African Journal of Biotechnology Vol. 11(13), pp. 3153-3159, 14 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1244
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Long-term effects of water pH changes on hematological parameters in the common carp (*Cyprinus carpio* L.)

Mahdi Ghanbari[1,2]*, Mansoureh Jami[1,2], Konrad J. Domig[2] and Wolfgang Kneifel[2]

[1]Department of Fishery, Hamun International Wetland Research Institute, University of Zabol, Zabol, Iran.
[2]BOKU — University of Natural Resources and Life Sciences, Department of Food Sciences and Technology, Institute of Food Sciences; Muthgasse 18, A-1190 Vienna, Austria.

Accepted 27 September, 2010

The aim of this study was to examine the effects of water pH changes on certain hematological parameters of fingerlings of common carp (*Cyprinus carpio*), in water with different pH (acidic and alkaline). Fingerlings of common carp were subjected to acidic (pH 5.5 and 6.5) and alkaline (pH 8.0, 8.5 and 9.0) water for 21 days. Control groups were maintained at neutral pH. The result showed that exposure to both acidic and alkaline water exerted stress on fish and considerably affected the hematology of fingerlings of common carp. Cytological study revealed that such swollen erythrocytes with centrally located swollen nucleus and also immature erythrocytes were observed in fingerlings exposed to pH 8.5 - 9.0. Exposure to the two extreme pHs (pH 5.5 and 9.0) caused significant reductions ($P < 0.05$) in total erythrocyte count (TEC) in common carp. A significant reduction ($P < 0.05$) in the hemoglobin content was observed at the two extreme pHs 5.5 and 9.0. The total leukocyte count (TLC) was reduced at pH 5.5, but only after a brief increase at pH 6.5. Alkaline range also experienced a reduction in TLCs at pH 8.0 and underwent further reduction with increased pH. Blood glucose was significantly higher ($P < 0.05$) in fingerlings exposed to both acidic and alkaline waters and the increase was proportional to the degree of pH change. Serum protein levels were also significantly reduced ($P < 0.05$) following exposure of the fingerlings to both acidic and alkaline conditions. The changes in hematological parameters of the fingerlings of common carp indicated that the change in water pH might have caused the ion regulatory and acid–base disturbances originating at the gill leading to the altered internal pH, electrolyte and osmotic balances that imply an increase in energy consumption to restore homeostasis instead of other physiological functions, weight gain and growth.

Key words: Hematology, pH, stress, *Cyprinus carpio*.

INTRODUCTION

Water pH plays an important role in maintenance of the homeostasis in aquatic animals and increases or decreases in pH are reported to cause disturbances in acid-base and ion regulation and ammonia excretion (Wood et al., 1989; Wilkie and Wood, 1991; Wilkie et al., 1993; Jensen and Brahm, 1995; Das et al., 2006). The major nitrogenous waste metabolite is ammonia (T_{amm}, the sum of NH_3 and NH_4^+), which mainly exists in teleost tissues and blood as NH_4^+ but crosses the branchial epithelium as NH_3, down favourable blood-to-water diffusion gradient (Smutna et al., 2002; Wilkie, 2002). Toxicity of ammonia to fish has been intensively investigated in numerous fish species. It was demonstrated that the toxicity of ammonia depends principally on the presence of NH_3 and concentration of NH_3 was directly dependent on the change in water pH and reversibly

*Corresponding author. E-mail: ghanbari.msc@gmail.com. Tel: +98-542-2232961-2. Fax: +98 - 542-2226765.

Abbreviations: Hb, Hemoglobin; **TEC,** total erythrocyte count; **TLC,** total leukocyte count; **RBC,** red blood cell counts.

Table 1. Quality parameters of water at the start of experiments.

Parameter	Range
pH	7.3 – 7.4
Temprature	30 – 32°C
Total alkanity	118 – 132 mg $CaCO_3$ l^{-1}
Total hardness	122 – 136 mg CaCO3 l^{-1}
DO	5.6 – 6.3 mgl^{-1}
Chloride	0.4 – 10.8 mgl^{-1}
Total ammonia-nitrogen	0.02 mgl^{-1}
Nitrite-nitrogen	<0.01 mgl^{-1}

affects the rate of T_{amm} excretion in fish (Evans et al., 2005; Lemarié et al., 2004). 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 noted by Claiborne et al. (2002) 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 effects of long-term exposure to high and low water pH on ion balance and ammonia excretion in freshwater fish is mainly limited to salmonids such as the rainbow trout (*Oncorhynchus mykiss*). In this fish, alkaline water (pH 9.5) causes increases in blood pH and higher plasma ammonia concentrations, which can be potentially lethal (Wilkie and Wood, 1991; Yesaki and Iwama, 1992; Wilkie et al., 1996; Wilson et al., 1998; Laurent et al., 2000), and also reduces plasma electrolytes such as Na^+ and Cl^-, brief increases, followed by recovery of plasma glucose concentrations and a reduction in the blood protein and hemoglobin concentration (Wood et al., 1989; Wilkie and Wood, 1991; Wilkie et al., 1993; Wilkie and Wood, 1996; Laurent et al., 2000). The effect of low environmental pH on fish includes increased branchial loss of ions, reductions in plasma pH, bicarbonate, Na^+ and Cl^- concentrations, slowing of apical H^+/Na^+ exchanger process (Ultsch et al., 1981), increased T_{amm} and increased urinary excretion of surplus H^+ ions for compensation of the net base loss at branchial epithelium (McDonald and Wood, 1981). Although numerous studies have investigated fish behavior in relation to a wide variety of environmental factors, information on the effect that changes in water pH have on fish physiology is sparse (Scott et al., 2003) and little is known about the physiological responses of non-salmonid fish to water pH changes (acidic and alkaline).

The aim of this study is to investigate the potential impacts of exposure to high and low pH on the physiology of common carp, *Cyprinus carpio*. The optimum pH range for culture of common carp is 7.5 – 8.5 (Banerjea, 1967) and the majority of the Iranian carp culture ponds have a pH in this range. The carp culture industry is currently the most important sub-sector of fisheries in Iran and its rapid development has attracted considerable attention in recent years. In situations 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). In addition, the hematological variables (size, shape and population of blood cells, concentrations of hemoglobin (Hb), blood sugar and serum protein) were measured since they are frequently used 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 changed water pH.

MATERIALS AND METHODS

Fish

Fingerlings of common carp, *C. carpio* (average weight = 10.3 ± 2.9 g) collected from the farm of the Central Institute of Freshwater Aquaculture, Zahak, Iran were used for the experiments. The fish were transported to the laboratory in plastic bags (5 L) filled with oxygenated water and acclimatized in a stock tank (capacity 400 L) to laboratory conditions for 2 weeks under normal conditions (23 ± 2°C). They were fed *ad libitum* with commercial fish feed throughout the period of study and water was changed once a day.

Experimental design

Tap water from the same overhead tank was used in all experiments. The water was stored in a continuously aerated storage tank in the laboratory for 3 days prior to its use for each experiment. Water quality parameters of the stored water were measured at the beginning of each experiment, following the standard methods (APHA, AWWA, WPCF, 1989) (Table 1). Stock solution of sodium bicarbonate (Na_2CO_3) and glacial acetic acid with sodium acetate (4%) were used to create the different alkaline and acidic conditions, respectively, in water of the experimental tanks (Das et al., 2006). The pH was measured with the help of a digital pH meter (Elico, L1-120). Exchange of water from the experimental tank was on a daily basis to remove the excreta and uneaten feed, and maintain the pH.

Prior to water exchange, required stock solution of acid and alkali were added to stored tap water kept in separate containers to create the desired pH of 5.5, 6.5, 8.0, 8.5 and 9.0. These waters with different pH were used to exchange about 90% water in the corresponding experimental tank with similar pH. Water exchange was given every day during morning hours (9.0 – 10.0 h) and was followed by final adjustment of pH with addition of stock solution of acid or alkali to the tank. The exchange was given slowly to avoid any stress to fishes. The tanks were continuously aerated all through the experiments.

Fingerlings of common carp in a prior experiment were exposed to pH 4.0, 5.0, 5.5, 6.0, 6.5, 7.5, 8.5, 9.0 and 9.5 in triplicate tanks to determine the lethal acidic and alkaline ranges. None of the fingerlings survived at pH 5.0 and 9.5, which led us to select the range of 5.5 – 9.0 for study of the hematological response. Three experiments, for common carp were conducted under similar experimental set up in 60 L glass jars for a period of 21 days each. Since majority of Iranian ponds have water pH falling in this alkaline range, three treatments were maintained at pH of 8.0, 8.5 and 9.0, while other two were maintained at pH 5.5 and 6.5. Another treatment with pH 7.4 (tap water) served as control. Three replications were used for each treatment. Ten fingerlings of common carp were put in each replication tanks.

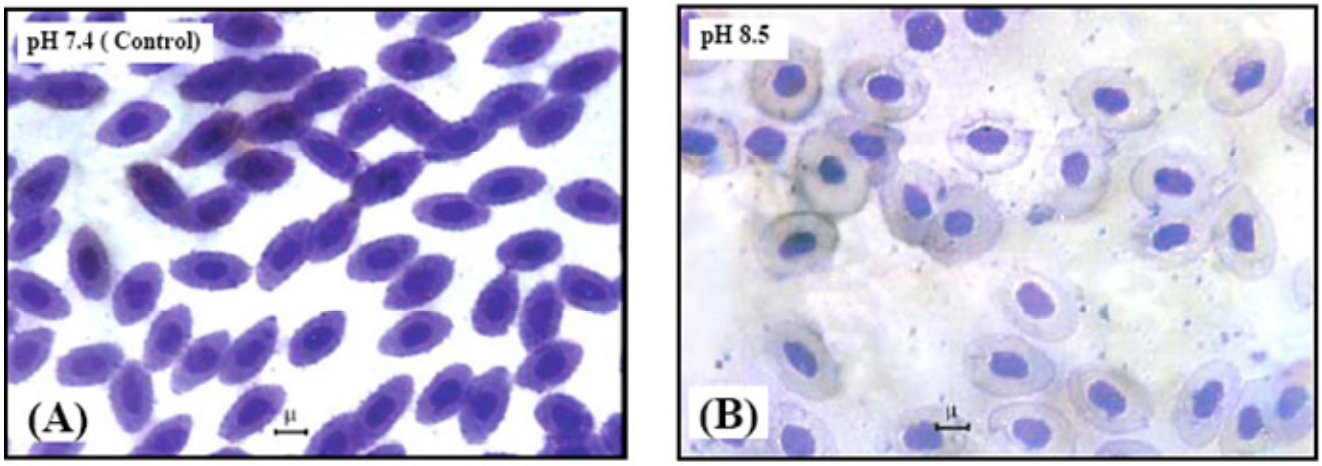

Figure 1. Blood smear of fingerlings exposed to different water pH. (A) pH 7.4 (control), (B) pH 8.5. Some erythrocytes are swollen and some are with distorted/ruptured plasma membrane.

Collection of blood sample

After 21 days of exposure to different pH, the sample blood was taken from one fingerling of each replication tank for measurement of hematological parameters. The samples for cytological study, total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin and blood glucose were collected through piercing in the ventro-lateral side nearer to the pelvic region with a 2 ml anticoagulant (Sequestrene: 10 g EDTA in 100 ml distilled water) rinsed disposable syringe and immediately transferred to a microcentrifuge tube.

Hematology indices

The red blood cell counts (RBC: 106 mm^{-3}) were determined in a 1:20 dilution of the blood sample in Hayem's solution and the white blood cell counts (WBC: 104 mm^{-3}) from a 1:200 dilution of the blood sample in Turke's solution with a Neubauer hemocytometer. The average of triplicate microhematocrits were used to determine the red blood cell volume at 10,000 rpm for 5 min (PCV: %) (Larsen and Snieszko, 1961). Hemoglobin (Hb: g/dl) was determined by the cyanhemoglobin method. A 20 µl blood sample was drawn from a heparinized capillary tube and mixed in 5.0 ml of cyanhemoglobin reagent (Hycel). Hemoglobin concentrations were determined at 540 nm with a Beckman DU spectrophotometer (Yokoyama, 1960; Larsen and Snieszko, 1961; Larsen, 1964; Hesser, 1960; Houston, 1990). TLC were done following the methods of Shaw (1930).

For serum protein estimation, blood was also drawn in anticoagulant free syringe and transferred to microcentrifuge tube, and kept at room temperature for 30 – 40 min for clotting. Thin uniform blood smears were prepared for each sample and stained with Wright-Giemsa (Hesser, 1960). These stained slides were observed under a research microscope (Zeiss, Axiophot, West Germany) for cytological study and relevant photomicrographs were made using a computerized photo-micrographic system. Serum protein and blood glucose content were measured spectrophotometrically following the diagnostic protocol of Boerhinger Manheim GmbH (Mannheim, Germany).

Statistical analysis

PC-SAS programme for Windows, release v6.12 (SAS Institute Inc., Cary, USA) was used for statistical analysis of the data at a significance level of 0.05. Analysis of variance was performed with the parametric procedure of General Linear Model. Duncan's multiple range test for variables was used for comparison of the treatments.

RESULTS AND DISCUSSION

Erratic swimming movement was observed in fingerlings when exposed to pH 5.5, 6.5 and 9.0. The photomicrograph of the blood smear of common carp kept in control (water pH 7.4) is presented in Figure 1. Cytological study revealed that such swollen erythrocytes with centrally located swollen nucleus were observed in fingerlings exposed to pH 8.5 – 9.0 (Figure 1B).

Some of these erythrocytes had distorted plasma membranes, which had ruptured in few of them. Immature erythrocytes, few macrophages, neutrophils and lymphocytes were also seen in fingerlings exposed to pH 8.5 to 9.0. Exposure to both acidic and alkaline water reduced the TECs of fingerlings of common carp in proportion with the change in water pH (Figure 2A). Exposure to the two extreme pHs (pHs 5.5 and 9.0) caused significant reductions ($P < 0.05$) in TEC in common carp. At pH 9.0, the maximum reductions in TECs were 22%.

A significant reduction ($P < 0.05$) in the hemoglobin content was observed at the two extreme pH 5.5 and 9.0 in which, hemoglobin content over that of respective controls were 23% lower at pH 5.5 and 24% lower at pH 9.0 in common carp (Figure 2B). The TLC were reduced at pH 5.5, but only after a brief increase at pH 6.5. Alkaline range also experienced a reduction in TLCs at pH 8.0 and underwent further reduction with increased pH.

Blood glucose was significantly higher ($P < 0.05$) in fingerlings exposed to both acidic and alkaline waters and the increase was proportional to the degree of pH

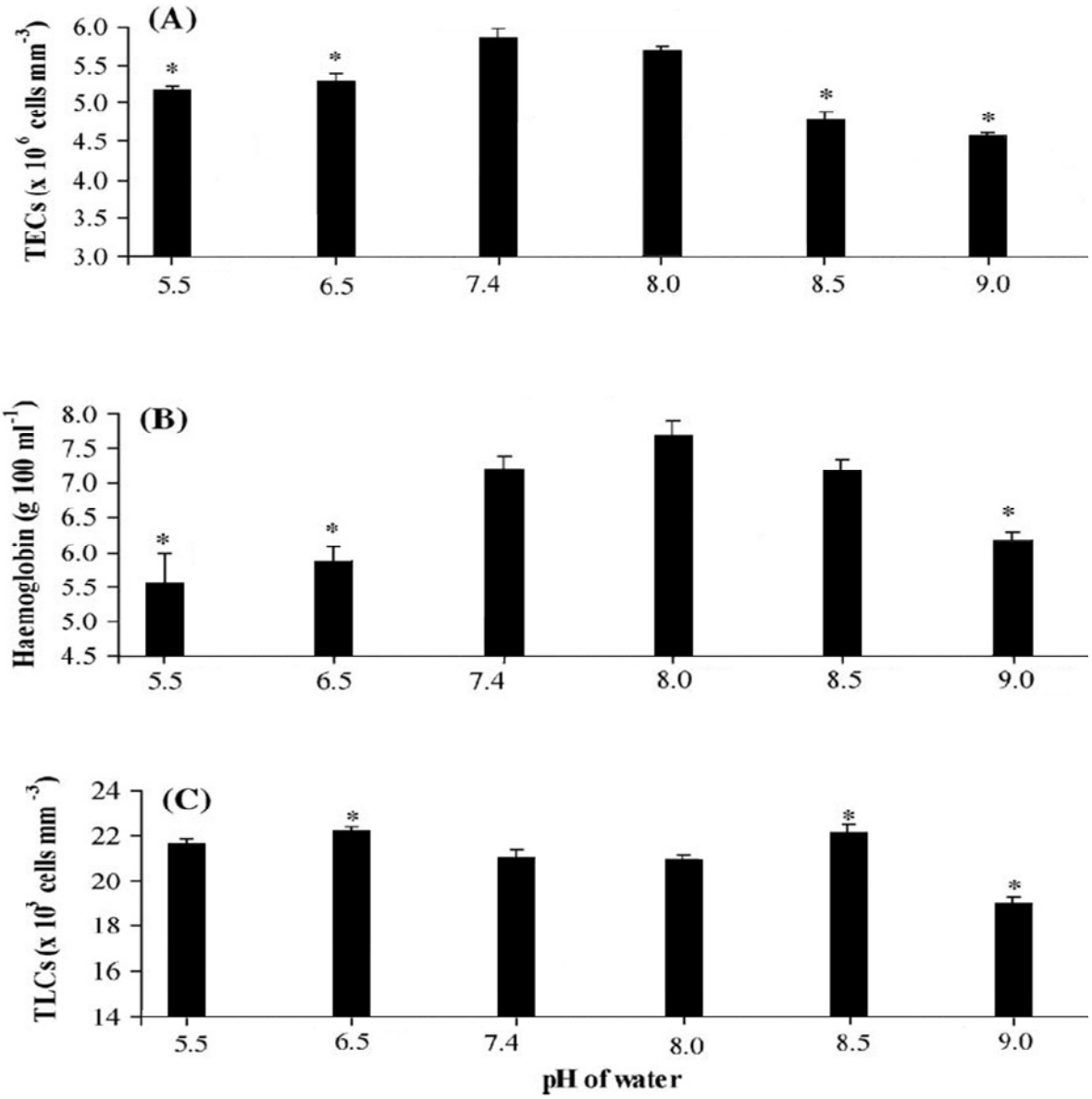

Figure 2. Fingerlings of the common carps exposed to different water pH showing changes in (A) total erythrocyte count, (B) total hemoglobin content and (C) total leukocyte count. Asterisks indicate a significant difference ($P < 0.05$) from control value ($n = 3$ for every species at each pH). pH 7.4 is the control.

change (Figure 3A). The maximum percentage elevations in the blood glucose were 50% in the acidic range and 58% in the alkaline range. Serum protein levels were reduced following exposure of the fingerlings to both acidic and alkaline conditions (Figure 3B). In the acidic range, the levels were significantly lower ($P < 0.05$) at pH 5.5. The serum protein was also significantly reduced at pH 8.0 and 8.5 in common carp.

Swelling of RBC was observed only in the fingerlings of common carp exposed to alkaline water. Such increase in the RBC volume occurs due to the regulatory volume increase mechanism of the cell, which requires an activation of the Na^+/H^+ with accompanying Cl^-/HCO_3^- exchanger at the membrane (Cossins and Gibson, 1997; Weaver et al., 1999).

In the present study, the change in water pH in the common carp might have caused the ion regulatory and acid-base disturbances originating at the gill leading to the altered internal pH, electrolyte and osmotic balances. Such disturbances in turn could lead to catecholamine mobilisation and disturbances in the red blood cell homeostasis leading to further activation of the catecholamine induced c-AMP sensitive activation of Na^+/H^+ pump at the RBC membrane surface for efflux of H^+ (Jensen et al., 2002; Das et al., 2006). Catecholamines were indeed released into the blood circulation as the

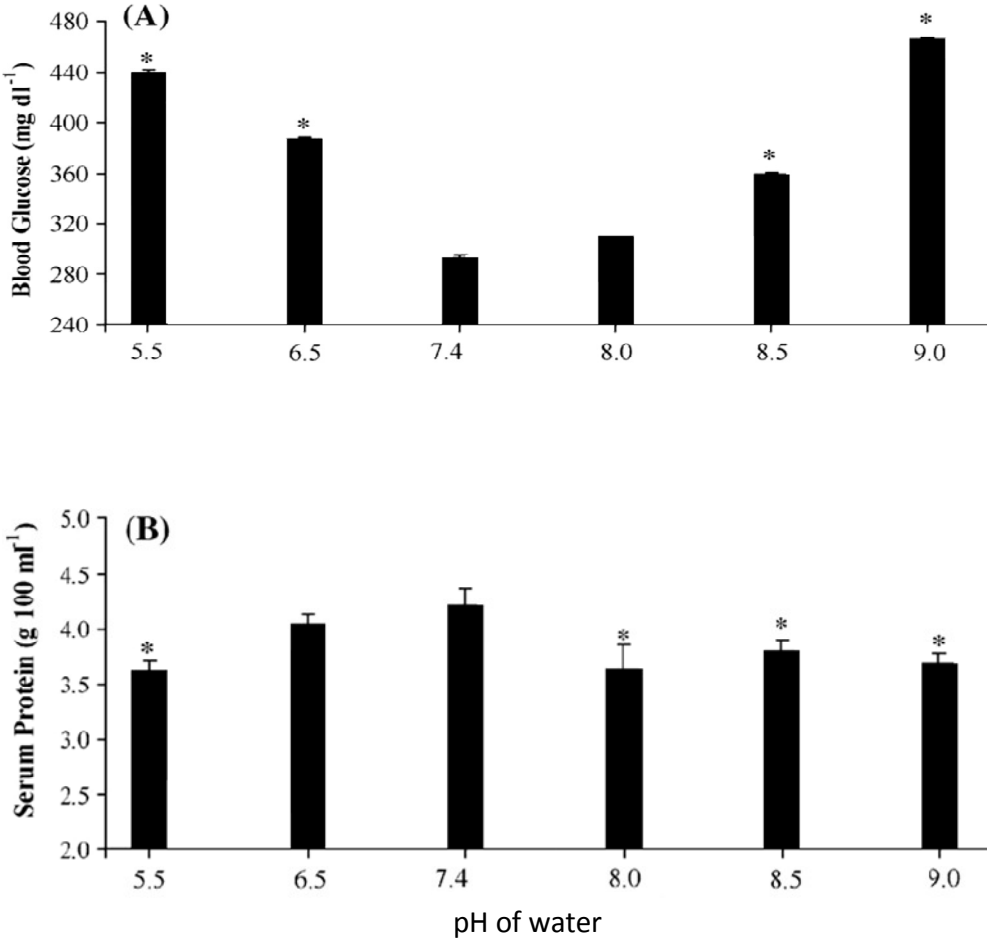

Figure 3. Fingerlings of the common carp exposed to different water pH showing changes in (A) blood glucose and (B) serum protein. Asterisks indicate a significant difference ($P < 0.05$) from control value (n = 3 for every species at each pH). pH 7.4 is the control.

fingerlings experienced elevated blood glucose contents (Figure 3A) following exposure to altered water pH. The lack of evidence for apical Na^+/H^+ exchange as the predominant acid transporter in freshwater fishes (Nelson et al., 1997) suggests that the common carp used an apical vacuolar proton-ATPase (VATPase) and an epithelial ENaC-like channel for the acid secretion and Na^+ absorption, as is hypothesised for freshwater salmonids, tilapia and flounders (Evans et al., 2005). This would ensure extracellular availability of Na^+ for entry into RBCs through Na^+/H^+ exchanger. The swelling of the carp RBC might therefore be due to obligatory water uptake accompanying the movement of Na^+ into the cell (Wood, 1989; Jensen et al., 2002).

In the present study, reduction in the TEC and Hb content in carps following exposure to both acidic and alkaline pH indicated a reduced blood oxygen carrying capacity (Stormer et al., 1996; Sawhney and Johal, 2000; Martinez and Souza, 2002; Jensen, 2003; Das et al., 2004a, b, 2006). In the context of distortion and lysis of certain RBC cells as observed at pH 5.5, 6.5 and 9.0, the reduced blood oxygen carrying capacity can be compensated either through increasing oxygen affinity and capacity of Hb and/or increase in RBC production (Das et al., 2006).

Though increased production of immature erythrocytes in this fish exposed to moderate pH change like those at pH 6.5, 8.0, and 8.5 explained a possible oxygen compensating mechanism against the stress at moderate pH (Gill et al., 1991), there was a lack of compensation at more extreme pH (pHs 5.5 and 9.0). The progressive reduction of the hemoglobin content in both acidic and alkaline conditions also indicated that there was a possibility of respiratory stress in this carp. Decreased RBC counts, hematocrit and hemoglobin concentration indicate that RBCs are being destroyed by the leucocytosis activity in an erythrocytic anemia with subsequent erythroblastosis (Haney et al., 1992). Haemodilution resulting from the impaired osmoregulation across the gill epithelium (Wedemeyer et al., 1984; Das et al., 2006) also might have contributed to such reduction in Hb content and TECs. Reduction in serum protein content in

fingerlings at these pHs also supports the view of erythrocytic cell lysis and haemodilution.

Das et al (2006) also found similar reduction in TECs and Hb after exposing three Indian carps to acidic and alkaline water pH and indicated that, the reduction of TECs as well as Hb content in all the carp species in both acidic and alkaline environment may been attributed to the erythrocytic cell lysis as well as exhaustion of the haemopoietic activity of the kidney (Das et al*.,* 2006). When the change in pH was moderate, that is, at pHs 6.5 and 8.5, immature erythrocytes could be produced through stimulated haemopoesis to compensate the oxygen carrying capacity of the lost erythrocyte and fish in these cases were experiencing haemolytic anaemia (Das et al*.,* 2006). But such compensatory mechanisms could not be sustained against the higher stress prevailed at pH 5.5 and 9.0. As a result, lysis of erythrocytes together with inhibition of erythrocyte production and significant reduction in the hemoglobin content at these pH caused a macrocytic type of anaemia (Barnhart et al*.,* 1983).

The leukocytes 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 enhanced release of leukocytes to the blood circulation. Stress also induces elevation of plasma catecholamines in fish within minutes (Mazeud and Mazeud, 1981). The released catecholamines, adrenaline and nor-adrenaline, increase the conversion of liver glycogen to blood glucose to satisfy the greater energy demands of the body to stress (Sriwastava and Srivastava, 1985). The variation of the TLC and increased blood glucose levels in the present study indicated elevated stress levels in the common carp (Salama and Nikinmaa, 1988; Harikrishnan et al., 2003) which were most likely due to the disturbance in the acid-base balance, respiratory homeostasis and ionic regulation during exposure to the changed water pH. Fingerlings of common carp showed protective responses at moderate pH change through increases in the TLC and blood glucose level. Though the blood glucose level increased further at pH 5.5 and 9.0, the TLC reduced in this species, most likely due to the greater stress level at such higher pH change, which weakened the leucopoiesis process. Such situation indicated susceptibility of common carp to greater change in water pH. The significant reduction in TLC and greater elevation in blood glucose in this species, particularly at higher water pH, could also be attributed to the additional stress due to possible accumulation of ammonia in fish body (Wilkie et al*.,* 1993). The higher serum protein reduction in fingerlings of common carp in all the acidic and alkaline pH exposures may be attributed to protein catabolism, the process converting blood and structural protein to energy, to meet the higher energy demand during the prevailing stress. Haemolysis and shrinkage of the erythrocytes, already been implicated in this species in the present study, also might have caused dilution of the plasma volume contributing to some extent, such reduction of serum protein content (Das et al*.,* 2004a*,* 2006). The changes in hematological variables in finger-lings of common carp exposed to different water pH indicate ionoregulatory or respiratory disturbances that imply an increase in energy consumption to restore homeostasis instead of other physiological functions and weight gain and growth.

REFERENCES

APHA, AWWA, WPCF (1989). Standard Methods for the Examination of Water and WasteWater, 17th edition. American Public Health Association, Washington, DC, pp. 10-203.

Cossins AR, Gibson JS (1997). Volume sensitive transport systems and volume homeostasis in vertebrate red blood cells. J. Exp. Biol. 200: 343-352.

Das PC, Ayyappan S, Jena J (2006). Haematological changes in the three Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) exposed to acidic and alkaline water pH. Aquaculture, 235(1-4): 633-644.

Das PC, Ayyappan S, Jena JK, Das BK (2004a). Acute toxicity of ammonia and its sub-lethal effects on selected haematological and enzymatic parameters of mrigal, *Cirrhinus mrigala* (Hamilton). Aquacult. Res. 35: 134-143.

Das PC, Ayyappan S, Jena JK, Das BK (2004b). Nitrite toxicity in *Cirrhinus mrigala* (Ham.): acute toxicity and sub-lethal effect on selected haematological parameters. Aquaculture, 235(1-4): 633-644.

Evans DH, Piermarini PM, Choe KP (2005). The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85: 97-177.

Gill TS, Pande J, Tewari H (1991). Haemopathological changes associated with experimental adicarb poisoning in fish (*Puntius conchonius* Ham.). Bull. Environ. Contam. Toxicol. 47: 628-633.

Harikrishnan R, Nisha Rani M, Balasundaram C (2003). Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for Aeromonas hydrophila infection. Aquaculture, 221: 41-50.

Jensen FB (2003). Nitrite disrupts multiple physiological functions in aquatic animals. Comp. Biochem. Physiol. 135: 9-24.

Jensen FB, Brahm J (1995). Kinetics of chloride transport across fish red blood cell membranes. J. Exp. Biol. 198: 2237-2244.

Jensen FB, Lecklin T, Busk M, Bury NR, Wilson RW, Wood CM, Grosell M (2002). Physiological impact of salinity increase at organism and red blood cell levels in the European flounder (*Platichthys fleus*). J. Exp. Mar. Biol. Ecol. 274: 159-174.

Martinez CBR, Souza MM (2002). Acute effects of nitrite on ion regulation in two neotropical fish species. Comp. Biochem. Physiol. 133: 151-160.

Mazeud MM, Mazeud I (1981). Adrenergic response to stress in fish. In: Pickering AD (Ed.). Stress and Fish. Academic Press, London, pp. 49-75.

Salama A, Nikinmaa M (1988). The Adrenergic responses of carp *(Cyprinus carpio)* red cells: Effects of P_{O2} and pH. J. Exp. Biol. 136: 405-416.

Santhakumar M, Balaji M, Ramudu K (1999). Effect of sub-lethal concentration of monocrotophos on erythropoietic activity and certain haematological parameters of fish *Anabus testudineus* (Bloch). Bull. Environ. Contam. Toxicol. 63: 379-384.

Sawhney AK, Johal MS (2000). Erythrocyte alterations induced by malathion in *Channa punctatus* (Bloch). Bull. Environ. Contam. Toxicol. 64: 398-405.

Scott DM, Lucas MC, Wilson RW (2003). The effect of high pH on ion balance, nitrogen excretion and behaviour in freshwater fish from an eutrophic lake: A laboratory and field study. Aquatic Toxicol. 73: 31-43.

Smutna M, Vorlova L, Svobodova Z (2002). Pathobiochemistry of ammonia in the internal environment of fish (Review) Acta Vet. Brno 71: 169-181.

Sriwastava UMS, Srivastava DK (1985). Effect of urea stress on fish. In: Trivedy RK, Goel K (Eds.). Current Pollution Researches in India. Environmental Publication, Karad, India.

Stormer J, Jensen FB, Rankin JC (1996). Uptake of nitrite, nitrate and bromide in rainbow trout, *Onchorhynchus mykiss*: effects on ionic balance. Can. J. Fish. Aquat. Sci. 53: 1943-1950.

Ultsch GR, Ott ME, Heisler N (1981). Acid base and electrolyte status in carp (*Cyprinus carpio*) exposed to low environmental pH. J. Exp. Biol. 93: 65-80.

Weaver YA, Kiessling K, Cossins AR (1999). Responses of the Na^+/H^+ exchanger of European flounder red blood cells to hypertonic, β-adrenergic and acidotic stimuli. J. Exp. Biol. 202: 21-32.

Wedemeyer GA, McLeay DJ, Goodyear CP (1984). Assessing the tolerance of fish and fish population to environmental stress. The problems and methods of monitoring. In: Cairns WV, Hodson PV, Nriagu JO (Eds.). Contaminant effects on fisheries. John Wiley and Sons Inc, New York, pp. 164-195.

Wilkie MP (2002). Ammonia excretion and urea handling by fish gills: present understanding and future research challenges. J. Exp. Zool. 293: 284-301.

Wilkie MP, Wood CM (1991). Nitrogenous waste excretion, acid-base regulation and ionoregulation in rainbow trout (*Onchorhynchus mykiss*) exposed to extremely alkaline water. Physiol. Zool. 64: 1069-1086.

Wilkie MP, Wood CM (1996). The adaptation of fish to extremely alkaline environments. Comp. Biochem. Physiol. 113: 665-673.

Wilkie MP, Wright PA, Iwama GK, Wood CM (1993). The physiological responses of the Lahontan cutthroat trout (*Onchorhynchus clarki henshawi*), a resident of highly alkaline Pyramid lake (pH 9.4) to challenge at pH 10. J. Exp. Biol. 175: 173-194.

Wood CM (1989). The physiological problems of fish in acid waters. In: Morris R, Taylor EW, Brown DJA (Eds.). Acid Toxicity and Aquatic Animals. Cambridge University Press, Cambridge, pp. 125-152.

Wood CM, Perry SF, Wright PA, Bergman HL, Randall DJ (1989). Ammonia and urea dynamics in the lake Magadi tilapia, a teleost fish adapted to an extremely alkaline environment. Respir. Physiol. 77: 1-20.