Alteration in haematological parameters in fresh water cat fish *Pangasius pangasius* in different water pH range.

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Abstract: Present study was design to record the haematological parameters of *Pangasius pangasius* in different water pH environment.The experimental sets were having different water pHviz.,pH4.50,(experiment-I),5.00(experiment-II),6.00(experiment-III),8.00(control,experiment-IV),9.5(Experiment-V).The experiment was conducted for a period of 10 days. Non significant decline in RBCs, Hb, PCV, and MCHC was recorded in experiment-I,II,III and V, but MCV and ESR level increased non significantly while TLC increased singnificantly when compared with control.The data was statistically analyzed by graphped prism5 at different significant level.

Key wards: *Pangasius pangasius* ,ESR,PCV, MCHC,TLC.

1.INTRODUCTION: As there is little evidence of pollution affecting the health of fish and shellfish on a global scale, Although there is no dispute that pollution can affect the health of aquatic organisms under experimental conditions and may be responsible for the decline of populations of such animals in some inland waters and some estuaries, most of the evidence for pollution causing or increasing disease in fish in open waters is circumstantial(D.Bucke,1993).

There is no dispute that during last 50 year,industrialization and urbanization is increased consequently anthropogenic activity increased and improper or unplanned drainage system ruined the naturally occuring physicho-chemical properties of fresh water bodies which were helpful for the growth of aquatic organism. The pH of water is primary indicator of pollution similarly haematological and biochemical parameters viz; RBC, Hb, PCV, MCV, MCHC, TLC, and ESR can be a useful tool for monitering the health status, detecting illness, and following the progress of disease and response to therapy. If hematological and biochemical parameters are within the normal range (reference range) it is the indicator of good health, but when these parameters are below or above the normal range, it is an indicator of diseases or poor health, which is directly associated with the growth of all vertebrates (Clauss,et al., 2008) and loss and profit for fish farmers. How ever considerable work has been done by many in the area of fish toxicology, Nagarajan et al., (2014) Studied the effect of cadmium chloride on *Oreochromis mossambiccus*, Das et al.,(2006) studied the haematological changes in the three Indian major carps, *Catla catla, Labeo rohita* and *Cirrhinus mrigala* when exposed to acidic and alkaline water pH. In Uttarakhand, *Pangasius pangasius* is in first priority for consumer due its good taste,low price and high food conversion ratio.This study was conducted to determine the haematological parameters of fresh water cat fish *Pangasius pangasius* in different water pH environment viz., pH 4.50,5.00,6.00, 8.00 and 9.50.

2. MATERIAL AND METHODS:

i.Transportation of Fingerlings:200 fingerlings of *Pangasius pangasius* were brought from Roorkee having weight 20-26gm and length 10-17 cm. During transportation, tobacco leaf dust @50ppm was used as sedative and brought to the fishery laboratory,Uttaranchal College Science and Technology,Dehradun(Dinesh et al., 2017),.fingerlings of fishes are transferred to large plastic tank ,dead fishes were eradicated.

ii.Preperation of experimental sets: The glass aquariums (experiment-I pH4.50,II-pH5.00,III- pH6.00,IV-pH8.50,V-pH 9.50)were filled with water(17 lit.each,size1.5x1.5 meter).After recovery the fishes were starved for 24 hrs.than 10 fishes from the stock are transferred to each glass Aquarium and acclimatized for one week. *Pangasius pangasius* is carnivorous cat fish and fed with commercial feed twice/day, 3.00% body weight(agrifarming2019) before feeding the pallets of feed was semi grinded according to mouth size of finger lings.The feed was manufactured by Manasarowar Agro Trades,Assam and the ingredients are is given in table –I. For the maintenance of dissolved oxygen(6.00ppm) and photoperiodism the aerator and ordinary bulb was arranged in each aquarium throughout the experiment.the temperature was maintained (26-28^{o}C) by replacing one half part of old water simultaniously acidity and alkalinity was maintained by adding 40% hydrochloric acid(V/V) and 20% sodium hydroxide (W/V) and required pH was measured by digital pocket pH meter manufectured by HANNA instruments, Delhi.Before the maintenance of required pH the of water LD_{50} and margin of safty was determined.The total hardness of water 180ppm was(general + carbonate hardness) maintained according to APHA (2009) by the help of pocket hardness tester manufactured by HANNA instruments,Delhi.

iii.Collection and estimation of Blood samples: For the haematological study, the blood sample was collected after end of 10 days(excluiding acclimatization period one week) from each fishes from all experimental sets (experiment-I,II,III,IVandV). The blood was collected from caudal veins using sterile needle in separate vails each containing 1.00 mg ethylene-diamine tetra-acetic acid before collection of blood sample fishes were anesthetized by MS222 @50 mg/L. Samples were coded and then kept in ice box and stored at 5°C(Bermudez et al., 2003).The haematological parameters were analyzed by complete blood count machine (hematology analyzer, BC2300) supplied by Mindray, Delhi(Chaves 2005).The Data were subjected to one-way analysis of variance (ANOVA) by software Graphpad Prism-5.

3.Result: The hematological alteration in *P.pangasius* obtained from exposure to different water pH for 10 days. The non-significant decline was recorded in RBCs,Hb,PCV and MCHC in experiment-I(pH4.50),II(pH5.00),III(pH6.00) and V(pH9.50) when compared with control(pH8.00).The three significant figure was considered as P<0.05*, P<0.01**,P<0.001***. non significant increament was noticed in MCV and ESR but TLC increased significantly.The all obtained haematological values with their measurement unit are given in table-2.The values of RBCs for experiment-I,II,III and V was recorded 2.60±0.04, 3.48±0.02, 3.92±0.06 and 2.20±0.04 respectively while for control(experiment-IV) it was recorded 4.50±0.06 million/mm^3 and shown in figure-1.The decreased values of Hb was recorded for experiment-1,II,III and V and was 4.20±0.10, 5.62±0.20, 6.20±0.24 and 3.88±0.02 respectively while for controled experiment it was recorded highest(6.44±0.20gm/dL) and shown in figure-2. The PCV% was recorded for experiment-I(26.63±2.42),II(27.64±1.80),III(29.45±2.00) and V(26.80±2.00) while for control it was recorded highest(30.20±4.00) shown in figure-3.Decreased MCHC level was also observed in Experiment-I(15.84±1.60),II(20.33±1.86),III(21.05±2.20) and V(14.47±1.6) while for control it was noticed highest(21.32±1.42 gm/dL) and shown in figure-4.Highest increased values for MCV(fl) was noticed in experiment-V(121.81±6.8) while in experiment-I,II and III it was recorded 102.31±5.50,79.42±6.00,72.57±5.60 and was higher than control(67.11±8.64)and given in figure-5.Similarly the highest increased values of TLC was recorded in experiment-V(25640±250) while in experiment-I,II,III it

was recorded 22400±175, 20644±210, 17422±225 and was higher than control(15700±150) and shown in figure-6.The significant increment was observed in ESR level, in experiment-I,II,III and V when compared with control. It was observed highest in experiment-V (7.60)and lowest in experiment-IV(1.60) and shown in figure-7.

Table-1: Composition and ingredients of commercially prepared food for experimental sets.

S.No	Name of ions,vitamins and substances	Amount/%	S.No	Name of ions/vitamin substances	Amount/%
1	Protein	12%	10	Selenium	0.60-0.61ppm
2	Fat,minimum	5.%	11	Vitamin E, minimum	225.00IU/lb
3	Fibre maximum	23.00%	12	Vitamin A, minimum	3500.00 IU/lb
4	Lysine minimum	0.70%	13	Biotin, minimum	3.60mg/lb
5	Calcium	0.8-1.00%	14	Starch, maximum	7.00%
6	Phosphorous minimum	0.50%	15	Sugar, maximum	4.00%
7	Magnesium minimum	0.50%	16	Ferrous Carbonate	QS
8	Zinc minimum	220ppm	17	Cod liver oil	1.00%
9	Copper minimum	65.00ppm	18	Manganous Oxide	QS

Ingredients: Alfalfa, Shredded Beet Pulp, Wheat Midlings, Ground Oat Hulls, Ground Soy Hulls, Ground Flaxseed, Soy Oil, Calcium Lignin Sulfonate, Calcium Carbonate, Mono-dicalcium Phosphate, Salt, Vitamin A, Natural falvour, Vitamin C, Biotin, B-12 Concentrate, Calcium Pentothenate, Choline Chloride, Natural Vitamin E, Tecopherols, Vitamin D,L-Lysine, Magnesium Oxide, DL- Methionine, Niacin, Riboflavin, Selenium, Thiamine, Cobalt Carbonate, Copper Sulphate, Manganous Oxide, Calcium Iodate, Zinc-Oxide

Table-2:Showing changes in haematological values in different water pH:

parameters	pH4.50 (Exp-I)	pH 5.00, (Exp-II)	pH 6.00 (Exp.-III)	pH8.00 control, (Exp.IV)	pH9.50 (Exp.-V)
RBCs 10^6/µl	2.60 ± 0.04^{ns}	3.48 ± 0.02^{ns}	3.92 ± 0.06^{ns}	4.50 ± 0.06^{ns}	2.20 ± 0.04^{ns}
Hb gm/dL	4.20 ± 0.10^{ns}	5.62 ± 0.20^{ns}	6.20 ± 0.24^{ns}	6.44 ± 0.20^{ns}	3.88 ± 0.02^{ns}
PCV%	26.63 ± 2.42^{ns}	27.64 ± 1.80^{ns}	29.45 ± 2.00^{ns}	30.20 ± 4.00^{ns}	26.80 ± 2.00^{ns}
MCHC gm/dL	15.84 ± 1.60^{ns}	20.33 ± 1.86^{ns}	21.05 ± 2.20^{ns}	21.32 ± 1.42^{ns}	14.47 ± 1.60^{ns}
MCV(fl)	102.31 ± 5.50^{ns}	79.42 ± 6.00^{ns}	72.57 ± 5.60^{ns}	67.11 ± 8.64^{ns}	121.81 ± 6.80^{ns}
TLC/mm^3	$22400\pm175^{***}$	$20644\pm210^{***}$	$17422\pm225^{***}$	15700 ± 150^{ns}	$25640\pm250^{***}$
ESR in first hrs	7.20 ± 0.40^{ns}	4.42 ± 0.24^{ns}	2.80 ± 0.28^{ns}	1.21 ± 0.04^{ns}	7.60 ± 0.42^{ns}

Each haematological value is the mean of 10 fishes, ± is S.D.and compared with control an marked with significant at *** P< 0.001, ns (non-signific at P<0.05, P<0.01,P<0.001 level)

Figures:

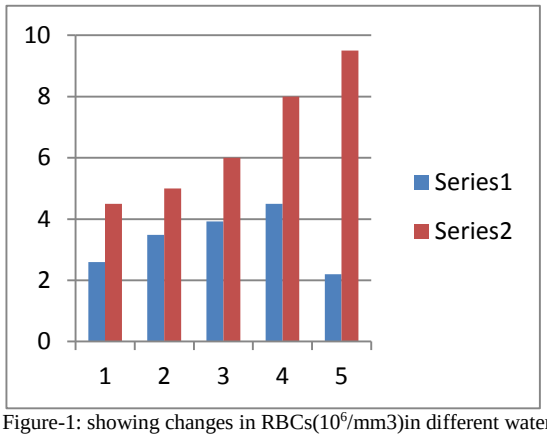

Figure-1: showing changes in RBCs(10^6/mm3)in different water pH

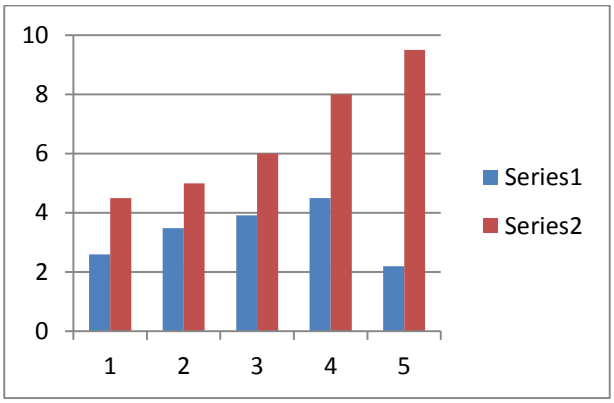

Figure-2: showing changes in RBCs(gm/dL)in different water pH

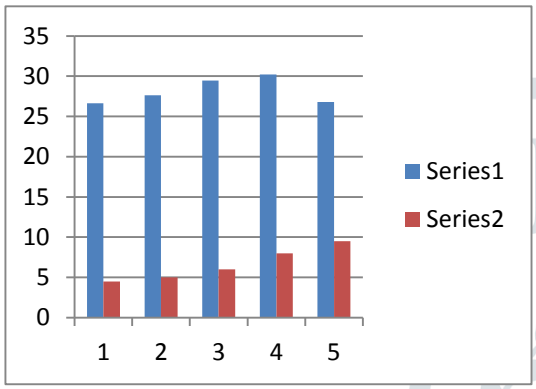

Figure-3: showing changes in PCV %in different water pH

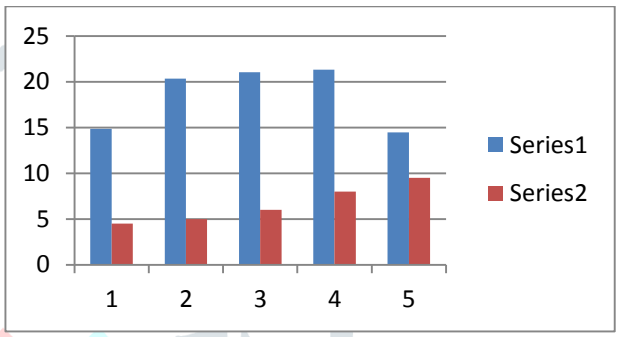

Figure-4:showing changes in MCHC(gm/dL) in different pH

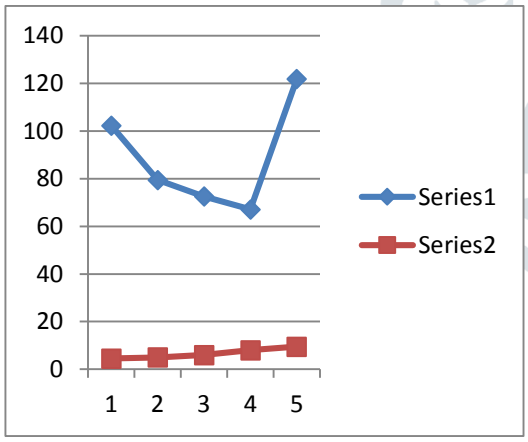

Figure-5: showing changes in MCV(gm/dL)in different water pH

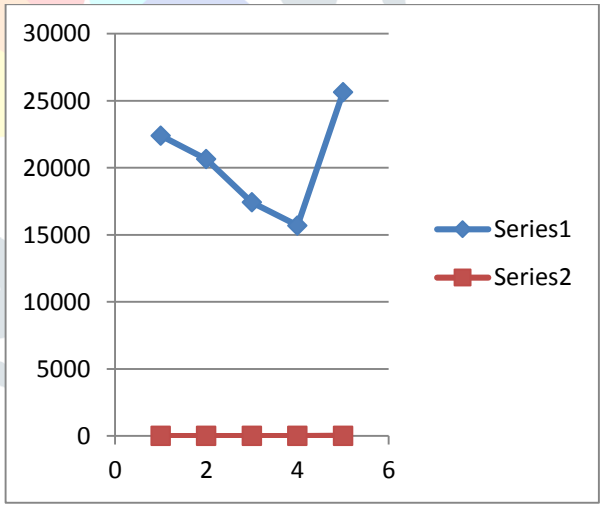

Figure-6: showing changes in TLC(10^3/mm^3)in different water pH

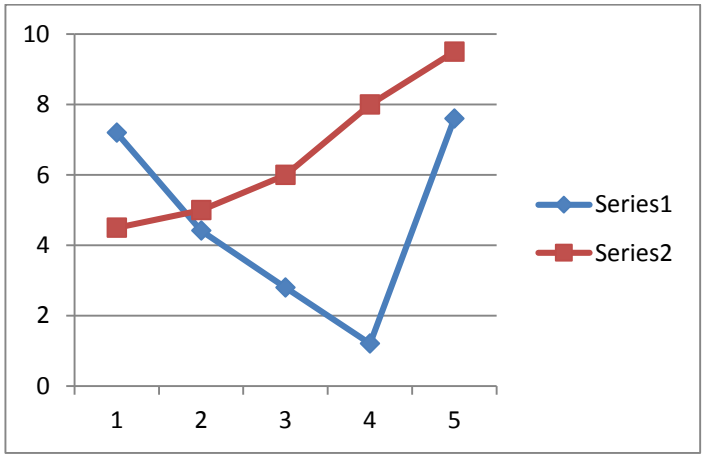

Figure-7: Showing changes in ESR(in first hrs)in different water pH

4.Disucussion: It has been proved that wter pH between 6.50 -8.50 is beneficial for the growth of fishes(fishsite.com,2015).In present study the non significant decreased in red blood cells count(RBCs), haemoglobin(Hb),packed cell volume(PCV), and mean cell haemoglobin concentration(MCHC) were observed. In experiment-I and V these indices reduced sharply during experimental period when compared to control while in experiment-II and III these values were not greatly reduced because pH 5.00 and 6.00 was under tolerable range but could be reduce greatly for long term exposure and it was primery sign anaemia. In experiment –I and V decreased RBCs,Hb,PCVand MCHC confirmed the anaemia in experimental fish *P.pangasius* because extreame change in water water pH caused environmental stress in experimental fishes and consequently haeamopoitic system of experimental organisms was negatively effected. Further non significant increased in mean cell volume (MCV) reconfirmed the acute macrocytic anaemia in experiment-I, and V and it might be due to extreame change in water pH consequently red blood cells swelled but could be shrink under long term exposure(microcytosis) in similar water pH.In experiment-II and III, the values for MCV increased less when compare to control because the water pH was in tolerable range(shown in table-2).Similar kind of results were obtained by Rokade et al.,(2018)when fresh water fish *R.daniconius* was exposed to Tributyltin oxide. Kumar and Ramulu (2013) also observed lowered RBCs,Hb,PCV and MCHC when *P.hypopthalmus* was infacted with *Aeromonas hydrophila*. Further highest erythrocyte sedimentand rate(ESR) and total leucocytes count (TLC) was observed in experiment-I,and V and was the final confirmation of environmental stress(inflammation) caused by extreame change in water pH. ESR increases because during infaction or inflammation the red blood cells settle at a faster rate in the presence of an increased level of proteins, particularly proteins called acute phase reactants.The level of acute phase reactants such as C-reactive protein (CRP) and fibrinogen increases in the blood in response to inflammation (Harrison 2015) and the TLC increases due the induced immune response against stress.

5.Conclusion: On the bases of obtained haematological data it can be concluded that the water pH below than 6.00 and above than 9.50 is not suitable for the growth and survival of fish *Pangasius pangasius*. In present study the water pH below 6.00 and above than 9.50 exterted negative impact on the health of experimental fishes.

6.Acknowledgement: The authors are thankful to the,Director of Uttaranchal College of Science & Technology,Dehradun for the necessary support and encouragement.

References:

1.APHA. (1998):Standard methods for the examination of water and weste water, 17th edition. American Public of Health Association,Washington DC.

2.https//:www.agrifarming.in/fish-farming(2019).

3.Bermudez AJ, and Stewart-Brown B.(2003):Diseases of Poultry. (11thedn), Iowa State Press, a Blackwell Publishing Compan, USA.

4.Bucke D.(1993):Aquatic pollution:Effects on the health of fish and shellfish.*Parasitology*,volume 106,Issue-S1,pp S25-S37.

5.Chaves FMD(2005):Quantitatative determination of neutrophil VCS parametes by the Coulter automated hematology analyzer. *American Journal of Clinical Pathology*, 2005; 124(2005): 440-444.

6.Clauss T. Dove, A. and Arnold, J. (2008):Haematological disorder of fish. *Veterinary clinics of North America Exotic Animal Practice*, 11(3): 445 - 462.

7. Das PC, Ayappan S, Jena JK.(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*. 2006; 256:80-87.

8.Dinesh R, Prakash C, Chadha NK, Poojary N, Abraham S. (2017): Does Tobacco (Nicotiana tabacum) Leaf Dust Save the Life of Rohu (Labeo rohita) Fingerlings During Transport*? J Aquac Res Development* Vol.8,Issue.3,pp 1-8.

9.Harrison M.(20015): Erythrocyte sedimentation rate and C-reactive protein. Aust Prescr. 38(3): 93–94.

10.Kumar PM and Ramulu SK (2013): Haematological chnges in *Pangasius.hypopthalmus* infacted with *Aeromonas hydrophila*. International Journal of Food, Agriculture and Veterinary Sciences. Vol. 3 (1), pp. 70-75.

11.Lucy T,(2018): the fishsite.com/articles/how to achieve good water quality management in aquaculture.

12.Rokade KB, Kharat TL. and Shejule K B(2015): Changes in some hematological and biochemical indices in fresh water fish *Rasbora daniconius* exposed to tributyltin. *Trends in fisheries Research*. Volume 7, Issue 2.pp 16-23.