ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(1): 155-159

www.fisheriesjournal.com
Received: 14-11-2015
Accepted: 16-12-2015

Mohan Arthanari
Research scholar,
Department of Zoology,
Kandaswami Kandar's College,
Tamil Nadu, India.

Senthilkumar Dhanapalan,
Associate Professor,
Department of Zoology,
Kandaswami Kandar's College,
Tamil Nadu, India.

Correspondence
Mohan Arthanari
Research scholar,
Department of Zoology,
Kandaswami Kandar's College,
Tamil Nadu, India.

Assessment of the haematological and serum biochemical parameters of three commercially important freshwater fishes in river Cauvery Velur, Namakkal district, Tamil Nadu, India

Mohan Arthanari, Senthilkumar Dhanapalan

Abstract
Haematological parameters have been recognized as valuable tools for monitoring fish health. Haematological and serum biochemical parameters were studied and compared with different feeding behaviour of freshwater fishes. Three freshwater fishes, *Ctenopharyngodon Idella* (herbivore), *Cyprinus Carpio* (omnivore) and *Pygocentrus nattereri* (carnivore) were carried out in order to find out a normal range of blood parameters which would serve as baseline data for assessment of the health status of the fish as well as a reference point for future comparative surveys. Blood parameters such as Red Blood Cell count (RBCs) and White Blood Cell count (WBCs), RBC/WBC ratio, Haemoglobin (Hb), Haematocrit (Hct), Erythrocyte Sedimentation Rate (ESR), Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Serum Glucose, Serum Protein and Serum Cholesterol were estimated from freshwater fishes of same trophic level. Statistical analysis confirmed the differences in hematological parameters between all the species were significant at $p<0.01$ level. The RBC / WBC ratio was more due to the decreased level of WBC during the study period. These differences can be attributed to the physiological acclimatization of the fish to their living condition and feeding regime, which influences the energy metabolism and consequently, the health of the fish. Blood serum biochemical parameters can be used for confirming the maturity and monitoring any changes in the quality of water and related soil environment.

Keywords: Ctenopharyngodon Idella, Cyprinus Carpio, Pygocentrus nattereri

1. Introduction
The blood parameters in fishes are influenced by many factors Mishra *et al*., (1977) [29]. Quality of water, temperature, food availability and physiological status of fish either or indirectly influence on blood constituents of fish Iqbal *et al*., (1997) [24]. According to Bhagat and Banerjee, (1986) [5] the sex, size, season and age of fishes are directly reflected in blood parameters. Changes in physicochemical parameters may be reflected haematological parameters of the fishes Abdul Naveen *et al.,* (2011) [1]. Holmes and Donaldson, (1969) [22]; Goel and Sharma, (1987) [18] studied the comparative aspects of haematological parameters. Basic ecological factors, such as feeding regime and stocking density, also have a direct influence on certain biochemistry parameters Coz-Rakovac *et al*., (2005) [9]. The variation in protein, cholesterol and glucose level is directly to sex, size and age of the fishes (Khanna and Singh, 1973 [26]; Dharan Sing *et al*., 2008) [12]. The main objectives of this study is to compare the Haematological and Serum biochemical parameters of three different feeding regime commercial freshwater fishes *Ctenophryngodon idella* (Herbivore), *Cyprinus carpio* (Omnivore) and *Pygocentrus nattereri* (Carnivore) in River Cauvery to know their health status during the study period.

2. Materials and Methods
2.1. Sampling area
Freshwater fishes were collected from River Cauvery in Namakkal district of Tamil Nadu (Latitude 11° 09' 67.84" N and Longitude 78° 00' 72.86" E; MSL 418 ft). Fish samples were collected from January to May 2014.

2.2. Fish

A total of three species of freshwater fishes namely *C. idella*, *C. carpio* and *P. nattereri* were collected, acclimated and brought to the laboratory. Ten fishes from each species select ranged from 90 to 200g in weight and 15 to 20 cm in body length were used for this study. The blood samples were drawn by caudal vein puncture using 21 gauge hypodermic needle in two different vials, one containing the anticoagulant EDTA (Heparin sodium 1%) for blood cell studies and the other without EDTA allowing the clot and serum to separate for studying some biochemical constituents. The collected blood samples were immediately subjected to haematological analysis.

2.3. Hematological parameters

The blood was diluted with appropriate diluting fluids for RBCs and WBCs counts were determined using improved Neubauer haemocytometer and calculated Blaxhall and Daisley, (1973) [6], replicated counts were made for each blood samples. Sahli's haemoglobinometer was used to estimate haemoglobin percentage (Hb%). Haematocrit (Hct) was determined using micro haematocrit capillaries filled with blood and centrifuged at 8,700×g for 5 min and expressed as percentage of the total blood volume (Wintrobe, 1974) [38]. Mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated from the average values of Hb% (Dacie and Lewis, 1984) [10]. For the determination of erythrocyte sedimentation rate (ESR), the blood was mixed well and 200 mm was drawn into a Westergren's tube. The tubes were placed vertically and were left undisturbed for 60 min; after that, the level of the column of sediment was noted as an ESR. Biochemical estimation of blood serum glucose, serum protein and serum cholesterol were determined by following standard methods (Folin and Wu, 1920; Fawcette and Scott, 1960; Henry, 1968; Annino, 1976) [2, 14, 16, 19].

2.4. Statistical Analysis

Differences in haematological parameters between the three freshwater fishes were statistically analysed by one-way analysis of variance (One way ANOVA) without replication. All these statistical analysed were performed using the SPSS Statistical software (Version. 21 for Windows 7 Ultimate). Bray-Curtis similarity was performed using the PAST statistical software (Version 2.17c for Windows 7 Ultimate).

3. Result

The haematological and biochemical parameters of three different feeding behaviour freshwater fishes *C. idella*, *C. carpio* and *P. nattereri* were given in the Table 1, 2 and Fig.1, 2, 3 and 4. An elevated RBC, RBC/ WBC ratio and MCHC% was observed in Carnivores fish *P. nattereri* and least in Omnivores fish *C. carpio*. High level of WBC, Hb, Hct, MCV and MCH was observed in *C. carpio*. A maximum ESR was registered in Herbivores fish C. idella. Among the haematological parameters statistical significance was exist for Hb and ESR at $p<0.05$ and no statistical significance observed for RBC, WBC, RBC/WBC ratio, MCH, MCHC, MCV and Hct.

Table 1: Comparative haematological parameters of three commercially important freshwater fishes (mean ± SD)

Parameters	*C. idella*	*C. carpio*	*P. nattereri*
RBC $X10^{-6}$	16.57±0.71	8.50±0.72	22.57±0.70
WBC $X10^{-4}$	9.59±0.52	14.85±0.73	4.47±0.68
RBC/WBC (%)	1.74±0.10	0.58±0.06	5.15±0.77
Hb (%)	5.54±0.62	5.68±0.97	5.47±0.59
Hct (%)	13.68±1.02	21.58±0.74	17.66±0.86
MCV (fl)	7.67±0.99	23.64±0.89	7.37±0.87
MCHC (%)	52.94±0.98	30.59±1.08	66.35±1.71
MCH (pg)	4.02±0.78	7.41±1.10	3.82±0.67
ESR (mm/h)	0.82±0.14	0.61±0.10	0.76±0.17

RBC- Red blood cells, WBC- White blood cells, HB- Haemoglobin, HCT- Haematocrit, MCV- Mean corpuscular, MCHC- Mean cell haemoglobin concentration, MCH- Mean cell haemoglobin, ESR- Erythrocyte sedimentation rate.

Maximum serum glucose and serum cholesterol was observed in Herbivores fish *C. idella* and serum protein in Omnivores fish *C. carpio*, and minimum in Carnivores fish *P. nattereri*. Serum biochemical parameters show significance at $p<0.05$ for these freshwater fishes. Bray-Curtis similarities (Fig.5) show that Carnivores fish stand separate from both Herbivore and Omnivore in the haematological and biochemical values. The dendogram clearly reflects the change that occurs in the blood parameter and physiological response of fishes in adverse environmental conditions.

Table 2: Comparative serum biochemical parameters of three commercially important freshwater fishes (mean ± SD)

Parameters	*C. idella*	*C. carpio*	*P. nattereri*
Glucose (mg/dl)	227.49±0.75	208.52±0.85	47.50±1.71
Protein (mg/dl)	4.55±0.81	11.47±0.77	6.32±0.65
Cholesterol (mg/dl)	596.37±0.89	128.44±0.82	29.53±0.81

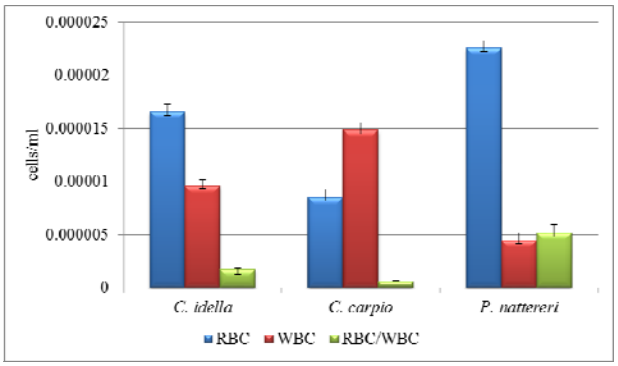

Fig 1: Haematological parameter of the Fresh water fishes

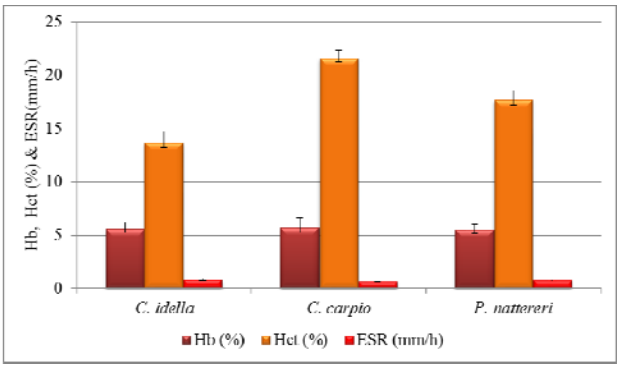

Fig 2: Hb, Hct and ESR of the freshwater fishes

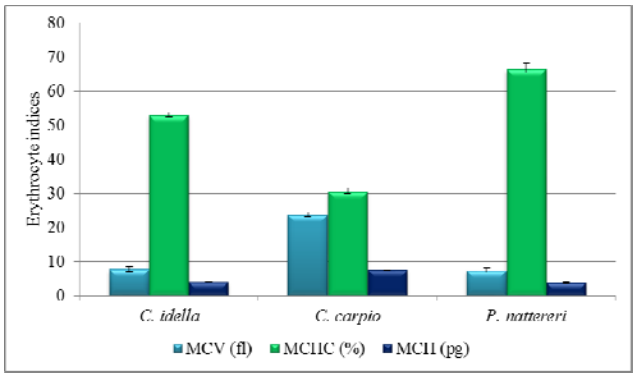

Fig 3: Erythrocyte indices of the Fresh water fishes

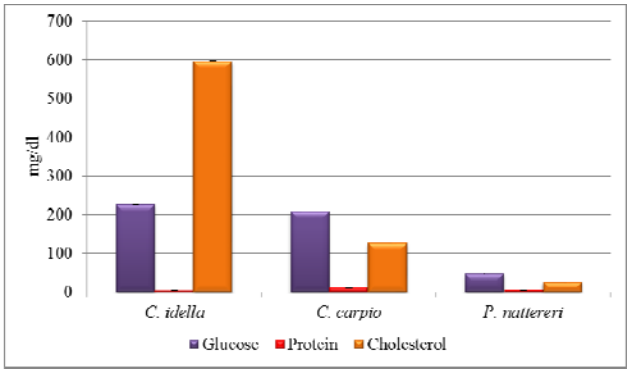

Fig 4: Serum Biochemical parameters of the Fresh water fishes

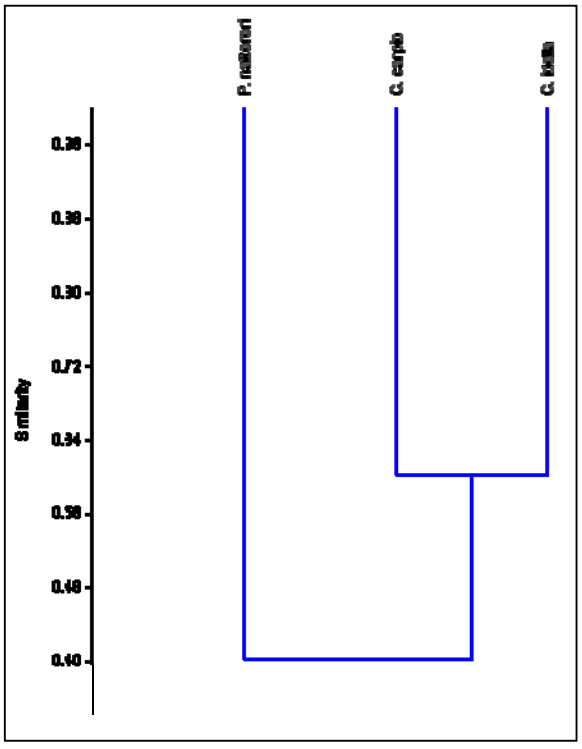

Fig 5: Bray - Curtis similarity of three freshwater fishes

4. Discussion

The haematological and biochemical characteristics of some fish species have been investigated with the aim of establishing normal blood values and ranges respect to sex, age, and size, environmental and physiological conditions (Kori-Siakpere, 1985; Sowunmi, 2003; Gabriel *et al*., 2007) [17, 27, 35]. Also, comparative studies on blood parameters of fish have been carried out to determine the systematic relationship among certain species.

Svobodova *et al*., (2008) [37] was reported active species displayed high values of all haematological parameters studied when compared to low active forms. RBC is the dominant cell type in the blood of the majority of fish species. However, significant differences in the values of erythrocyte volumes between the three species were observed in this study, suggesting that, in the intensive river environments, the elevated RBC counts and Hct concentration are a response to the higher metabolic demand and have no impact on erythrocyte volume. The increased number of RBC indicates the oxygen requirement at higher metabolic rates (Engel and Davis, 1964) [13]. In this present study, Hb and ESR seemed to vary, significant at the $p < 0.05$ level between species. The low Hb value was associated with low active fishes; similar results were already reported by Engel and Davis, (1964) and Rambhaskar and Srinivasa Rao, (1986) [13, 32].

RBC and Hb concentration tend to increase with length and age Das, (1965) [11]; Hct value in the present work was within the range of 13% to 21%. Blaxhall and Daisley, (1973) [6] has reported the possibility of using haematocrit as a tool in the aquaculture and fishery management for checking the anemic condition. Fish haematocrit values were usually between 20% to 35% and rarely attain greater than 50% Clark *et al*., (1976) [8], and haemoglobin values of 14.0–12.7 g 100 ml^{-1} Larsson and Johansson-Sjobeck, (1976) [28], but with comparatively low values of haematological parameters particularly Hct recorded in the 13.68% for *C. idella* the present study. There is an inverse relationship between WBC and RBC count in all the fishes of the present investigation. Decreased WBC count was reported in the present study was in consistence with Shakoori *et al.,* (1996) [33]. The environmental changes and the pollutants in the river water may increases the RBC/WBC ratio and also alters the physiological and chemical properties of fish blood parameters (Hughes and Nemcsok, 1988 [23]; Yanik and Atamanalp, 2001) [39]. Environmental changes may also cause differences in the Physiological and chemical properties of fish blood (Hughes and Nemcsok, 1988; Yanik and Atamanalp, 2001) [23, 39]. The ESR level may be a consequence of alternation in blood plasma of different fish species or may be due to the stress condition (Joseph John, 2007) [25].

No difference was observed in MCH in *C. idella* and *P. nattereri*, whereas a high MCH value was noticed in *C. carpio*. This may be due to the overall oxygen consumption rates and swimming performance under normal condition (Stillwell and Benfey, 1995) [36]. MCHC in all the three commercially important freshwater fishes ranged between 30 to 66% and this was similar to Peruzzi *et al*., (2005) [31]. The MCV of *C. carpio* was higher, and the remaining two species were ranged between 7.37 and 7.67fl.

Biochemical analysis can provide valuable information for monitoring the health conditions of fishes. Biochemical changes depend on the fish species age, the cycle of sexual maturity and health condition. Moreover, analysis of serum biochemical constituents level have shown useful information in detection and diagnosis of metabolic disturbances and diseases in fishes (Ferrari *et al.,* 2007) [15]. An increased blood glucose and protein level was recorded in *A. subrostratus*; this may be probably due to an increased depletion of liver glycogen Ojolick *et al*., (1995) [30]. In the present study, it is very likely that the physical processes involved in transporting the fish, and administering anaesthesia for blood sample

collection caused physical stress and affected hormone and glucose levels in the plasma. Since glucose in serum is a major metabolite of carbohydrate metabolism Artacho *et al*., (2007) [3], the higher glucose concentration detected in cultured strains should be attributed, in part, to the higher glycogen reserves in artificially cultured fish than in their wild counterparts. Increased concentrations of total protein can be caused by structural liver alterations reducing amino transferase activity, with concurrent reduced de-amination capacity Burtis and Ashwood, (1996) [7] and impaired control of fluid balance Coz- Rakovac *et al*., (2005) [9]. Haemato-biochemical constituents of *C. punctatus* and *C. striatus* are directly related to their behavioral physiology (Hickely, 1982; Holmes and Donalson, 1996) [20, 22]. The cholesterol concentration of *C. idella* was higher due to the high proportion of fat in the chemical composition of their food. Hill (1982) [21], reported that cholesterol concentration increase as fish size increased. The elevated blood cholesterol levels may be due to mobilization of stored cholesterol from tissue. The availability of more cholesterol in the serum during the breeding season could be due to its decrease conversion into gonadal steroids (Singh and Singh, 1997) [34]. Bano (1985) [4], observed an increase in serum cholesterol level after the administration of pesticides.

5. Conclusions

The study is the first to characterize and provide a comparative physiological account of Loach in wild ecological systems. Based on our results, it appears that human manipulation has a physiological effect on the fish haematological and biochemical parameters are useful tools for detecting. The River Cauvery receives large amount of polluted water from upstream alters the physiological activities of animals result in poor health status of the commercial fishes. From the above work it's clearly visible that alteration in water quality will affects the fish health. The approach may provide a rapid means for determining the physiological status of the fish, thereby enabling changing conditions to be diagnosed early, which in turn would facilitate the implementation of remedial measures during culture operations.

6. References

1. Abdul Naveen P, Venkaeshwaslu and Janaiah C. Biochemical alteration induced by triazophos in the blood plasma of fish *Channa punctatus* (Bloch.). Annals Biol. Res. 2011; 2(4):31-37.
2. Annino JS. Clinical chemistry principles and procedures, 4thedn. Little Brown and Company, Boston, 1976.
3. Artacho P, Soto-Gamboa M, Verdugo C, Nespolo RF. Blood biochemistry reveals malnutrition in black-necked swans (*Cygnus melanocoryphus*) living in a conservation priority area. Comp Biochem Physiol, 2007; A 146:283-290.
4. Bano Y. Sub lethal stress of DDT on biochemical composition of catfish *Clarias batrachus*. Indian J Environ Health. 1985; 27:230-236.
5. Bhagat RP, Banerjee V. Haematology of an Indian freshwater cel *Amphipnous cuchia* (Hamilton) Erythrocyte count related parameters with special reference to body length sex and seasons. Compt. Physiol. Ecol. 1986; 2(1):21-27.
6. Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood, J Fish Biol. 1973; 5:771-781.
7. Burtis CA, Ashwood ER. Tietz fundamentals of clinical chemistry. WB Saunders, Philadelphia, 1996.
8. Clark S, Whitemore DH, Mc Mahon RF. Consideration of blood parameters of large mouthbass, Micropterus salmoides, J Fish Biol. 1976; 14:147-154.
9. Coz-Rakovac R, Strunjak-Perovic I, Hacmanjek M, Topic PN, Lipez Z, Sostaric B. Blood chemistry and histological properties of wild and cultured sea bass (*Dicentrachus labrox*) in the North Adriatic Sea. Vet Res Commun, 2005; 29:677-687.
10. Dacie JV, Lewis SM. Practical haematology, 6th edn. ELBS and Churchill, Livingston, 1984.
11. Das BC. Age related trends in the blood chemistry and haematology of the Indian carp (*Catla catla*). Gerontologia, 1965; 10:47-64
12. Dharan Sing, Kamlesh Nath SP, Thrivedi and Sharma YK. Impact of copper on haematological profile of freshwater fish *Channa punctatus,* Journal of Environmental Biology. 2008; 29(2):253-257.
13. Engel DM, Davis EM. Relationship between activity and blood composition in certain marine teleosts. Copeia, 1964; 3:586-587.
14. Fawcette JK, Scott JE. Practical clinical biochemistry, 4th edn. Arnold Harold Varley, 1960, 119-122.
15. Ferrari A, Venturino A, Pechén AM, D'Angelo. Effects of carbaryl and azinphos methyl on juvenile rainbow trout (*Oncorhynchus mykiss*) detoxifying enzymes. Pesticide Biochemistry and Physiology. 2007; 88(2):134-142.
16. Folin O, Wu H. Span Diagnostic Kits, J Bio Chem. 1920; 9:341-367.
17. Gabriel UU, Anyanwu PE, Akinrotimi OA. Blood characteristics Associated with confinement stress in Black chin Tilapia, *Sarotherodon melanotheron*, Journal of Fish International. 2007; 2:186-189.
18. Goel KA, Sharma SD. Some haematological characteristics of *Clarias batrachus* under metallic stress of arsenic comp. Physiol. Ecol. 1987; 12:63-66.
19. Henry RJ. Clincal chemistry, principles and techniques. Harper and Row, New York, 1968, 664-666.
20. Hickely CR. Comparative hematology of wild and captive cunners, Trans Am. Fish Soc. 1982; 111:242-249.
21. Hill S. A literature review of the blood chemistry of rainbow trout, *Salmo gairdneri*. J Fish Biol. 1982; 20:535-569.
22. Holmes WN, Donaldson EM. The body compartments and the distribution of electrocytes. In Fish Physiology [Hoar, W.S. and Randall, D.J. (eds.)], New York: Academic Press, 1969; 1:1-89.
23. Hughes GM, Nemcsok J. Effects of low pH alone and combined with copper sulphate on blood parameters of rainbow trout. Environ. Pollutant, 1988; 55:89-95.
24. Iqbal MJ, Ali SS, Shakoon AR. Toxicity of lead in freshwater fish *Cirrhinus mrigala* Haematological changes, J Ecotoxi. Environ. Moni. 1997; 7(12):139-143.
25. Joseph John P. Alteration of certain blood parameters of fresh water teleost Mystus vittatus after chronic exposure to Metasystox and Sevin. Fish Physiol Biochem, 2007; 33:15-20.
26. Khanna S, Singh J. Studies on the blood glucose level in *Channa punctatus* (Bloch.). Acta Zool, 1973; 52:97-101.
27. Kori-Siakpere P. Haematological characteristics of *Clarias isheriensis*, Journal of Fish Biology. 1985; 27:259-263.
28. Larsson A, Johansson-Sjobeck FML. Comparative study

of some haematological and biochemical blood parameters in fishes from Skagerrak, J Fish Biol. 1976; 9:425-440.
29. Mishra N, Pandey PK, Datta Munshi S. Haematological parameters of an air breathing mud eel *Amphipnous cuchia* (Ham.), J Fish Biol. 1977; 10:567-573.
30. Ojolick EJ, Cusack R, Benfey TJ, Kerr SR. Survival and growth of all female diploid and triploid *Clarias macrocephalus,* Fish Genet Biotrop Spec Publ, 1995; 52:79-86.
31. Peruzzi S, Varsamos S, Chatain B. Haematological and physiological characteristics of diploid and triploid sea bass, *Dicentrarchus labrax*, J Aquac. 2005; 244:359-367.
32. Rambhaskar B, Srinivasa Roa K. Comparative hematology of ten species of marine fish from Visakhapatnam Coast, J Fish Biol. 1986; 30:59-66.
33. Shakoori AR, Mughal AL, Iqbal MJ. Effects of sublethal doses of fenvalerate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver and muscles of a freshwater fish, *Ctenopharyngodon idella*. Bull. Environ. Contam. Toxicol, 1996; 57:487-494.
34. Singh AK, Singh TP. Seasonal fluctuation in lipid and cholesterol content of ovary, liver and blood serum in related to annual sexual cycle in *Heteropneustes fossilis* (Bloch); Endokrnologie, 1997; 1:47-54.
35. Sowunmi AA. Haematology of the African catfish, *Clarias gariepinus* (Burchell, 1812) from Eleyele Reservoir Ibadan, Southwest Nigeria. Zoologist, 2003; 2:85-91.
36. Stillwell EJ, Benfey TJ. Haemoglobin level, metabolic rate and swimming performance of triploid brook trout *Salvelinus fontinalis,* J Aquac. 1995; 137:355-358.
37. Svobodova Z, Kroupova H, Modra H, Flajshans M, Randak T, Savina LV, Gela D. Haematological profile of common carp spawners of various breeds, J Appl Ichthyol. 2008; 24:55-59.
38. Wintrobe MM. Clinical haematology. Lea and Febiger, Philadelphia, 1974.
39. Yanik T, Atamanalp M. Bal›k Yetiştiriciliğinde Su Kirliliğine Giriş. Atatürk Üniversitesi Ziraat Fakültesi Ofset Tesisi Yay›n Erzurum, 2001; 226:322.