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Effect of Artificial Environment on the Microbial Quality and Haematology of *Clarias gariepinus* (Catfish)

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A B S T R A C T

Introduction/Aim: Microbiology and heamatology of natural population of Clarias gariepinus from three ponds (earthen, concrete and plastic) were investigated with a view of establishing species specific baseline values for these parameters

Method: Total Coliform Count (TCC), Salmonella-Shigella Count (SSC), Vibrio Count (VC), Total Bacterial Count (TBC) and Total Enterobacteriaceae Count (TEC) were done with selective agars using pour plate techniques. Also, blood was collected from caudal vein and haematologic parameters such as Red Blood Cell (RBC), White Blood Cells (WBC), Haemoglobin (Hb) and Packed Cell Volume (PCV) were determined using autoanalyser.

Result: The microbial count did not vary significantly (P<0.05) between the ponds except in Salmonella-Shigella Count (SSC) where plastic pond recorded the least count. Nine different genera of bacteria including Escherichia coli, Staphylococcus sp., Salmonella sp., Micrococcus sp., Bacillus sp., Pseudomonas sp., Enterobacter sp., Klebsiella sp., and Streptococcus sp. However, Escherichia coli, Salmonella sp., Enterobactersp., Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumonia were present in the three ponds. In the haematological assay, the haemoglobin concentration, PCV and RBC were significantly (P<0.05) higher in fish grown in earthen pond than others while the WBC were lower in earthen pond sample compared to the other ponds.

Conclusion: In conclusion, the results of the present study indicated that the type of pond used to rear fish can alter microbiological quality of pond water and hematological parameters of fish culture.

Keywords: Catfish, Ponds, Haematology, water quality, microorganisms

1. INTRODUCTION

Fish farming (aquaculture), involves the growth and cultivation of fish to be harvested as food, as opposed to catching fish in the wild. It provides profitable returns to the farmers, employment to the unemployed in rural areas and supplying good quality protein diet for people (Onoja, 2005). In addition to fish protein which is ranked the cheapest among animal protein sources, fish provides high-quality calories, fats and vitamins. More so, fish culture generates income for all categories of people involved in it as well as providing foreign exchange for the nation (Samson, 2007).

Among the culturable species of food fish in Nigeria are carp, tilapia, catfish, etc. Meanwhile, catfish (*Clarias gariepinus*) is the most sought after. It is very popular among fish farmers and commands very good commercial value in the markets. Consequently, the *Clarias gariepinus* is vital to the sustainability of the aquaculture industry in the country having in possession the following good qualities identified by Osawe (2007) as they survive in different culture systems and diverse environments, grow very fast and have high fecundity, improved survival of the fry and adaptation to supplemental feed.

As observed by Ajao (2012), many African farmers are still using low-yielding agricultural technologies, which lead to low productivity. Also, it is always argued that the relevant question for agricultural policy makers is whether the agricultural sector can be made more efficient, by achieving more output with the current input level, or achieving the current output with less input use than is currently observed. An important step in answering this question is to identify the behaviour of productivity and its components. The concept of efficiency is at the core of economic theory. The theory of production economics is concerned with optimization and this implies efficiency. The crucial role of efficiency in increasing agricultural output has been widely recognized by researchers and policy makers alike (Battese, 2002; Bravo-ureta and Pinheiro, 2007).

Generally, fish farming as an industry is faced with some problems which include inadequate supply of fishing inputs such as fingerlings and feed, which causes rising cost of trawling operation, lack of sufficient least cost-effective feed for fish culture, insufficient production of fingerlings of cultivable fish species, etc. (Adeniyi, 2002).

The better performance in quality and quantity of fish nutrient is responsible for its increase in demand and investment in Nigeria. However, the higher demand has not been met by the supply of the product because most ground fish pond could not operate due to some technical inefficiency that exists among the fish farmers in Nigeria. There have been wide differences of production systems being exploited for culturing of fish. These production systems include: ponds, cages, tanks and raceways. Fish farming in earthen ponds remains the most used method in Nigeria. With increased urbanization and the attendant increase in fish demand, large expanse of land required for intensive aquaculture in earthen pond is becoming seemingly unavailable in many areas. Similarly, earthen pond system of production is characterized with low productivity (Wang *et al.*, 2016). Therefore, for Nigeria to make significant contribution in fish farming at global level and meet her Millennium Development Goals (MDGs) of increasing fish production by over 250 % by 2025, efforts need to be geared towards achieving higher production intensities. One way of achieving this is through the encouraging of urban aquaculture system. This system of production makes use of varieties of water and culture facilities that provide needed environment for the growth of the fish. Many researchers have studied the effects of artificial ponds in relation to growth performance of fish (Kolkovski *et al.*, 2005; Gonzales *et al.*, 2005). However, effects of these artificial ponds on the quality of the fish have not been adequately investigated. The fact that a vessel holds water does not necessarily make it a good fish-rearing habitat. Therefore, this study was designed to assess the effect of artificial environment on the pond water quality and haematology of catfish..

2.0 MATERIALS AND METHODS

2.1 Collection of Sample:

Pond water samples were collected from Opomulero and Ogbomo areas of Owo township, a pond each for Earthen, Concrete and Plastic type were sampled into different previously labeled 1L sample bottles. The bottles were rinsed thoroughly with the water to be sampled before sampling. Moreover, five fish were collected from each pond into appropriate containers. They were transported to the laboratory immediately for analysis.

2.2 Media Preparation:

The media that were used for the work include Nutrient agar for isolation of total bacteria, Salmonella-Shigella agar for isolation of Salmonella and Shigella species, Violet Green Lactose Bile agar for the isolation of Enterobacteriaceae and Eosin Methylene Blue for the isolation of coliforms. They were prepared strictly according to the manufacture's specifications and then autoclaved at 121 °C for 15minutes.

2.3 Serial Dilution:

The sample (1 ml) was measured into a sterile test tube containing 9 ml of sterile distilled water. Other test tubes were arranged each with 9 ml of sterile distilled water. A sterile syringe was used to measure 1 ml of the mixture into the second test tube in the series. The second tube now has a total dilution factor of 10^{-2}. The same process was then repeated for the remaining test tubes upto the fifth test tube to make 10^{5} dilution (Cullen and Maelntyre, 2016).

2.4 Isolation of Microorganisms and Maintenance of Pure Culture:

The pour plate method was used in isolating bacteria in the samples and pure culture was obtained by repeated streaking. Colonies that appear on agar plate were sub-cultured by streaking into newly prepared agar plates using sterile wire loop. The streaked plates were incubated for 24 hrs in 37 °C. Pure colonies of bacteria were gotten by sub-culturing the bacteria on a Nutrient Agar plate after which it was streaked onto slants and incubated for 24 hrs at 37 °C and were kept in a refrigerator to keep the culture viable for further use (APHA, 2005).

2.5 Haematological Analysis:

Blood samples of about 2.0 ml were collected from the ventral region near the anal opening using a 2.0 ml syringe and hypodermic needles. The blood samples were introduced into heparinized Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant tubes and capped sealed effectively to avoid escape for haematological analysis. The packed cell volume (PCV), haemoglobin concentration (Hb), Red Blood Cell (RBC), White Blood Cell (WBC) count and other blood parameters of each of the blood sample, were determined in Haematology Laboratory of the Federal Medical Center (FMC), Owo, using 5-part differential Haematology Auto-analyzer (Mindray BC 5300 model).

2.6 Statistical Analyses:

Data from blood and growth assays were subjected to one-way analysis of variance (ANOVA) to evaluate mean differences at 0.05 significant levels. Results with $P \leq 0.05$ were considered significantly different. The statistical analyses were done using IBM SPSS Inc. (Windows version 22).

3.0 RESULTS

The result of the microbiological evaluation of water sampled from Earthen, Concrete and Plastic fish ponds are shown in Table 1 and 2.

Table 1: Bacterial count in the fish pond water in Owo (x 10^5cfu/ml)

Organism	Earthen	Concrete	Plastic
TBC	13.2±0.20^b	9.40±0.58^a	8.7±0.52^b
TCC	25.2±0.07^d	29.2±1.07^c	26.1±1.50^c
TEC	17.7±0.10^c	32.5±2.50^d	23.7±0.37^c
SSC	8.2±0.02^a	12.1±0.30^b	2.4±0.01^a
VC	Nil	Nil	Nil

Key: TCC - Total ColiformCount; SSC -*Salmonella-Shigella* Count; VC - *Vibrio* Count; TBC – Total Bacterial Count and TEC – Total Enterobacteriaceae Count.

Table 1 shows microbial count of the three fish ponds in Owo. The microbial count did not very significantly between the ponds except in *Salmonella-Shigella* Count (SSC) where plastic pond recorded the least count (2.4x 10^5 cfu/ml) against 8.2x 10^5 cfu/ml and 12.1x 10^5cfu/ml for Earthen and Concrete respectively. Total bacteria count (TBC) was highest inearthen pond (13.2x10^5 cfu/ml) and lowest in plastic pond (8.7x 10^5 cfu/ml).TCC and TEC were ranged (25.2 - 29.2 x 10^5 cfu/ml) and (17.7 - 32.5 x 10^5 cfu/ml) respectively with concrete pond recording the highest in both while earthen pond recorded the least. Vibrio count was empty; no growth (nil)

Bacteriological analysis of the watersamples showed nine different genera (Table 2) which are *Escherichia coli, Staphylococcus* sp.*, Salmonella* sp.*, Micrococcus* sp.*, Bacillus* sp.*, Pseudomonas* sp.*, Enterobacter* sp.*, Klebsiella* sp.*, and Streptococcus* sp. However, *Escherichia coli, Salmonella* sp., *Enterobacter* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were present in the three ponds. Furthermore, *Streptococcus faecalis and Bacillus subtilis*were only present in earthen pond but absent in the other two ponds (concrete and plastic). While *Micrococcus luteus* was only absent in concrete pond but present in the other two ponds (earthen and plastic).

Table 2: Occurrence of isolates in the fish pond water in Owo(x 10^5cfu/ml)

Metal	Earthen	Concrete	Plastic
Escherichia coli	+	+	+
Salmonella spp	+	+	+
Streptococcus faecalis	+	-	-
Enterobacter sp.	+	+	+
Pseudomonas aeruginosa	+	+	+
Staphylococcus aureus	+	+	+
Micrococcus luteus	+	-	+
Bacillus subtilis	+	-	-
Klebsiella pneumoniae	+	+	+

+ = Present, - = Absent

Haematological parameters of catfish harvested from the different types of fish ponds (earthen, concrete and plastic) in Owo were shown in Table 3. Fish from earthen pond recorded the highest haemoglobin (8.33 g/dL) followed by concrete (6.33 g/dL) and plastic (6.00 g/dL). Packed cell volume recorded the highest in earthen pond (25.01 %) followed by plastic (20.6 %) and concrete (20.02 %). Earthen pond recorded the least (5300.30 x10^3/L) white blood cell (WBC) count while both concrete and plastic ponds recorded same count (6100.00 x10^3/L). Red blood cell (RBC) count was recorded the highest in earthen pond (2.80 x10^6/L) followed by concrete (2.15 x10^6/L) and plastic (2.05 x10^6/L). Table 3 also contains white blood cell types counted in cat fish harvested from the three ponds. Amongst the white blood cell types, Eosinophil and basophil were not detected. Concrete recorded the highest neutrophils (60 %) followed closely by plastic pond (59 %) and earthen pond (54 %). Earthen pond recorded the highest leucocytes (44 %) followed by plastic pond (41 %) and concrete pond (38 %). Monocytes counts in both earthen pond and concrete pond were (2 %) while it was absent in plastic pond.

Table 3: Haematologicalparameters of catfish harvested from different types of fish pond in Owo.

TREATMENT	HB (g/dL)	PCV (%)	WBC (x10^3/L)	RBC (x10^6/L)	Neu (%)	Leu (%)	Mono (%)	Eos (%)
Earthen	8.33±0.50^b	25.01±0.20^b	5300.30±34.50^a	2.80±0.01^a	54	44	2	-
Concrete	6.33±0.00^a	20.02±0.07^a	6100.00±18.00^b	2.15±0.00^a	60	38	2	-
Plastic	6.00±0.02^a	20.6±0.15^a	6100.00±25.02^b	2.05±0.00^a	59	41	-	-

Key: HB: Haemoglobin, PCV: Packed cell volume, WBC: White blood cell, RBC: Red blood cell,Neu: Neutrophils, Leu: Leucocytes, Mono: Monocytes, Eos: Eosinophil

4.0 DICUSSION

The diverse groups of bacteria isolated from these ponds are in line with the report of Okpokwasili and Ubah (2009) who worked on pond water suggesting that allochthonous bacteria from feed added to the ponds are the principle source of bacteria of health importance and Dabbor (2008) who reported similar organisms in the microbiological study of El-quanter fish pond. The presence of pathogenic microorganisms especially *E. coli* and *Salmonella* spp can lead to the transmission of water borne diseases such as, Typhoid fever, Cholera, food poisoning and gastroenteritis (Piet, 2009; Aklakur, 2016) on consumption of improperly cooked fish cultivated in these ponds. The presence of *E. coli* in water or food indicates the possible presence of causative agents of many gastrointestinal diseases (Ampofo and Clerk, 2010). *Pseudomonas, Staphylococcus* species have been implicated in food poisoning (Oni *et al.*, 2013; Barragán-Méndez *et al.*, 2019).

The haematological characteristics observed in the present study showed marginal differences when compared with that of *C. isheriensis* (Kori-Siakpere, 2007) and *C. buthupogon* (Kori-Siakpere and Egor, 2007; Friel and Lundberg, 2016). A high RBC and haemoglobin values for earthen pond in this study agreed with earlier works as expected for fast swimming tropical species (Kori-Siakpere and Egor, 2007; Annune and Ahuma, 2008) inhabiting pond water (Fänge, 2002; Alkahem *et al.*, 2008). Similarly, the high WBC values agreed with Fänge (2002) submission of remarkable richness of fish blood in leucocytes, although it can be an indication of diseased conditions. The derived variable of PCV (or haemocrit) was observed to be lower than expected the reason could be as a result of size of fish used. Kori-Siakpere and Egor (2007) and Annune and Ahuma (2008) earlier observations of low PCV values for both *C. buthupogon* and *C. gariepinus* respectively were consistent with my observations on *C. gariepinus*. It is therefore possible that the genus Clarias might have PCV value lower than other teleostan species. Changes in differential leukocyte count are recognized as a sensitive indicator of environmental stress and provide an overview of the integrity of the immune system (Cole *et al.*, 2001; Teles *et al.*, 2019).

5.0 CONCLUSION

In conclusion, the results of the present study indicated that the type of pond water used to rear fish can alter microbiological and hematological parameters. Findings from this work revealed that regularly monitoring water parameter such as microbiological and hematological provide insight to the health of the aquatic ecosystems. The microbiological and hematological analysis of the earthen concrete and plastic fish ponds showed that the values fell slightly below the acceptable limit especially those of concrete and plastic ponds while that of earthen pond was in the range of generally acceptable limit. This indicates the water quality of earthen pond may not have adverse effect on fish health. The detection of pathogenic bacteria that may be multi-drug resistant may suggest the need for formulation and implementation of code of practice for fish farmers. This is to ensure appropriate fish management and prevention of transmission of potential pathogens which is important in the part of food safety plan.

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