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Effects of pH Variations on Growth and Haematological Parameters of *Pangasianodon hypophthalmus* (Sauvage, 1878)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

An experiment was carried out with an aim of enhancing culture of *Pangasianodon hypophthalmus*. Juveniles with an average length of 13.35±0.14 cm and 17.52±0.16 g of body weight were subjected to various pH levels of 7.5 (Control), 6, 9 and 9.5 to determine their growth and hematological changes. The control group exhibited the highest growth in both length (14.70±1.04 cm) and weight (21.12±2.67 g), while the lowest length (13.87 ± 0.43 cm) and weight (18.92 ± 1.11 g) in pH 9.5. The highest (1.24 ± 0.15) and lowest (0.56 ± 0.15) value of SGR was noticed in control and pH 9.5 respectively. The highest value of PER (3.79 ± 0.38) was observed in control and the lowest (1.66 ± 0.31) in pH 9.5. The highest (2.59 ± 0.47) and lowest (1.11 ± 0.16) value of FCR observed in pH 9.5 and control respectively. The highest count (26.80 ± 1.01 (10^3/µl)) of TLC was observed in pH 9.5 and the least (22.26 ± 0.53 (10^3/µl)) in control. The highest count (2.47 ± 0.15 (10^6/µl)) of TEC was observed in control and the least (1.97 ± 0.23 (10^6/µl)) in pH 9.5. The highest value (9.42 ± 0.14 g/dL) of Hb was observed in control and the least (9.5 with 5.77 ± 0.56 g/dL) in pH 9.5. The highest value (29.06 ± 0.46%) of Ht was observed in control and the least (22.41 ± 2.11%) in pH 9.5. The highest value (124.93 ± 9.99 fL) of MCV was observed in pH 9 and the lowest (114.45 ± 11.98 fL) in pH 9.5. The highest value (32.42 ± 0.76 g/dL) of MCHC was observed in control and the lowest (25.73 ± 0.87 g/dL) in the pH 9.5. The study revealed that *P.hypophthalmus* juveniles showed significant difference (p < 0.05) between the control and treatments. Study inferred that alterations in the growth and haematological parameters were occurred at higher pH levels compared to lower pH levels.

Keywords: Growth; haematology; Pangasianodon hypophthalmus; pH.

1. INTRODUCTION

Striped catfish, *Pangasianodon hypophthalmus* farming is one of the flourishing types of aquaculture in the world. The world aquaculture production in 2018 is 114.5 million tonnes in which *P. hypopthalmus* alone contributed about 4.3% with 2359.5 thousand tonnes of total production [1] and it increased to 5.1% with 2520.4 thousand tonnes of production in 2020 [2]. This striped catfish is known to culture in diverse environmental circumstances and is generally considered as very hardy in nature. In spite of these, certain water quality parameters play a major role in survival, growth and physiology of the fishes. A number of physiological changes like activation of osmoregulatory activities, disturbances in body fluid and alteration of energy metabolites were observed when *Acipenser naccarii* were stocked in full seawater (35 ppt) [3]. Excessive accumulation of ammonia in fish causes significant reduction and growth and survival of yellow catfish, *Pelteobagrus fulvidraco* [4]. In view of the current and future climate variables, more coastal areas are going to become vulnerable to brackish water inundation, where fluctuations in the physico-chemical parameters of water will likely to occur frequently. In particular, the alteration in water pH causes disturbances in acid-base, ion regulation, ammonia excretion and state of homeostasis in fish [5].

Many studies have been focused on the acid and alkaline pH limits at which fish growth and reproduce rapidly since they usually have narrow tolerance range. Research on the effects of acidic and alkaline water pH (5.5, 6.0, 7.0, 8.0 and 8.5) on the growth and survival of silver catfish, *Rhamdia quelen* larvae indicated that optimal pH range for survival and growth was between 8.0–8.5 [6]. The hybrid juveniles of *Heterobranchus bidorsalis* (♂) X *Clarias Gariepinus* (♀) exhibited reduced growth at pH 6 and 8, while higher growth rates were observed at 7 and 7.5, inferring that the optimum pH range for rearing hybrid catfish juveniles is between 7.0 – 7.5 [7]. A significant (P≤0.05) reduction in growth of Nile tilapia (*Oreochromis niloticus*) fingerlings was observed at pH 6 and 9 while no significant (P≥0.05) difference was noted at pH levels 7 and 8 when fingerlings were maintained at pH levels of 6, 7, 8 and 9 [8].It has been observed that the acid and alkaline lethal points for fish are about pH 4 and 11 respectively [9] and growth is mostly affected at pH below 6.0 or above 9.0 [10]. However, the preferred range of pH for better growth of fish is about 6.5–9.0 [11]. Certain fishes like rainbow trout can be able to live in high alkaline condition by reimbursing physiological adjustments to maintain the respiratory homeostasis. This physiological adjustment can be done through influx of ion mediated by branchial chloride cells [12,13]. Further long term exposure of fish to acidic or high alkaline pH causes stress in fish and in turn causes changes in blood components [14].

Haematological parameters are generally considered as indicator in determining the well being of the fish as well as to assess the alteration in circulation system when it is exposed to polluted or toxic environment [15,16]. Studies on lead (Pb) toxicity showed a negative effect on growth performance, erythrocyte morphology on *Oreochromis niloticus* [17]. When Clarias batrachus was exposed to Acute toxicity of mercury, showed a siginificant ($p < 0.05$) differences on haematological indices like TEC, hemoglobin and hematocrit values [18].

Besides, it helps in understanding the correlation of blood characteristics to the phylogeny, activity, habitat and adaptability of fishes to the environment [19,20,21]. Various studies have been carried out to determine the pH tolerance limits of various fish species. Studies on long term effects water pH changes on certain hematological parameters of common carp (*Cyprinus carpio*) fingerlings showed that exposure to pH levels of 8.5 - 9.0 resulted in swollen erythrocytes with centrally located swollen nucleus and along with the presence of immature erythrocytes [14]. A study demonstrated that a changes in water pH whether in acidic or alkaline conditions, exerted stress in Indian Major Carps (IMC) leading to swelling of erythrocytes, production of immature erythrocytes, reduction in the TEC, haemoglobin and serum protein content. Additionally, the study found an increase in total leukocyte count and blood glucose levels [22]. In this context the study was aimed to find out the haematological changes of fish *P. hypopthalmus* when exposed to different acidic and alkaline pH.

2. MATERIALS AND METHODS

The study was conducted from December, 2019 to January, 2020 for about 30 days. Juveniles of *P.hypophthalmus*, having length and weight of 13.35±0.14 cm and 17.52±0.16 g respectively were collected from the commercial nursery, Narukur, SPSR Nellore District, Andhra Pradesh and brought to the wet laboratory of Department of Fisheries Resource Management, College of Fishery Science, Muthukur. The fish were acclimatized to laboratory conditions for one week in fresh tap water in 500 liters collapsible tarpaulin tanks and fed with floating pellet feed having 24% protein. Faecal matter and unutilized feed materials were siphoned out every day and 25-30% of water was replenished on every alternative day.

Prior to the experiment the fish were subjected to different acidic (5.5, 6.0, 6.5) and alkaline waters (8.5, 9.0, 9.5, 10.0) in triplicates to determine the LC_{50}. For conducting lethal test, 10 numbers of fish having size of 15 to 17 gm were randomly selected and stocked in each collapsible circular tank having 250 lit capacity with different acidic and alkaline pH. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were used to prepare different alkaline and acidic waters for the experiment respectively. The pH was measured by using digital pH meter (eco Tester pH1). The lethal study was conducted for 96 h exposure with an observation for every 6 h interval. Based on LC_{50} values obtained, the pH range below the lethal levels i.e, 6.0, 9.0 and 9.5 were chosen to study the effect of pH on growth and haemtological parameters of *P. hypophthalmus.*

The experimental tanks were cleaned and filled up to a depth of 50 cm with acidic (pH 6.0) and alkaline waters (pH 9.0 and 9.5) and keeping fresh water as control (pH 7.5). The acclimatized fish (length 13.35±0.14 cm; weight of 17.52±0.16 g) in 10 numbers were randomly selected and stocked in each experimental tank and fed with commercial pellet feed twice a day. During the experiment, water quality parameters of experimental units viz., Temperature, DO, pH, Ammonia, Total Alkalinity, Total hardness and salinity were measured every day using standard methods [23]. Similarly, blood samples were collected on weekly basis in the morning hours to evade diurnal variations in the blood components. The blood was collected using 1 ml sterile syringe rinsed with anticoagulant (Heparin) fitted with 26G needle from the caudal peduncle of the fish. Blood was immediately flushed into the K2 EDTA coated blood collection vials of 2 ml capacity for haematological analysis. To calculate the growth performance, the parameters like Weight Gain (WG), Specific Growth Rate (SGR), Protein Efficiency Ratio (PER), and Feed Conversion Ratio (FCR) were estimated by using the following standard formulae [24,25]. Similarly, for cytological study, Total Erythrocyte Count [26], Total Leukocyte Count [26], Haemoglobin [22], Total Haematocrit [27], Mean Corpuscular Volume [28] and Mean Corpuscular Haemoglobin Concentration [28] were analysed every week.

2.1 Statistical Analysis

Statistical tool of Two-way Analysis of Variance (Univariate) was used to assess the effect of interaction between different pH levels and their respective time intervals on all the haematological parameters. Statistical analysis were performed using the SPSS (16.0) Statistical software.

3. RESULTS AND DISCUSSION

Decreased growth rate was observed in acidic pH 6 as well as alkaline pH 9 and 9.5 when compared to control (7.5) (Fig. 1). The highest value of length and weight observed were 14.70±1.04 cm and 21.12±2.67 g in control respectively. Similarly, the lowest length and weight observed were 13.87 ± 0.43cm and 18.92 ± 1.11g in 9.5 respectively. The growth in terms of length differed significantly ($p < 0.05$) between treatments (6, 9.0 and 9.5) and among weeks with control (7.5). However, in the case of weight, no significant increment could be found between pH 6 and control (pH 7.5) while it varied significantly in pH 9 and 9.5 and among weeks. Likewise, reductions in growth rate were recorded in *Clarias gariepinus* when exposed to pH 5.0, 8.0 and 9.0 [29]. The reduction in growth of fish might be due to the demand for energy to detoxicate the ammonia content in the body that increased due to raise in pH, which in turn drops the energy spent for growth [30].

Both SGR and PER were in declining trend in pH 6 to 9.5 when compared to control (Fig. 2). The study reported the highest value of SGR (1.24 ± 0.15) and PER (3.79 ± 0.38) in control and the lowest SGR (0.56 ± 0.15) and PER (1.66 ± 0.31) in pH 9.5. Both SGR and PER varied significantly ($p < 0.05$) between treatments (pH 6, 9 and 9.5) and weeks with control. The findings of present study were similar to significant ($p<.05$) reduction in SGR and PER of *Labeo rohita* when exposed to stress environment [31]. The reduction in SGR and PER rates in experimental fish might be due decrease in food intake [32] and approximately 10-50% of their available energy would be directed to regulate their homeostatic balance [33].

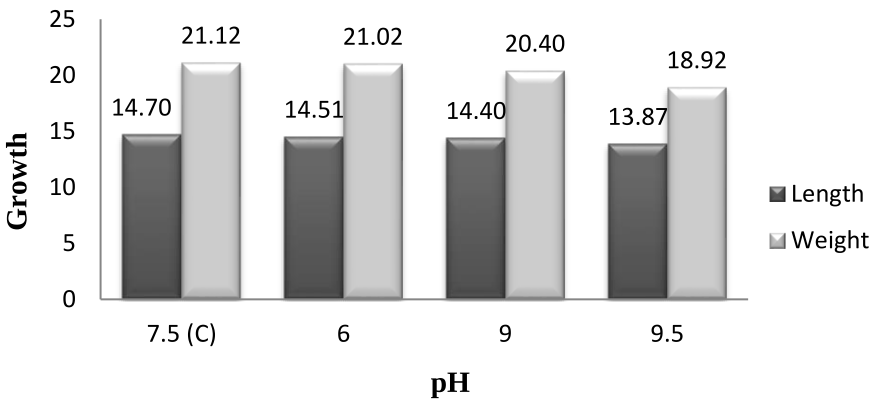

Fig. 1. Mean of Growth (Length and Weight) of *P. hypophthalmus* juveniles at different pH

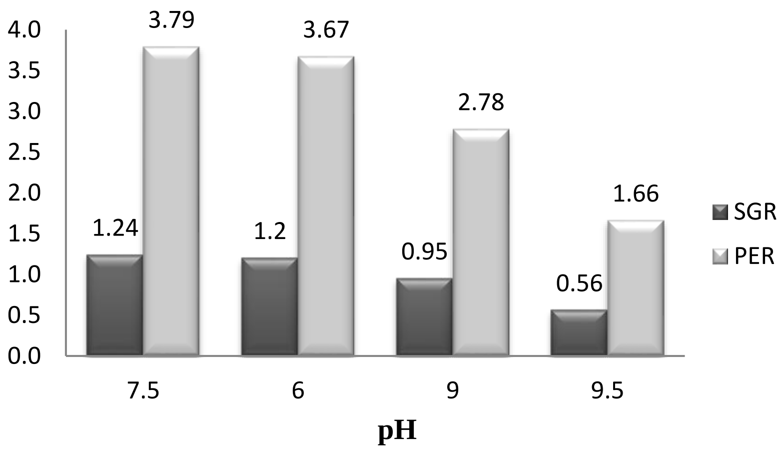

Fig. 2. Mean Specific Growth Rate (SGR) and Protein Efficiency Ratio (PER) of *P. hypophthalmus* juveniles at different pH

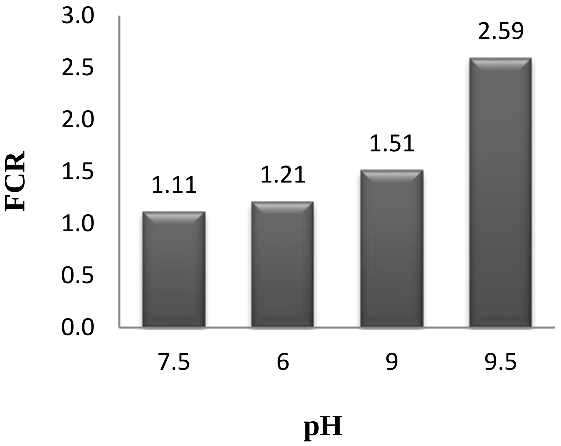

Fig. 3. Mean variation of Feed Conversion Ratio of *P. hypophthalmus* juveniles at different pH

Table 1. Mean variation of Haematological parameters of *P. hypophthalmus* juveniles exposed to different pH levels in different week periods

pH / Parameters	7.5 (C)	6.0	9.0	9.5
TLC (10^3/µl)	$22.26^a \pm 0.53$	$22.81^b \pm 0.24$	$24.75^c \pm 1.00$	$26.80^d \pm 1.01$
TEC (10^6/µl)	$2.47^b \pm 0.15$	$2.39^b \pm 0.14$	$2.09^a \pm 0.22$	$1.97^a \pm 0.23$
Haemoglobin (g/dL)	$9.42^a \pm 0.14$	$9.14^b \pm 0.22$	$7.40^c \pm 0.56$	$5.77^d \pm 0.56$
Haematocrit (%)	$29.06^d \pm 0.46$	$28.60^c \pm 0.66$	$25.97^b \pm 1.75$	$22.41^a \pm 2.11$
MCV (fL)	$117.98^a \pm 8.04$	$120.13^a \pm 8.75$	$124.93^b \pm 9.99$	$114.45^a \pm 11.98$
MCHC (g/dL)	$32.42^c \pm 0.76$	$31.95^c \pm 0.94$	$28.51^b \pm 1.25$	$25.73^a \pm 0.87$

FCR was increased in*P. hypophthalmus* from pH 6 to 9.5 (Fig. 3). The highest and lowest value of FCR observed were 2.59 ± 0.47 in pH 9.5 and 1.11 ± 0.16 in control respectively. However, there was no significant ($p > 0.05$) difference in FCR between pH 6 and control, while significant difference ($p < 0.05$) could be observed between alkaline pH 9 and 9.5 with control as well as between weeks. The observations were similar to the results of tilapia when exposed to increased concentration of unionized ammonia [8]. It is thus opined that raise in FCR in the pH 9.5 in the present study might be due to diverted energy away from growth to other activities, in particular to deal with ion regulation [34].

Haematological characteristics generally act as an effective tool in monitoring the physiological and health status in fishes. The mean haematological parameters of *P. hypophthalmus* when they exposed to acidic and alkaline pH are given in Table 1. In the present study, the TLC increased with pH and the mean value of TLC (10^3/µl) was high in pH 9.5 (26.80 ± 1.01) while low in Control (22.26 ± 0.53) (Fig. 4A). TLCs results showed statistical significance ($p < 0.05$) between treatments of 6, 9 and 9.5 and also between weeks with control. It indicates that the fish will be susceptible to stress when exposed to any abnormal environment. The likewise increase of leukocyte count was recorded in tilapia when exposed to various ammonia levels [35]. Besides, the increase in the count of TLC might also be due to release of cells, accumulated in the spleen to combat the stressors [36] or stimulation of leucopoietic process might have enhanced the release of leucocytes in blood [22].

In contrary, TECs of the striped catfish decreased with high alkaline and acidic condition. The highest count of TEC observed was 2.47 ± 0.15 (10^6/µl) in control and the lowest was 1.97 ± 0.23 (10^6/µl) in pH 9.5 (Fig. 4B). TECs did not differ significantly ($p > 0.05$) between pH of 7.5 and 6 and between pH 9 and 9.5. However, pH 9 and 9.5 varied significantly ($p < 0.05$) when compared to control. Similar decrease of TEC in common carp, *Cyprinus carpio* was noted when exposed to ammonia [37]. The reduction in TEC might be due to decrease in haemopoietic activity of the kidney [38] or erythrocytic cell lysis when the fish are exposed to acidic and alkaline pH [22].

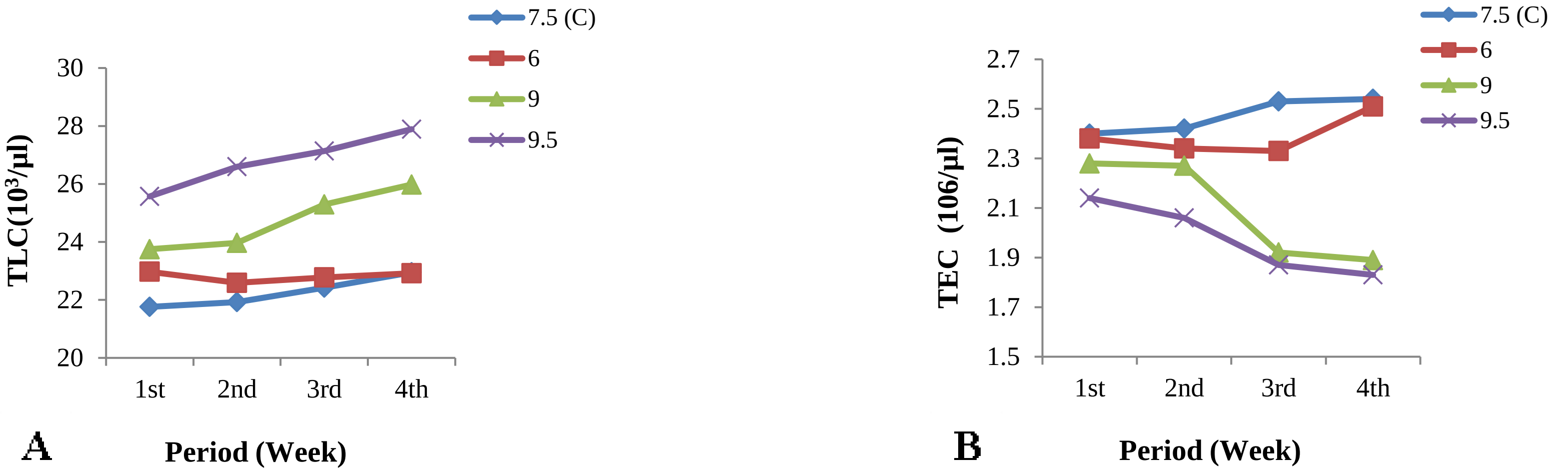

Fig. 4. Weekly analysis of blood composition of *P. hypophthalmus* subjected to different pH; (A) Total Leukocyte Count (TLC), B) Total Erythrocyte Count (TEC)

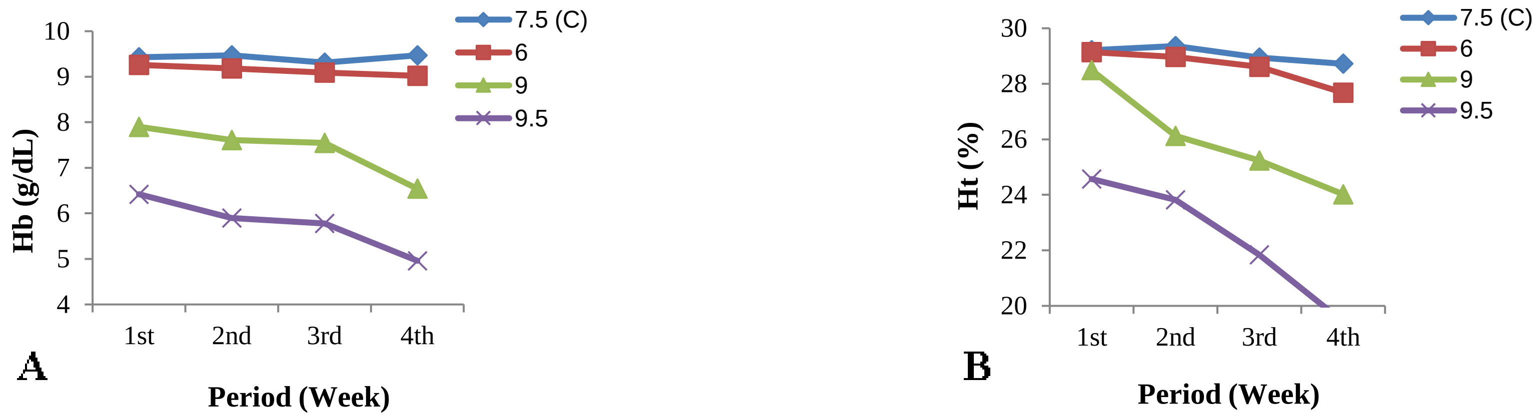

Fig. 5. Weekly analysis of blood composition of *P. hypophthalmus* subjected to different pH; A) Total Haemoglobin (Hb), B) Total Haematocrit (Ht)

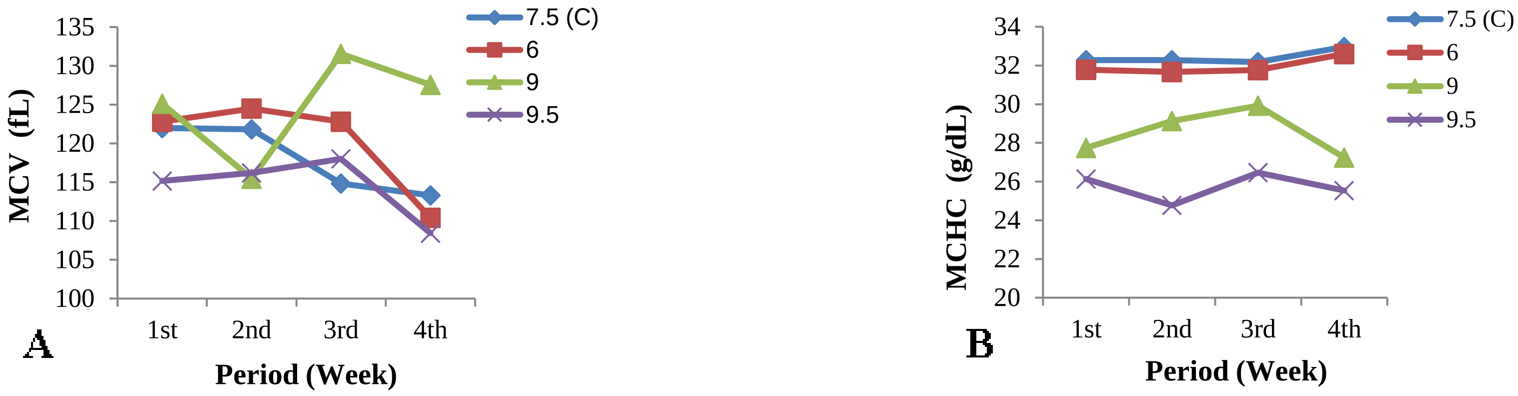

Fig. 6. Weekly analysis of blood composition of *P. hypophthalmus* subjected to different pH; A) Mean Corpuscular Volume (MCV), B) Mean Corpuscular Haemogolobin Concentration (MCHC)

Decreasing trend of haemoglobin (Hb) and haematocrit (Ht) was observed in treatments from acidic pH (6.0) to alkaline pH (9 and 9.5) (Fig. 5A&B). The highest haemoglobin (9.42±0.14 g/dL) and haematocrit (29.06% ± 0.46) was observed in control while the lowest haemoglobin (5.77±0.56 g/dL) and haematocrit (22.41% ± 2.11) was observed in pH 9.5. The Significant difference ($p < 0.05$) in Hb levels and Ht values was observed between treatments of pH 6, 9 and 9.5 with control (7.5). The progressive reduction in haemoglobin could be due to respiratory stress [14] or the increase of NH_3 in the blood circulation which might have ruptured high percent of RBCs and/or Haemodilution resulting from the impaired osmoregulation across the gill epithelium [39]. The decrease in haematocrit could be due to shrinkage or decrease of erythrocytes production in the hematopoietic tissue [40].

Undulated fashion of MCV was observed among treatments (Fig. 6A). The highest value of MCV observed was 124.93± 9.99 fL in pH 9 while the lowest was 114.45±11.98 fL in pH 9.5. MCV results showed no significant difference ($p > 0.05$) between treatments of 6 and 9.5; except pH 9 where significant difference ($p < 0.05$) was observed when compared with control. The increase in MCV in the experimental fish of pH 9 might be due to the swelling of red blood cells or the release of large red blood cells into the circulation by which blood O_2 transport capacity can be increased to combat stress [41]. On other hand, gradual decrease in pH 9.5 might be due to high percentage of immature red blood cells in the circulation [42]. In the case of MCHC, declining trend was observed from acidic pH to alkaline pH (Fig. 6B). The high and low MCHC observed was in control (32.42 ± 0.76 g/dL) and pH 9.5 (25.73 ± 0.87 g/dL) respectively. Experiment results of MCHC showed that no significant difference ($p > 0.05$) could be observed between pH 6 and control. However significant ($p < 0.05$) difference in MCHC values could be observed between the treatments of 9 and 9.5 when compared to control. The decrease in MCHC might be attributed to the hemodilution and/or the lack of production of hemoglobin in circulation [35].

4. CONCLUSION

To summarise, the present observations revealed that the raise in pH reduces the growth, SGR and PER while increases the feed conversion ratio since a lot of energy is diverted for ion regulation in the fish that leads to imbalanced homeostatis. Enhanced stimulation of leucopoietic process causes the increased count of TLC with increasing pH to combat the stress. TEC, Hb, Ht decreased with increasing pH due to erythropoietic dysfunction. The alterations in MCV and MCHC could be due to reduction in Ht and Hb concentrations respectively. Further, the results conclude that *P. hypophthalmus* can survive upto pH 9.5 despite feed intake was low by the fish which ultimately lead to poor growth. It is thus, inferred that pH 7.5 to 9.0 would be ideal pH for obtaining good growth of *P. hypophthalmus*. Hence it can be concluded that this fish had good survival, growth and health in alkaline pH levels upto 9 ppt and helps for farmers community to expand the culture of *P. hypophthalmus* and it may also be helpful to understand the ecological adaptations and evolutionary adaptations among fish species.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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ETHICAL APPROVAL

No animals were sacrificed in this study and animals are were handled with utmost care.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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