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Effects of prophylactics on growth, and hematology of African catfish, *Clarias gariepinus* (Burchell, 1822) fingerlings

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ABSTRACT

Clarias gariepinus fish were treated with 2 ppm, 150 ppm, and 100 ppm of potassium permanganate, hydrogen peroxide, and formalin respectively. After a 70-day culture period, growth and hematological indices were measured. Data were subjected to one-way ANOVA and the mean were separated using the New Duncan Multiple range test at $p \leq 0.05$. Results indicated that fish treated with potassium permanganate had the highest weight gain (5.31 ± 1.66 g) while the least (3.70 ± 0.28 g) was recorded in fish treated with hydrogen peroxide. The control fish had the highest (1.91 ± 2.28) feed conversion ratio while the least (1.47 ± 0.41) was recorded in catfish fingerlings treated with potassium permanganate. White blood cells were more (6.23 ± 0.25) in fish treated with formalin and lowest in control fish. Hemoglobin values were similar in control fish (12.45 ± 2.03) and potassium permanganate (13.09 ± 0.98) treated fish. Apart from PCV that decreased in fish treated with H_2O_2 and formalin, other red blood indices increased significantly ($p \leq 0.05$) against the control. Potassium permanganate appeared to have the least negative effect on the fish. The study, therefore, recommends the use of potassium at 2 ppm for treatments in catfish fingerlings aquaculture.

1. INTRODUCTION

African catfish is an important aquaculture species cultured in various regions in the world (**Akeem *et al.* 2018**). The African catfish is highly appreciated as good aquaculture species because of its resistance to disease, the ability to tolerate a wide range of environmental parameters and relatively fast growth rate (**Adeyemo *et al.* 2012**). Despite the wide acceptability of *Clarias gariepinus*, its full aquaculture potential has not yet been realized (**David, 2017**). There are many factors that have been attributed to this,

but high mortality in the fingerling stage and the resulting seed scarcity are prominent (**Mylonas *et al.* 2010**). Catfish farmers using different culturing systems often face the challenge of high fingerlings mortality. Fingerlings are most vulnerable when they are less than 10g in size, thus the highest mortality occurs during this period (**Adebisi, 2018**). One probable cause of fingerlings mortality is disease infection. Fish diseases constitute one of the most important problems and challenge confronting fish culturists. According to **Adebisi, (2018),** fish are subjected to infection by disease-causing viruses, bacteria, and fungi.

Chemicals and aqua drugs are important components for a successful aquaculture practice according to **Sharker *et al.* (2014)**. They further stated that the most commonly used chemicals are sodium chloride, formalin, malachite green, methylene blue, potassium permanganate, hydrogen peroxide and glutaraldehyde. However, owing to the carcinogenicity of malachite green, its use was discontinued for oomycete control in edible aquatic animals (**Schreck *et al.*, 1993**). Standard treatments with sodium chloride (NaCl) and potassium permanganate (KMnO4) are now effectively used in aquaculture to treat external parasites.

$KMnO_4$ is used for the treatment of ponds water. It is also potent against protozoan infiltrations on skin, gills and fins (**Adhikary *et al.* 2018**). Hydrogen peroxide (H_2O_2) is a disinfectant; it has antimicrobial effects and easily degrades to harmless by-products (**Shehab *et al.*, 2017**). Formalin is effective in treating fungi, external parasites, including protozoans and monogenic trematodes and is widely used in therapeutic and prophylactic treatment by aquaculturists (**Floyd, 1996**). Successful treatment of diseases depends on selecting the most effective therapeutic drug or chemical and applying it in the most appropriate legal manner (**Helfrich, 2009**). However, misuse of approved drugs or chemicals in aquaculture may pose potential hazards to fish. These substances may be toxic, allergenic, or carcinogenic, or may cause antibiotic resistance in pathogens (**Hossain *et al.*, 2018**). Formalin has been in use in Nigerian aquaculture industries for fish and egg disinfection without much attention being paid to the fact that the therapeutant might also be absorbed systemically and may produce significant internal effects (**Okomoda *et al.*, 2010**). Keeping these in mind, this research was conducted to determine the effects of formalin, hydrogen peroxide and potassium permanganate used as prophylactics on the growth performance and hematology of *Clarias gariepinus.*

2. MATERIALS AND METHODS

2.1 Experimental Procedure

The experiment was conducted at the laboratory of the Department of Fisheries and Aquaculture, Federal University, Oye Ekiti, Nigeria from August 2018 to October 2018. One hundred and eighty fingerlings (2.44±0.05g) were procured from a reputable farm within the state. They were transported to the laboratory in an open plastic bucket containing aerated water. The *C. gariepinus* fingerlings were acclimatized in plastic holding tanks for at least 21 days as previously done by **Andem *et al.* (2015).** Fifteen fish

were randomly distributed into 12 plastic aquaria (36 x 25 x 25 cm) in triplicates. They were fed twice daily at 3% of body weight with commercial feed containing 40% crude protein. The experiment was a complete randomized design (CRD) consisting of four treatments (control, $KMnO_4$, H_2O_2 and formalin) designated as T1, T2, T3, and T4 respectively. $KMnO_4$ was applied at 2 ppm (**Ovie, 2008**), H_2O_2 at 150 mg/L (**Bowman *et al.* 2008**) and formalin at 100 ppm (**Andem *et al.* 2015**). The treatments were given on the first day of the experiment and then repeated biweekly for 10 weeks (70 days). All used water was diluted and drained into a septic tank for proper disposal as effluent. Weights of fish were taken fortnightly using an electronic weighing balance Kerro BL10001 compact scale according to **Shehab *et al.* (2017)**. Aquaria water was changed every other day while uneaten feeds were daily siphoned. Water temperature, pH, and dissolved oxygen were monitored daily. Temperature was measured using a mercury-in-glass thermometer, pH was measured with a pH meter (Jenway model 9060) while the dissolved oxygen was measured with an oxygen meter (Hanna model H1-9142).

2.2 Growth Parameters:

Growth performance of *C. gariepinus* fingerlings was evaluated using weight gained, specific growth rate, and feed conversion ratio using the formula specified by **Jauncey and Rose, (1982)** and **Abiodun (2016)** in equations 1 and 2 respectively

2.2.1 Mean weight gained (MWG) = $W_2 - W_1$

Where W_2 = the final weight, W_1 = the initial weight

2.2.2 Specific growth rate (SGR)

$$SGR = Log_e(W_2) - Log_e(W_1) x\ 100/t_2 - t_1\ x\ 100 \quad (1)$$

Where W_2 = the final weight, W_1 = the initial weight while $t_2 - t_1$ = duration between W_2 and W_1 (days).

2.2.3 Feed conversion ratio (FCR)

$$FCR = Feed\ consumed\ (g)/Weight\ gained\ (g) \quad (2)$$

2.3 Haematological Analysis

Blood sample from each treatment group was collected via caudal peduncle puncture as described by **Stockopf, (1993)** and emptied into EDTA bottles for haematological analysis. Haemoglobin (Hb) was estimated by cyanomethemglobin method. Red blood cells (RBV) and white blood cells (WBC) were counted by Naubauer's improved haemacytometer using Hyem's and Turks solution as diluting fluids respectively. Packed cell volume (PCV) was estimated by using haematocrit method (**Joshi *et al.* 2002**). Mean corpuscular haemoglobin (MCH), mean cell

haemoglobin concentration (MCHC) and mean cell volume (MCV) were calculated using standard formula described by **Soyinka and Boafo, (2015)** in equations 3, 4 and 5 while the differentials in the white blood cell were also determined using methods described by **Joshi *et al*. (2002)**. All procedures were in accordance with the ethical standard of the animal ethics committee of Federal University, Oye Ekiti, Nigeria.

$$MCV\ (fl) = PCV\ x\ 10/RBC \quad (3)$$

$$MCH\ (pg) = Hb\ x\ 10/RBC. \quad (4)$$

$$MCHC\ (g/dl) = Hb\ x\ 100/PCV \quad (5)$$

2.4 Statistical Analysis

Growth, haematological and water quality parameters were subjected to one-way analysis of variance (ANOVA) using the Statistical Analysis System (University Edition). Means were separated using the New Duncan's Multiple Range Test at 5% level of probability.

3. RESULTS

3.1 Water quality parameters

The results of this study indicated that the mean pH value ranged from 6.92 to 7.00 while temperature of water is within the range of 22.63^0C and 22.70^0C. Mean dissolved oxygen varied from 9.40mg/l to 9.43 mg/l. There was no significant difference among all the tested water quality parameters in this study (Table 1).

TABLE 1. Water quality parameters during rearing of *Clarias gariepinus* under different prophylactic treatments

Parameters	**T1**	**T2**	**T3**	**T4**
Temperature (^{0}C)	22.70±0.03^a	22.70±0.06^a	22.70±0.10^a	22.63±0.33^a
DO (mg/L)	9.42±0.02^a	9.40±0.00^a	9.42±0.02^a	9.43±0.02^a
pH	6.92±0.03^a	7.00±0.06^a	7.00±0.50^a	6.98±0.03^a

Mean ± S.E with the same superscripts along the same row are not significantly different at $p \leq 0.05$

3.2 Growth performance of *Clarias gariepinus* fingerlings

The best weight gain (5.31±1.66g) was observed in the potassium permanganate treated fish (T2), while the hydrogen peroxide treated fish (T3) gained the least weight (6.13±0.29g). There was a significant difference between the weight gain of all the treated fish and the control (Table 4.1). The feed conversion ratio (FCR) of the fish in this

study ranged btween 1.47±0.41 and 1.91±0.28 with treatment 2 (T2) having the best FCR while the least was seen in the control experiment (T1), however, there was no significant difference in the FCR of all the fish used in this study. The specific growth rate (SGR) ranged between 0.57 %/day and 0.68 %/day. Although Treatment 2 had the best SGR, there was no significant difference in the SGR value across all the treatments (Table 2).

TABLE 2. Growth performance of the *C. gariepinus* fingerlings under different prophylactic treatments

Parameters	**T1**	**T2**	**T3**	**T4**
MIW (g)	2.43±0.04[a]	2.45±0.03[a]	2.43±0.01[a]	2.46±0.10[a]
MFW (g)	6.39±0.68[a]	7.76±1.65[a]	6.13±0.29[a]	7.73±1.94[a]
WG (g)	3.96±0.67[a]	5.31±1.66[b]	3.70±0.28[a]	5.27±1.84[b]
FCR	1.91±0.28[a]	1.47±0.41[a]	1.82±0.07[a]	1.59±0.16[a]
SGR (%/day)	0.57±0.06[a]	0.68±0.14[a]	0.62±0.03[a]	0.67±0.14[a]

Mean ± S.E with different superscripts along the same row are significantly different at $p \leq 0.05$. MIW – Mean Initial Weight, MFW – Mean Final Weight, WG – Weight Gained, SGR – Specific Growth Rate

3.3 Haematological parameters of *Clarias gariepinus* fingerlings

There were significant differences among the white blood cell (WBC) count across the treatments. The highest count ($6.23 \pm 0.25 \times 10^9$/L) was observed in the treatment 4 fish while the least ($4.13 \pm 0.32 \times 10^9$/L) was recorded in treatment 1 (Table 3). The Haematological analyses revealed a significant reduction in red blood cell (RBC) of fish treated with $KMnO_4$ from 27.60 ± 0.88 to 9.20 ± 9.40 in fish treated with formalin. Also, a significant decrease was recorded in haemoglobin (Hb) from 13.09 ± 0.98 in treatment 2 ($KMnO_4$) to 10.52 ± 3.04 in treatment 4 (Formalin). A significant decrease was also recorded in Packed Cell Volume (PCV) from 40.33±0.67 in treatment 2 ($KMnO_4$) to 27.83 ± 1.88 in treatment 4 (Formalin). Treatment 4 (formalin) had the highest MCV (mean corpuscular volume) and MCH (Mean Corpuscular volume). Significant differences were noticed between the MCV values in treatment 4 and the other three treatments. A significant variation was observed between the MCH values in treatment 1 and treatment 2. The Mean Corpuscular Haemoglobin Concentration (MCHC) values of the entire treatments were statistically the same (Table 3).

TABLE 3: Haematological indices of the *Clarias gariepinus* fingerlings under different prophylactic treatments

Treatments	**T1**	**T2**	**T3**	**T4**
WBC (10^9/L)	4.13±0.32^c	4.57±0.21^c	5.50±0.50^b	6.23±0.25^a
RBC (10^{12}/L)	26.70±4.04^a	27.60±0.88^a	14.00±13.54^b	9.20±9.40^c
Hb (g/L)	12.45±2.03^a	13.09±0.98^a	11.34±1.76^{ab}	10.52±3.04^b
MCV (10^{-15}L/cell)	138.58±4.53^c	146.12±3.45^{bc}	202.36±8.34^b	356.85±2.45^a
MCH (10^{-12}g/cell)	46.63±0.56^a	47.43±0.76^a	81.00±7.04^b	114.35±2.34^c
MCHC (g/L)	33.65±0.45^b	32.46±0.55^b	40.03±2.45^c	37.80±0.45^a
PCV (%)	37.00±1.15^b	40.33±0.67^b	28.33±1.76^a	27.83±1.88^a
N	65.00±10.40^c	26.67±1.20^b	27.03±1.67^a	27.33±5.75^a
L	30.00±10.40^a	69.33±1.20^b	70.33±1.67^c	70.67±2.33^c

Mean ± S.E with different superscripts along the same row are significantly different at $p \leq 0.05$

4. DISCUSSION

All the water quality parameters measured in this study were within the levels recommended by **Viveen *et al*. (1985)** and **Boyd, (1983)**, for general fish survival and growth. The positive trend in all the growth indices across the treatments negates the findings of **Jimmy *et al.* (2014)** where African catfish exposed to sub-lethal concentrations of formalin recorded reduced weight gain. The specific growth rate (SGR) observed in this study are better when compared to what was observed by **Walakira *et al.* (2014)** in *C. gariepinus* larvae treated with salt and formalin at 3000 ppm and 400 µL/L concentration respectively but they are similar to the ones observed in banana leaf extract treated fish. As observed in the growth indices of this study, the use of synthetic chemicals in aquaculture to boost productivity might not be a major threat to the growth of fish as claimed by **Chitmamatic and Nunsong, (2009)** and **Okomoda *et al*. (2010).**

It is important to state that haematological characteristics are essential tools that are used as indicators for monitoring physiological status and changes in fish **(Erhunmunse and Ainerua, 2013, Ogueji *et al.* 2017).** These characteristics have provided reliable

information on metabolic disorders, chronic stress status and health status before and after clinical examination of specimens **(Bahmani *et al.*, 2001)**. In this study, a significant increase in WBC of the fish exposed to the chemicals could be as a result of an immune system response to the toxic effect of the chemicals as WBCs are involved in immune function regulation in many organisms (**Nwani *et al.* 2013**). **Saravanan *et al.* (2012)** reported a significant increase in WBC in *Cirrhinusm rigala* exposed to various concentrations of ibuprofen drug. A similar result has been reported in *Oreochromis. niloticus* exposed to verapamil (**Ajima *et al.* 2016**).

The sharp decrease in RBC values in *Clarias gariepinus* treated with the chemicals except for potassium permanganate might be due to an impairment of the erythropoietic process. **Ogueji *et al.* (2017)** reported a significant reduction in RBC when African catfish (*Clarias gariepinus*) were exposed to diazepam. The reductions in PCV might be as a result of significant decrease in hematopoietic activity. In other studies involving the use of prophylactics, **Nwani *et al.* (2013)** reported significant reduction in PCV and Hb values across diazepam treated fish specimens when compared to the control. **Ajima *et al.* (2016)** also reported a significant reduction in RBC, Hb and PCV values of *O. niloticus* juvenile exposed to varying concentrations of verapamil. The decrease in Haemoglobin (Hb) values could also be as a result of the adverse effect on Hb biosynthesis. This was supported by **Nwani *et al.* (2013)** as they reported that Hb biosynthesis when adversely altered, could limit the oxygen-carrying capacity of the fish blood. Generally, red cell indices are important for the diagnosis of anemia in most animals including fish (**Cole, 1986**). Alterations in the values of these red cell indices (MCV, MCH, MHCH) may indicate macrocytic anemia (**Dacie and Lewis, 2011; Iheanacho *et al.* 2017**). **Cole, (1986)** reported an increase in MCV, MCH, and Hb values when *Oncorhynchus mykiss* was exposed to pharmaceutical drug (verapamil). Similar report was made by **Saravanan *et al.* (2012)** indicating a significant increase in MCV and MCH values when Indian major carp (*Cirrhinus mrigala*) were exposed to Ibuprofen drug.

Alteration in WBC differential count was observed in this study. WBC differential count is an insightful indicator of environmental stress (**Cole *et al.* 2001**). **Barros-Beeker *et al.* (2012)** reported a significant increase in neutrophil values when Zebrafish (*Danio rerio*) larvae were exposed to oxytetracycline. **Ogueji *et al.* (2017)** also reported a significant increase in neutrophil and lymphocytes values when African catfish (*Clarias gariepinus*) juveniles were exposed to diazepam.

CONCLUSION

This study revealed that exposure of *Clarias gariepinus* to potassium permanganate, hydrogen peroxide and formalin can produce significant changes in the growth and physiology of *Clarias gariepinus* as manifested in the growth parameters, and haematological characteristics of the fish. On the other hand, persistent exposure of fish formalin and hydrogen peroxide is capable of altering the haematology of the exposed

fish. Potassium permanganate's effect on the other hand was not as severe when compared to the other two chemicals. From these findings, it could be recommended that potassium permanganate at 2 ppm be used as a prophylactic for disease prevention in *Clarias gariepinus* fingerlings.

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