatchery C	14.0±11.7			

Table 4. Mean total bacterial load count in selected organs of cat fish fed hatchery waste

	DESCRIPTIVE	ANOVA		
Organs	Mean± SD(n=20)	F-value	P-value	Remarks
Skin	33.1±9.0	2.02	0.14	Not Significant
Stomach	37.1±7.3			
Intestine	32.7±6.3			

Table 5. Mean enterobacteria load count in selected organs of cat fish fed hatchery waste

	DESCRIPTIVE	ANOVA		
Organs	Mean± SD(n=20)	F-value	P-value	Remarks
Skin	17.5±8.6	1.38	0.26	Not Significant
Stomach	14.1±9.0			
Intestine	13.3±8.2			

Discussion and Conclusion

The species of bacteria isolated are those in the genera *Citrobacter, Escherichia, Staphylococcus, Pseudomonas, Salmonella, Bacillus, Leclercia, Klebsiella. There was frequent occurrence of Escherichia, Staphylococcus, Pseudomonas, Salmonella, and Klebsiella*.in the sample analysed. This shows that these organisms are likely to have been transferred to the catfish from the hatchery waste through feeding or through the contaminated aquatic environment where these catfish were cultured as well as through unhygienic processes being employed in the food chain. There is also the chance that the organisms could have been passed to the catfish and hatchery waste by fish handlers and workers working in the hatchery through routine operations in the hatchery. The bacterial organisms isolated from the hatchery waste agreed with some of that isolated by other workers who determine the bacterial flora within unhatched eggs (Seviour et a., 1972; Bruce and Johnson 1978; Rosario et al., 2004). From their studies, the most recovered bacterial flora are: *Enterobacteriaceae* (*E. Coli, Salmonella, Klebsiella and Proteus*) *Staphylococcus spp, Streptococcus enterococcus spp, Micrococcus spp Pseudomonas spp* and *Bacillus spp.* The bacterial load observed in the hatchery waste was in the range of 1.9 x 10^5 to 4.6 x 10^6, this was higher than what Tadtiyanant et al., (1993) found in their studies. The higher range found could be associated with the level of compliance with biosecurity measures in the hatchery unit.

Bacterial growth is the main cause of fish spoilage therefore it is logical to use bacterial number as an index of fish quality. In this study, the total number of bacterial count for fresh catfish (*Clarias gariepinus*) was in the range of 1.2 x 10^5 cfu/g and 5.6 x10^5 for fish organs, and this number fell within acceptable limit according to Anon (1991) who gave a limit of 10^6 cfu/g for mesophilic aerobic bacteria. These results agree with finding of Chou (1993) who reported that the total aerobic plate count of unwashed and washed catfish frame mince without cryoprotectants were 5x10^6 cfu/g and 10^6 cfu/g, respectively. The total viable count of bacteria of refrigerated catfish *(C.lazera)* for 4 days was 8.8x106 cfu/g, is regarded in normal of freshness as stated by

Liston (1980) which was 102-107 cfu/g of fish meat, and the total viable count of refrigerated catfish for 7 days was $1.6x10^7$ cfu/g of fish meat. This is considered in critical point compared to (Olafdottir 1997) who reported that the total viable count of fish products is 10^7-10^8 cfu/g at the point of sensory rejection. However, in spite of the normal bacterial load seen in this study, bacterial organisms isolated are pathogenic and capable of causing diseases in both fishes and humans under poor hygiene practice and in immune-compromised individual and animals. These bacteria isolated recovered in the fish samples calls for concern and provides an early warning since the catfish industry stands the potential risk of being devastated by disease outbreak with time if the feed materials being fed to the catfish are not properly screened for pathogenic organisms. Fishes could be contaminated by the water in which they are grown (Alcaide det al., 2005). Although the bacterial species found in the present study did not cause mortality to the fishes in the studied farms probably because the fishes have strong host defense response yet the species are both opportunistic and pathogenic species which could be involved in causing fish diseases to human beings. Fish and their products have been reported as vehicles of foodborne bacterial infections in humans (Novotyn et al., 2004; Hastein et al., 2006). This constitutes a food safety problem because catfish could be a potential agent of transfer of these species to unsuspecting consumers.

It is suspected that the hatchery wastes improve considerably the nutrients levels in the ponds, a situation that will increase the population of bacteria present in the ponds (Olayemi et al., 1991; Ogbondeminu, 1993). This is ideal for the growth of the fish, as food will be in abundance. However, with conditions where pathogenic bacteria are introduced into the ponds with the wastes, the risk of infection in fish and humans is high (Herbs et al., 2008). The safety of products for consumption is prime concern from the point of view of managing of the fish culture systems, as well as ensuring public health (Schotissek and Naylor, 1988). Official regulatory bodies in many countries specify maximum permissible concentrations of toxic substances or the number of harmful bacteria that a product may contain, in order to ensure that unfit or unwholesome food does not reach the consumer.

Corresponding Author:
Olufemi Adedeji,
Faculty of Veterinary Medicine,
University of Ibadan, Nigeria.
Oluadedeji2001@yahoo.com

References

1. Adedeji, O.B. Tiamiyu A.M. and Emikpe B.O.: The Antibiotic Resistance Patterns of Bacterial Flora of Fish from Different Aquatic Environment from Ibadan, Southwest Nigeria. *Advances in Environmental Biology,* 5(8) 2039-2047 2011.
2. Adejimi, O.O. 2000. The chemical composition and nutritional potential of soldier flylarvae *(Hermetia eluucens)* in poultry rations. University of Ibadan, Ph.D Thesis. Pp292.
3. Adikwu, I. A. 2002. A review of aquaculture: Nutrition in aquaculture development in Nigeria. In-Proceeding of the Joint Fisheries Society of Nigeria/NIFFR/FAO-National Special Programmme for food security. National Workshop on Fish Development and Feeding Practices in Aquaculture (Eyo, A.A. Ed.) held at National Institute for Freshwater Fisheries Research, New Bussa. 15th -19 September 2003. Pp 33-42.
4. Alcaide,E.., M.D. Blasco and C. Esteve, (2005). Occurrence of drug resistant bacteria in two European ell farms. *Appl. Environ. Microbiol.,* 71: 3348-3350.
5. ANONYMOUS. 1991. Su Ürünlerinde Mikrobiyolojik ve Agˇir Metal Kabul Edilebilir Degˇerler. Resmi Gazete, 28 Mayis 20884, p. 5
6. APHA (1995). Standard Methods for the examination of Water and Wastewater. 19th ed. American Public Health Association.
7. Ayoola, S.O. 2010. Modern fish farming techniques (Aquaculture) (2nd edition). Glamour books Publishing, Ibadan; pp 180.
8. Ayoola, S.O. 2011. Haematological characteristics of Clarias gariepinus (Buchell, 1822) Juvellines fed with Poultry hatchery waste. *Iranical Journal of Energy and Environmental* 2(1); 18-23.
9. Baron, E.J. and P.R. Murray 1999. Bacteriology. In Manual of clinical microbiology, 7th Edition P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (eds). ASM Press, Washington, D.C., pp 246-831.
10. Bruce, J., Johnson, A.L. (1978): The bacterial flora of unhatched eggs. *Br Poult. Sci.* 19, 681-689.
11. Chelossi, E., Vezzulli, L., Milano, A. Branzoni, M. Fabiano, M., Ricarrdi, G. And Banat, I.M. (2003) Antibiotic O.L. Akinbowale et al. Antimicrobial resistance aquaculture resistance of benthic bacteria in fish farm and control sediments of Western Mediterranean. Aquaculture 219, 83-97.
12. Chou C. 1993. Evaluation of Quality Changes in Frozen Catfish (Ictalurus punctatus) Mince[M.S. thesis]. Baton Rouge, La.: Louisiana State University.
13. Edwards P (2008). Key issues in the safe use of wastewater and excreta in aquaculture. Guidelines for the Safe Use of Wastewater, Excreta and Greywater in Agriculture and Aqualcuture, 3. WHO/FAO/IDRC/IWMI document 3.

14. Erondu, E.S. and Anwanwu, P.E. 2005. Potential hazards and risks associated with the aquaculture industry. *African Journal of Biotechnology* 4 (1)' 1622-1627.
15. Fagbenron A.O. and E.Adeparusi (2003): Feedstuff and dietary sudstitution for farmed fish in Nigeria. Paper presented at Pan African Fish and Fisheries Conference Cotonou, Benin Rebpublic. Book of Abstracts pp. 276.
16. Filani, M.O. 1994. Ibadan Region R-Charles Publications in Conjunction with Connell Publications, Ibadan, Nigerian Health Protection Agency. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods. London: Health Protection Agency, November 2009.
17. Guardabassi, L., Petersen, A., Olsen, J.E. and Dalsgaard, A. (1998): Antibiotic resistance in Acinetobacter spp. Isolated from sewer receiving waste effluent from hospital and a pharmaceutical plant. *Appl Environ Microbiol* 64, 3499-3502.
18. Hatha, M. Vivekanandam, A.A., Joice, G.J. and Christol, G.J. (2005) Antibiotic resistance pattern of motile aeromonads from farm raised freshwater fish. *Int. J. Food Microbiol* 98, 131-134.
19. Horsely, R.W. (1977). A review of bacterial flora of teleosts and elasmobranches, including methods for its analysis. *Journal of Fish Biology* 10, 529-533.
20. McKoen, D.M., Calabrese, J.P. and Bissonnette, G.K. (1995) Antibiotic resistant Gram-negative bacteria in rural ground water supplies. Water Res 29, 1902-1908.
21. McPhearson, R.M., DePaopla, A., Zywno, S.R., Motes, M.. Jr and Guarino, A.M. (1991) Antibiotic resistance in Gramanegative bacteria from cultured catfish and aquaculture.
22. Michel, C., Kerouault, B. And Martin, C. (2003) Chloramphenicol and florfenicol susceptibility of fish-pathogenic bacteria isolated in France: comparison of minimum inhibitory concentration, using recommended provisory standards for fish bacteria. *J. Appl Microbiol* 95, 1008-1015.
23. Mirand, C.D. and Zemelman, R. (2002): Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms. *Sci Total Environi,* 293, 207-218.
24. Ndifon, P.M. 1987. Studies on the nutritive value of chicken offal meal with emphasis on its production shelf life stability and its biological evaluation. Ph.D Thesis, University of Ibadan, pp 215.
25. Novotyn, L., L. Dvorska, A. Lorencova, B. Beran and I. Pavlik (2004): Fish: A potential source of bacterial pathogens for human beings. Vet. Med. Czech., 49: 343-358.
26. Ogbodeminu FS (1993): The occurrence and distribution of enteric bacteria in fish and water of tropical aquaculture ponds in Nigeria. *J. Aquac Trop ;* 8: 61-6.
27. Olafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Underland, I., Mackie, I.M., Henehan, G., Nielsen, J., Nilsen, H. (1997) Methods to evaluate fish freshness in research and industry. Trends in Food Science and Technology. 8: 258–26
28. Olayemi AB, Adebayo P, Ojo A.O. (1991): Microbial flora of six freshwater fish species from Asa River, Ilorin, Nigeria. *Rev Biol Trop* 1991; 39: 165-7.
29. Phil Glatz, Zhihong Miao and Belinda Rodda 2011. *Sustainability*2011 3 (1); 216-237.
30. Rosaria Cortes, C., Tellez Isaias, G., Lopez Cuello, C., Villaseca-Flores, J.M., Anderson, R.C., 2004. Bacterial isolation rate from fertile eggs, hatching eggs, and neonatal broilers with yolk sac infection. *Rev. Lat. Microbiol.* 46. 12-16.
31. Saviou4, E.M. Sykes F.R., Board, R.G. (1972): A microbiological survey of the incubated eggs of chickens and waterfowl. Brit. Pult. Sci. 13, 549-556.
32. Schotissek C, Naylor E.F. (188): Fish farming and influenza pandemic. Nature 1988; 33:215. Senanayake S.N. Ferson M.J., Botham S.J. Belinfante R.T. (2004): A child with Salmonella enteric serotype Para-typhi B infection acquired from a fish tank. *Med. J. Australlia,* 180. 250.
33. Tadtiyanant, C., Lyond, J.J. Van De populiere, J.M. (1993): Extrusion processing used to convert dead poultry, feathers, eggshells, hatchery waste, and mechanically deboned residue into feedstuff for poultry. *Poult. Sci.* 72, 1515-1527.
34. Tendencia, E.A. and de la Pena, L.D. (2001) Antibiotic resistant bacteria from shrimp ponds. *Aquaculture* 195, 193-204.

6/7/2013