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Influence of different water pH on packed cell volume, total erythrocyte count, total leucocyte count, blood glucose and blood protein of laboratory reared Heteroclarias fingerlings in Minna, Nigeria

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Abstract

A twelve weeks study was carried out to determine the influence of different levels of water pH on selected haematological parameters of *Heteroclarias* fingerlings under laboratory conditions. *Heteroclarias* fingerlings were subjected to water pH of 5.00, 7.00 (control), 9.00 and 11.00 with three replicates each respectively. The monitoring of the pH treatments was done on daily basis to maintained the tested pH levels while the initial pH of the treatments were also adjusted to desired pH levels by following the standard experimental procedures. An average of nine (9) *Heteroclarias* fingerlings from each treatment were bled on the first, sixth and twelve weeks of the experiment to determine the Packed Cell Volume (PVC), Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Blood Glucose (BG) and Blood Protein (BP) based on standard experimental procedures. The physico chemical parameters were also determined weekly based on standard experimental procedures. The results showed that water pH had no significant influence ($p>0.05$) on the PVC, TEC, TLC, BG and BP respectively at the first week of the experiment. However, at the sixth (6) week of the experiment, the PCV increased from 19.05 ± 0.75 to $21.30 \pm 2.40\%$ of *Heteroclarias* fingerlings exposed to pH 9.00 and 7.00 were significantly higher ($p< 0.05$) when compared with pH 5.0 treatment. Similarly, the TEC ($15.35 \pm 1.55 \times 10^{12}$/L) of the fingerlings cultured in pH 9.0 was significantly different ($p< 0.05$) at the 6th week of the study. The PCV of the fingerlings exposed to pH 9.0 was significantly reduced ($p< 0.05$) from $19.05 \pm 0.75\%$ in week 6 to $10.30 \pm 5.60\%$ at the end of the experiment. Water pH had no significant influence ($p>0.005$) on the TEC, TLC, BG and BP of *Heteroclarias* fingerlings at the end of the study. The physicochemical parameters measured such as water temperature, dissolved oxygen concentration, biochemical oxygen demand, ammonia concentration were all within the recommended range for fish growth in the tropics. Electrical conductivity ($304.42 \pm 47.20\mu s/cm$) of water at pH 7.0 was significantly low ($p< 0.05$) when compared with pH 5.00 and 9.0 treatments. Most of the physiochemical and hematological parameters studied were not significantly ($p> 0.05$) influenced by water pH ranged from 5.0 to 9.00 indicating no physiological stress. However, farmers are advised on routinely evaluation of haematological parameters of *Heteroclarias* fingerlings so as to provide essential information on their physiological status.

Keywords: Haematological parameters, *Heteroclarias* fingerlings, pH, physicochemical parameters

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Introduction

Blood is a medium for all biological activities in animal body and a feature of all vertebrate animals and some invertebrates (Taio and Anosa, 1995). It is one of the most important tissues of the body. It circulates through the walls of the intestine and transport nutritional materials in the plasma to other tissues throughout the body (Auta *et.al*, 2010). Haematological analysis will enhance fish cultivation by facilitating early detection of situation of stress and/or diseases that could affect production (Tavares-Dias and Moraes, 2007).

Haematological indices are of different sensitivity to various environmental factors and chemicals (Vosyliene, 1999). Haematology and clinical chemistry analysis, although not often used in fish medicine, can provide substantial diagnostic information. In addition, haematological studies will help to predict the physiological state of fish in natural water bodies (Abbas *et.al*, 2011). Haematological studies in teleost have indicated that haematocrit values might be useful as a general indicator of fish health, since fish given iron deficient diets, or those exhibiting anaemia, all possess reduced haematocrit values, haemoglobin concentration and red blood cell count (Osuigwe *et.al,* 2005).

A number of haematological indices such as haematocrit (Ht or PCV), haemoglobin (Hb), Total Erythrocyte Count (TEC) are used to access these functional status and oxygen carrying capacity of blood stream (Shah and Altindag, 2004).The evaluation of haematological parameters has provided a tool for facilitating fish health management (Chen *et al.*, 2004). Moreover blood chemistry parameters are used as indicators of physiological stress response in fish (Lermen *et al.*, 2004).Assessing a normal fish blood will provide a reference point whether there is a change in blood parameter as regard to age, size, diseases or stress condition (Auta *et al.,* 2010). Hardig and Hoglundix (1982) also demonstrated that haemoglobin and other blood parameters undergo seasonal variation co-committal to climatic changes, light, water, temperature and to a lesser extent influenced by age.

Water pH plays an important role in maintenance of the homeostasis in aquatic animal. Increases or decreases in pH are reported to cause disturbances in acid-base and ion regulation and ammonia excretion (Das *et al.*, 2006). Toxicity of ammonia to fish has been intensively investigated in numerous fish species. It was established that the toxicity of ammonia depends principally on the change in water pH and reversibly affects the rate of total ammonia excretion in fish (Evans *et al.*, 2005). The gill is the primary interface between fish and its environment for gas transfer, acid- base balance, ion regulation and ammonia excretion (Das *et al.*, 2006). As reported by Ghanbari, *et al.* (2012) in their recent review, fish must maintain a high ventilation rate to meet their oxygen demands and thus cannot significantly alter ventilation in response to changing pH. Information on the long term exposure to high and low water pH anion balance and ammonia excretion on freshwater fish is mainly limited to that of Salmonids such as the rainbow trout (*Oncorhynchus mykiss*). In this fish, alkaline water (pH 9.5) cause increase in blood pH and higher plasma ammonia excretion, this can be potentially lethal (Wilkie and Wood, 1996). The effect of low environmental pH on fish includes increased bronchial loss of ions, reduction in plasma pH, bicarbonate, Na^+ and Cl^- concentration. In situation of high nutrient, input, supplementary feed, manures and inorganic fertilizers are added to get higher production per unit area, however, a change in water pH, either to higher or lower levels, could cause stress in fish and affect its body physiology and growth (Das *et al.*, 2006).

Ghanbari *et al.* (2012) stressed that the haematological variables (population of blood cells, concentrations of haemoglobin, size, shape, blood sugar and serum protein) were measured to assess the state of fish health and also could be used as tools to indicate the stress

level in this fish during exposure to change in water pH. The authors also reported that fingerlings of common *Cyprinus carpio L.* exposed to both acidic and alkaline water had significant reductions ($P< 0.05$) in TEC and haemoglobin content (Hb) at the two extreme pH 5.5 and 9.0.The reduction in the TEC and Hb content in the carps following exposure to both acidic and alkaline water indicated a reduced blood oxygen carrying capacity. (Martinez and Souza, 2002; Jensen, 2003). The progressive reduction of the heamoglobin content in both acidic and alkaline conditions also revealed possibility of respiratory stress in fish or fingerlings. The decrease in TEC counts, haematocrit and haemoglobin concentration also indicate that the red blood cells are being destroyed by the leucocytosis activity in an erythrocytic anaemia with subsequent erythroblastosis (Ghanbari *et al.*, 2012).

The total leucocyte count (TLC) was reduced at pH5.5 but there was a brief increase at pH 6.5 in *C. carpio* fingerlings. However, alkaline range also experienced a reduction in TLCs at pH 8.0 and was further reduced with increased pH. The TLC reduction observed in the *C. carpio* fingerlings may be most likely due to the greater stress level at such higher pH change, which weakened the leucopoiesis process (Ghanbari *et al.*, 2012).The significant reduction in TLCs and greater elevation in blood glucose in *C. carpio* fingerlings, particularly at higher water pH (acid and alkaline) could also be attributed to the additional stress due to possible accumulation of ammonia in fish body (Wilkie *et al.* , 1993)

El-sheriff and El-feky (2009) documented that the average haematocrit value in the experimental group was not different from the control in performance of *Oreochromis niloticus* reared in pH 6, 7, 8 and 9 respectively for 60 days. Also it decreased at pH 6 as feed consumption decreased with appearance of anaemia. The authors also documented that, at pH 7 and 8, there were no significant ($P>0.05$) differences from control. However, significant differences ($p<0.05$) were obtained among the pH 6, 7, 8 and 9 but the difference was not significant ($p> 0.05$) between pH 8 and 9.

Pratap *et al.* (2004) observed that the average Hb concentration in the experimental groups at pH 7, 8 and 9 were not different from control, but the average Hb concentration decreased at pH 6. The above submission agreed with the works of El-sherif and El-feky (2009) who reported that there were no significant differences ($p>0.05$) in the haemoglobin concentration at pH 7, 8 and 9 while Ghanbari *et al.* (2012) documented that serum protein levels were significantly ($P<0.05$) reduced. When *C. carpio* fingerlings were exposed to pH 5.5, 8.0, and 8.5. The higher serum protein reduction in the fingerlings of *C. carpio* in all the acidic and alkaline pH exposures may be attributed to protein catabolism, the process of converting blood and structural protein to energy to meet the higher energy demand during the prevailing stress. Haemolysis and shrinkage of the erythrocytes may also lead to the dilution of the plasma protein volume contributing to some extent such reduction of serum protein content (Das *et al.*, 2004, and 2006).

The leucocytes are involved in regulation of immunological function in the organism (Santhakumar *et al.* 1999). As a protective response of the body during stress, TLC increases through stimulation of leukopoietic process and enhances release of leucocytes to the blood circulation. Stress also induces elevation of plasma catecholamines in fish within minutes. The released catecholamines, adrenalin and nor-adrenalin, increased the conversion of liver glycogen to blood glucose to satisfy the greater energy demands of the body to stress (Sriwastava and Srivastava, 1985).

The changes in haematological parameters in fingerlings of *C. carpio* exposed to different water pH indicated ion regulatory or respiratory disturbances that imply an increase in energy consumption to restore homeostasis instead of other physiological functions, such as weight gain and growth. This also confirmed the swelling of the fingerlings red blood cells which might be due to water uptake accompanying the movement of Na^+ into the cell (Ghanbari *et al.*, 2012; Jensen *et al.*, 2001).

The aim of this study is to investigate the potential effects of exposure to different levels of water pH on the haematological and physicochemical parameters of *Heteroclarias* fingerlings in Minna, Nigeria.

Materials and methods

Experimental Site

The study was conducted at the Biology Laboratory of the School of Life Sciences, Bosso Campus, Federal University of Technology, Minna. Niger State, Nigeria.

Source of the experimental fish

One thousand eight hundred, four weeks old Heteroclarias fingerlings with average weight of 1.01g were purchased from a private fish farm in Lagos, Nigeria. The fingerlings were transported to the Biology Laboratory in 50 litres Jerican with well-aerated water through openings at the top for ventilation.

Acclimatization of the fingerlings

The Heteroclarias fingerlings were acclimatized in rearing tanks ($55\times35\times35cm^3$) for a period of seven days to allow them to recover from transportation stress. They were also visually observed to ensure that there were no infections from the hatchery and also to select average weight of the fish to be cultured together (Adewolu *et al.*, 2008, Ayanwale *et al.*, 2014). During this period, the fish were fed on a commercial diet (Catco fish concentrate) by coppens international, Holland. They were fed to satiation, morning and evening following the method of Koeypudsa and Jongjareanjai, (2011). Water exchange was done when necessary in the morning. The left overfeed and faecal samples were siphoned immediately after feeding (Ghanbari *et al.*, 2012).

Experimental design

A Completely Randomised Design (CRD) with a total of 4 treatments replicated 3 times was adopted in this experiment.The experiment consisted of 4 treatments with 3 replicates each. Each treatment had a stocking density of four hundred and fifty fingerlings. Treatments 1, 2, 3, and 4 had water pH of 5.0, 7.0 (control), 9.0 and 11.0 respectively. The initial pH in all the experimental tanks was determined first by inserting the pH probe of the electrically – operated pH meter into the rearing tanks. The pH meter probe was inserted into ach experimental tanks for about 5minutes until it stabilized before the reading was taken. The meter was standardized with buffer solutions of pH 4.0, 7.0 and 9.0 before the readings were taken (Ayanwale *etal.* ,2015). The stock solution of the acid was prepared by measuring 3ml concentrated HCl and 97ml of distilled water into beaker, mixed thoroughly with a clean glass rod and stored in a reagent bottle. The stock solution of the alkaline was prepared by dissolving 0.4g of sodium hydroxide granules in 100ml of distilled water (Ivoke *et al.*, 2007). The initial pH of the borehole water was adjusted to pH 5 by the addition of 3% concentrated Hydrochloric acid. The water was made alkaline when the initial pH of borehole water was adjusted to 7, 9 and 11 by the addition of prepared 0.01M sodium hydroxide. To obtain the desired pH levels, drops of 0.01M sodium hydroxide or 3% concentrated HCl was added where necessary to obtain the most desire reading as displayed by the pH meter. The fingerlings were fed on a commercial diet (Catco fish concentrate) to satiation, morning and evening following the method of Koeypudsa and Jongjareanjai, (2011). Exchange of water from the experimental tanks was done twice a week, while the removal of faecal samples, uneaten feed and monitoring of the pH of experimental tanks with the aid of pH meter were done on a daily basis, so as to maintain the tested pH levels (Ghanbari *et al.* 2012) . The experiment lasted for a period of twelve weeks.

DETERMINATION OF SOME PHYSICO-CHEMICAL PARAMETERS

Water Temperature

Water temperature of the control treatment was determined with mercury in bulb thermometer (10-110^{0}C range). Temperature was determined by lowering the thermometer into the tanks in an inclined position for about 5 minutes to allow for equilibrium before taking the reading at 10.00am in the morning throughout the duration of the experiment.

Dissolved Oxygen

This was determined by using Winkler Azide method (American Public Health Association, 1995). Water samples from the control and treatment tanks were collected by inserting 250 ml water sample bottles into the tanks and sampled water was fixed right in the laboratory with 1ml of reagent (I) (Manganous sulphate) and 1ml of reagent (II) Alkaline iodide solution (KOH + KI). About 2 ml of concentrated sulphuric acid was added to each sample and 10ml of the sample was titrated with 0.025N sodium thiosulphate using starch as indicator until it turns colourless.
Calculation was based on the formula described by Boyd (1979) as follows:-

$$\text{Dissolved Oxygen (mg/L)} = \frac{\text{Volume}(Na_2SO_3) \times \text{Normality} \times 8 \times 1000}{\text{Sample volume (ml)}}$$

Where, normality= 0.025 ml of sodium sulphite (Na_2S0_3)
8 = Equivalent weight of oxygen in water, 1000 = Conversion to mg/litre.

Hydrogen Ion Concentration (pH)

The pH of the water samples were determined with Jenway 3305 pH meter model at room temperature. The pH meter probe was inserted into the sampled water for about 5minutes until it stabilized before the reading was taken. The meter was standardized with buffer solutions of pH 4.0, 7.0 and 9.0 before the readings were taken.

Ammonia (NH_3)

100ml of the water sample from control and treatment tanks was pipetted into a Markham distillation apparatus (Kjeldal flask) and there after 5ml of 40% NaOH was added. The flask was connected to the condenser and the cooling water was turned on. About 10ml of 40% boric acid (H_3BO_3) solution was placed under the condenser ensuring that the tip of the condenser was immersed in the receiving solution and distilled slowly until 50ml of the distillate was collected in the receiving flask. The ammonia was determined from the distillate by titrating with 0.01M HCl until the colour at the end point changed from green to pink (APHA, 1995). Calculation was based on the formular below : -

$$NH_3(mg/L) = \frac{\text{Titre value} \times 14 \times 0.01 \times 1000}{V}$$

Where 0.01 = molarity of HCl used as titrant; 14 is the molecular mass of nitrogen; 1000 is the conversion to mg /litre and V is the volume of sample used.

Electrical Conductivity

The manual electrical conductivity metre (CD 4303 Lutron) probe was inserted into the sampled water for about 5 minutes until it stabilizes before the reading was taken. The readings were expressed in microseimen (µs).

Haematological Analysis

The haematological analysis was done at Pathology Department General Hospital, Minna, Niger state. The fingerlings were bled from the ventral region near the heart by using a sterilized razor blade according to the method of Adeyemo *et al.* (2003). The blood was allowed to flow freely into sample bottles containing 6 % EDTA (Ethylene Diamine Tetra Acetic Acid) solution, an anticoagulant, and to the other plain sample bottles (without EDTA) according to the method of Haruna and Adikwu (2001). Owing to insufficient amount of blood, from each experimental treatment, blood was collected from pooled samples in triplicate (from an average of three fish each; (Ayuba, 2004). The blood samples collected in the EDTA bottles

were used for the determination of haematocrit or packed cell volume (PCV), erythrocyte count and leucocyte count. Serum was obtained from samples without EDTA by centrifugation and then transferred into non-heparinised bottle and stored in a refrigerator and later used for the determination of total protein and total glucose (Haruna and Adikwu, 2001). The haematological parameters were determined at the first week, sixth week and at the end of the experiment (12 week). The parameters determined were Packed Cell Volume (Haematocrit value), Erythrocyte count, Leucocyte count, Total Protein and Total Glucose.

Blood Glucose

The method of Sacks (1999) was used to determine blood glucose level. This involved the use of 10µl of sample mixed with 1,000 µl of the working reagent (a mixture of 250 mmol/L phosphate buffer, pH 7.5, 5mmol/L phenol, 0.5mmol/L 4-Aminoantipyrine, ≥ 10ku/L glucose oxidase, ≥ 1 ku/L peroxidase and stabilizer. It was then incubated for 10 minutes at 37^{0}C for 20 minutes at room temperature. Spectrometer at the wavelength 500nm was used to take the readings of the sample and the standard. Glucose level was calculated using the formulae: -

$$\frac{\text{SA.OD}}{\text{ST.OD}} \times 100 = \text{mmol/L} \quad \text{OR} \quad \text{mg/dl} \times 0.55$$

Where: -
SA. O D = Spectrometer reading of the sample
ST. O.D= Spectrometer reading of standard

Blood Protein

The serum total protein was determined using Biuret method (Johnson *et al.*, 1999). The process involved mixing 20.00µL of serum with 1000.00µL of Biuret solution into test tubes and incubated at 37.00^{0}C for 5 minutes in a water bath. Reading was taken at 540nm and at 1.00cm light path cuvette using spectrophotometer (GENESYS 10, Rochester NY USA). The total protein was calculated using the formula: -

$$\frac{Test\ blank}{Standard\ black} \times Concentration\ of\ standard = Total\ protein\ (g/dl)$$

Packed Cell Volume (PCV)

The packed cell volume was determined using the standard haematological procedures described by Svobodova *et al.* (1991). Whole blood was drawn in to a heparinised capillary tube, one end of which was sealed with plasticine and centrifuged for 5 minutes at 12,000 revolutions per minute. The PCV level was read using a haematocrit reader and was expressed in percentage.

Total Erythrocyte Count (TEC) and Total Leucocytes Count (WBC)

The TEC and TLC were determined using the method of Svobodova *etal.* (1993). Blood was drawn up to the 0.5 ml mark of pipette; it was then diluted to the 101ml mark using the diluting fluid as described by Svobodova *et al.* (1993). The counting chamber was filled with the mixture after placement of cover slip (charged) and the RBC counted under the microscope.

Total TEC = Number of TEC × 10^{12} / L

About 0.02ml of blood was added to 0.38ml of Turks solution (which destroys all TECs). The counting chamber was charged and the TLC counted under microscope.

Total TLC = Number of TLC × 10^9/L

Data analyses

The data collected were analysed for significant differences ($P < 0.05$) by the analysis of variance (ANOVA) using a Computer Statistical

Package for Social Sciences (SPSS). Duncan Multiple Range Test (Duncan, 1955) method was used to separate the means where there were statistically significant differences ($P < 0.05$).

Results

The results of the mean ± standard error of selected haematological parameters of Heteroclarias fingerlings exposed to different pH levels are presented in Table 1. There were no significant difference in the PCV (12. 30 ± 0.60 at pH 5.0 to 12.90.0 30% at pH 9.0), TEC (17.70 ± 1.55 at pH 9.0 to 18.55 ± 0. 65 x 10^{12}/L at pH 5.0), TLC (1.30 ± 0.00 at pH 5.0 to 1.40 ± 0.02 x 10^9/L at pH 7.0), BG (3.1 0 ± 0.10 at pH 9.0 to 3.35 ± 0.45mMol/L at pH 7.0) and BP (5.80 ± 0.20 at pH 5.0 to 6.15 ± 0.35 g/dL at pH 7.0) respectively at the first week. However, by the 6th week, the PCV values of fingerlings raised in pH 9.00 and control group) were significantly higher ($p< 0.05$) from those reared in pH 5.00. Similarly, at the 6th week of exposure, the TEC (15.35 ± 1.55 X 10^{12}/L) of the fingerlings cultured in pH 9.00 was significantly higher ($p<0.05$) from those of pH 5.00 and control (ranged from 12.20 ± 0.90 to 13.55 ± 1.25 x 10^{12}/L). But, the PCV (10.30 ± 5.60%) of the fingerlings cultured in pH 9.00 was significantly lowest ($p<0.05$) when compared with those fingerlings raised at pH 7.00 and 5.00 ranging from 13.95 ± 0.45 to 15. 60 ± 0.90% at the end of the study.

The TLC (ranged from 1.60 ± 0.10 at pH to 2.40 ± 0.30 x 10^9/L at pH 7.0), BG (ranged from 2.95 ± 0.15 at pH 5.0 to 3.60 to ± 0.10 mMol/L at pH 9.0) and BP (ranged from 6.55 ± 0.35 at control group to 6.60 ± 0.20 mMol/L at pH 9.0) were not significantly different ($p> 0.05$) at the 6th week of the study. Similar trend were also observed in the TEC (5.70± 0.40 at pH 5.0 to 7.15 ± 0.95 X 10^{12}/L at pH 9.0), TLC (ranged from 1.55± 0.05 at pH 7.0 to 1.80 ± 0.00 x 10^9/L at pH 9.0), BG (ranged from 2.80± 0.10 to 3.00 ± 0.00 mMol/L at pH 9.0) and BP (ranged from 4.60± 0.00 at pH 9.0 to 5.55 ± 0.35g/dL at pH 7.00) respectively at the end of the study. The results of physicochemical parameters of Heteroclarias fingerlings cultured at different pH levels are depicted in Table 2 .The water temperature (ranged from 26.21 ± 1.00 at pH 9.00 to 26. 51 ± 0.77°C at pH 5.0) , Dissolved Oxygen Concentration (ranged from 4.63 ± 3.37 at pH 5.0 to 5.90 ± 3.92 Mg/L at pH 7.0), Ammonia Concentration (ranged from 0.06 ± 0.0 at pH 5.0 to 0.06 ± 0.00 Mg/L at pH 9.0) and Biochemical Oxygen Demand Concentration (ranged from 2.00 ± 1.13 at pH 5.0 to 2.84 ± 1.13 Mg/L) were not significantly different ($P> 0.05$) among the fingerlings reared in all the pH treatments. However, the Electrical Conductivity (304.42 ± 47.20µs/cm) of the fingerlings cultured at pH 7.00 was significantly lower ($p<0.05$) than those fingerlings cultured at pH 5.0 to pH 9.00 ranged from 369. 44 ± 41.54 to 380. 94 ± 34. 80 µs/cm.

Table 1: Haematological parameters of *Heteroclarias* fingerlings exposed to different pH levels for a Period of 12 Weeks (Mean±SE) n=3.

pH Levels	Weeks 1st	6th	12th
	Packed Cell Volume(%)		
5	12.30±0.60[a]	13.35±0.75[a]	15.60±0.90[b]
7.00(Control)	12.90±0.30[a]	21.30±2.40[b]	13.95±0.45[b]
9	12.90±0.60[a]	19.05±0.75[b]	10.30±5.60[a]
11	-	-	-
	Total Erythrocyte Count($X10^{12}/1$)		
5	18.55±0.65[a]	12.20±0.90[a]	5.70±0.40[a]
7.00(Control)	18.25±0.15[a]	13.55±1.25[a]	7.15±0.95[a]
9	17.70±1.55[a]	15.35±1.55[b]	6.10±0.00[a]
11	-	-	-
	Total Leucocytes Count($X10^{9}/L$)		
5	1.30±0.00[a]	1.60±0.10[a]	1.65±0.05[a]
7.00(Control)	1.40±0.02[a]	2.40±0.30[a]	1.55±0.05[a]
9	1.40±0.10[a]	2.15±0.15[a]	1.80±0.00[a]
11	-	-	-
	Blood Glucose(mMol/L)		
5	3.20±0.10[a]	2.95±0.15[a]	2.80±0.10[a]
7.00(Control)	3.35±0.45[a]	3.50±0.10[a]	2.95±0.15[a]
9	3.10±0.10[a]	3.60±0.10[a]	3-00±0.00[a]
11	-	-	-
	Blood Protein(g/dl)		
5	5.80±0.20[a]	6.60±0.10[a]	5.15±0.45[a]
7.00(Control)	6.15±0.35[a]	6.55±0.35[a]	5.55±0.35[a]
9	6.00±0.10[a]	6.60±0.20[a]	4.60±0.00[a]
11	-	-	-

Values followed by the same superscript, in the same column, for each parameter are not significantly (P>0.05) different. *Not applicable, as 100% mortality was attained at day 1.

Table 2: Physicochemical Parameters Measured during Experiment on Influence of Different pH Levels on Heteroclarias Fingerlings (Mean±SE) n=3.

pH levels	Temperature (°C)	Dissolved Oxygen (mg/L)	Ammonia (mg/L)	Biochemical Oxygen Demand (mg/L)	Electrical Conductivity (µs/cm)
5.00	26.51±0.77[a]	4.64±3.73[a]	0.06±0.01[a]	2.00±1.13[a]	369.44±41.54[b]
7.00 (Control)	26.22±0.90[a]	5.90±3.92[a]	0.06±0.01[a]	2.14±1.47[a]	304.42±47.20[a]
9.00	26.21±1.00[a]	5.20±3.37[a]	0.06±0.01[a]	2.84±1.13[a]	380.94±38.40[b]
11.00	-*	-	-	-	-

Values are Mean ± Standard error, Values followed by the same superscript(s), in the same column, are not significantly different at (P >0.05) tested by DMRT. *Not applicable, as 100% mortality was attained at day one.

Discussion

The PCV values of the fingerlings cultured in all the treatments in week one were low, when compared with the normal range of 20.00 to 35.00% as documented by Ayandiran *et al.* (2010). This could be an indication of anaemia which is a condition characterized by a deficiency of haemoglobin, packed cell volume, and erythrocytes. Moreover, haematology studies in teleosts have indicated that haematocrit values might be useful as a general indicator of fish health, since fish given iron deficient diets, or those exhibiting anaemia, all possess reduced haematocrit (PCV) values (Gatlin and Wilson, 1986).The findings were also in agreement with the works of Osuigwe *et al.* (2005) who reported that fish with reduced PCV, haemoglobin concentration and total erythrocyte count exhibits anaemia. Similarly, the PCV values in all the pH treatments in week one including the control were in agreement with the works of El-Sheriff and El-Feckey (2009) who documented that the PCV of *O. niloticus* were not different from the control group and the experimental group. The significant increase in the packed cell volume (PCV) in the sixth week, especially in the fingerlings cultured in pH 7.00 and 9.00 treatments, indicated an increase in blood oxygen carrying capacity of the fingerlings (Jensen, 2003). This is possible through increasing oxygen affinity and capacity of the fingerlings to increase in TEC production (Das *etal.,* 2006). In addition, the PCV values of the

fishes cultured in pH 7.00 and 9.00 treatments were very close to the normal range of 20 – 35% (Ayandiran *et al.* 2010).This finding suggests that the fingerlings were in good health status as none of them suffered from anaemia during this period (Osuigwe *et al.*, 2005). Moreover; the reduction in the PCV of the fingerlings reared in pH 9.00 compared to pH 5.00 and control treatments at the twelveth week of the experiment indicated that the fingerlings red blood cells were being destroyed by leucocytosis activity in an erythrocytic anaemia with subsequent erythroblastosis (Haney *et al.* 1992).The TEC values of the fishes cultured in pH 5.00, 7.00 and 9.00 at the first and the twelveth week of the study were all in agreement with the reports of Haney *et al.*

(1992) who observed progressive reduction of the TEC and haemoglobin contents in both acidic and alkaline conditions. The significant increase ($p<0.05$) in the TEC of the fingerlings cultured in pH 9.00 among other treatments at week 6, is an indicative of high oxygen carrying capacity of the blood which is characteristic of fishes capable of aerial respiration and with high activity (Lenfant and Johasen ,1972). The finding contradicted the reports of Ghanbari *et al.* (2012) who documented a significant reduction in TEC at the two extremes pH 5.50 and 9.00 in the fingerlings of common *Cyprinus carpio L.* However, during the same period, the insignificant reduction in TEC of the fishes cultured in pH 5.00 and 7.00 were all in the agreement with the works of Das *et al.* (2006) who reported similar reduction in TEC after exposing three Indian carps to acidic and alkaline pH. The insignificant influence of pH on TLC, BG and BP may be attributed to pH, duration and species differences (Saad *et al.*, 2013). The insignificant influence of pH in the Total Leucocytes Counts (TLC) of fishes in all the pH treatments including control during the period of investigation were contrary to the reports of Ghanbari *et al.* (2012) who documented significant reduction in TLC of *Cyprinus carpio L.* fingerlings, particularly at higher water pH (acid and alkaline). However, the insignificant lower values of TLC in all the pH treatments and controlled fishes were in conformity with the works of Ghanbari *et al.* (2012) who also reported TLC reduction (lower values) in the *C. carpio L.* fingerlings. The insignificant influence of pH on blood glucose level of fishes cultured in all the treatments including control were contrary to the works of Wilkie *et al.* (1993) who observed greater elevation in blood glucose in *C. carpio* fingerlings particularly at higher water pH (acid and alkaline).The insignificant influence of pH on blood protein (BP) of the fishes in all the pH treatments including the control. These observations were contrary to the works of Ghanbari *et al.* (2012) who reported that serum protein levels were significantly reduced when *C. carpio* fingerlings were exposed to pH 5.50, 8.00 and 8.50.

The results of physiochemical parameters except electrical conductivity were not influenced by water pH. Most of the parameters were within the range approved for culturing fresh water fishes in the tropics. Water temperatures of 26.21 ± 1.00 to 26.51 ± 0.77^0c were within the range of 25.00-32.00^{0}c acceptable for good fish growth (Ayanwale *et al.*, 2014). Dissolved oxygen concentration of the water media of Heteroclarias fingerlings; (4.63 ± 3.37 to 5.90 ± 3.92mg/L) were also within the recommended value of 5.00mg/L required for healthy growth, tissue repairs and reproduction of warm water fish as reported by Svobodova *et al.* (1993). The Ammonia concentration of 0.06 ± 0.00 to 0.06 ± 0.00mg/L were within the range 0.01 to 1.55mg/L for freshwater fingerlings as documented by Kohinoor *et al.* (1994). Similarly, the Biochemical oxygen demand concentration of 2.00 ± 1.13 to 2.84 ± 1.13. Mg/L recorded in this study was also within the acceptable range of 1.0 to 5.00mg/L recommended for fish growth in the tropics (CIESE, 2010). These results suggest no organic pollution from left over feed or faecal matter in the rearing media of Heteroclarias fingerlings throughout the experimental period which in turn increased the Dissolved oxygen concentration. These findings might be attributed to constant changing of water in all the experimental tanks (Ayoola and Fredrick, 2012). The lowest value of electrical conductivity of *Heteroclarias* fingerlings cultured at pH 7.00 was normal since the medium was devoid of acid and base (Ivoke *et al.*, 2007). Although, the water conductivity ($304.42 \pm 47.20\ \mu s/cm$) of the fingerlings cultured at pH 7.00 was within the range of 120 to $340 \mu s/cm$ recommended for fish growth by Kolo (1996).However, the water conductivities of the fingerlings cultured at pH 5.0 and 9.0 were higher than the recommended range of 120 to $340 \mu s/cm$ as documented for fish growth by Kolo (1996).

Conclusions

Most of the physicochemical and haematological parameters studied were not significantly influenced by water pH indicating no physiological stress. However, it is recommended that farmers routinely evaluation of haematological parameters of Heteroclarias fingerlings so as to provide essential information on their physiological status.

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