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Serum Biochemical Parameter of Male and Female Rainbow Trout (*Onchorhynchus mykiss*) Cultured in Haraz River, Iran

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Abstract: The serum biochemical parameters of rainbow trout, one of the most cultured fish of the world were assessed using an automated blood analyzer. The study was carried out with 20 rainbow trout from a private fish husbandry in Haraz River, Iran. Fish were catched in summer 2008. Serum sample of rainbow trout were analyzed and their serum parameter value determined as mean ±SD in male and female fish. According to the present results, some parameters were significantly higher in males than females fish ($P \leq 0.05$), including; Total protein (7.7±3.1, 4.3±1.4 g/dl), Albumin (0.54±0.1, 0.34±0.9 mg/dl), Glucose (5.6±0.7, 4.0±0.8 mg/dl), Phosphorous (1.6±0.2, 1.3±0.1 mg/dl), Magnesium (4.1±0.6, 3.6±0.7 mg/dl). There were no significant differences with higher values in male than female of the C3 (38.8±8.5, 37.2 ±9.5 mg/dl), C4(24.5±10.2, 35.7±17.7 mg/dl), Alkaline phosphatase (22.3±6.9, 19.5±5.8 IU/L), Amylase (157.3±44.3, 140.4±17.5 IU/L), Cholesterol (53.3±13.0, 49.4±9.5 mg/dl) and Creatine phosphokinase (54.3±12.6, 50.0±9.4 IU/L), The other biochemical parameter were higher in female and the differences were not significant, in case of; Aspartate aminotransferase (67.6±25.6, 89.9±23.6 IU/L), Alanine aminotransferase (22.8±9.8, 30.2±15.9 IU/L) IgM (78.8±22.5, 91.2±6.3 mg/dl), Iron (26.5±6.4, 30.6±8.5 µg/dl), Calcium (6.8±0.6, 6.7±0.9 mg/dl), Blood Urea Nitrogen (2.7±0.6, 2.8±0.9 mg/dl), between male and female respectively. Cratinin (0.46±0.9, 0.5±0.7 mg/dl),Serum biochemical values reported here will be used as reference for the early detection, identification and monitoring of disease and sublethal conditions in brood rainbow trout cultured in Haraz river, Iran. It was concluded that the current findings can provide a helpful reference for evaluating the health, nutritional status, physiological status of individuals and routine metabolic levels of rainbow trout in Haraz river aquaculture condition.

Key words: Rainbow trout · Biochemical parameters · Serum · Haraz river

INTRODUCTION

Rainbow trout is one of the most commonly cultured fish in the world because it has a rapid growth and easy accommodation to environment conditions [1], so it is the common consumed and economical important fish and is a food staple in many parts of Europe, Asia and North America. Rainbow trout consumption has increased in the Iran, because its production has increased during the last decade. Modern intensive aquaculture tends towards higher stocking density, which often implies increased susceptibility to infections, nutritional diseases and various environmental stress reactions. Aquaculture of Rainbow trout, as with other species of finfish, is adversely affected by production related disorders and infectious diseases. Many of the clinical tools used to evaluate mammalian health are not developed for use in fish. As the aquaculture industry expands, there is an increasing need for improved diagnostic methods [2]. Payne and Colleagues in 1970s introduced for the first time the idea of a "metabolic profile," (MP). They provide a better understanding of the species-specific physiological characteristics, especially with respect to nutritional and farming aspects by haematological analysis [3]. The MP can be considered a valuable method in preventing deficiencies that affect

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the productive and reproductive performances of animals, at least in homeothermic animals [3]. The practical utility of this diagnostic technique is thus clear, as it permits the verification of possible errors in the farming practice so that they can be dealt with before they show up clinically [4]. So great attention has been recently paid to biochemical characterization of fish blood as to an index of the state of internal milieu [5].

The analysis of blood can reveal for us nutritional situation, physiological condition, situations of living habitat of fish, for example in response to stress, pollutants and nutrition as well as ecological and physiological conditions. Major changes occur in the fish blood compositions, such as fluctuations in the hormones levels of proteins, sugar, cholesterol and other basic components [6, 7]. In this study, we determined reference intervals for serum chemistry analyses in cultured rainbow trout. Our clinical chemistry results for rainbow trout, report the values as reference intervals suitable for diagnostic use.

MATERIALS AND METHODS

Rainbow trout were cultured in private center in Haraz River, Iran. Water temperature, Oxygen and pH ranged from 8-10°C, 7-9 mg/l and 6.8-7.2 respectively. Fish were not fed up to sampling to reduce any dietary influences on metabolic status. Clinical and pathological testing were performed avoiding to take sample of sick fish.

Individual rainbow trout was rapidly netted and carefully placed in a circular, tank and were anesthetized with MS222. Ten male and ten female average weirht of 1.950±50 were bleed using 2 ml syringes from the caudal vein. Serum was separated by centrifugation at 3000 g for 15 min at 4°C. After separation all sera maintained at -20°C until processed in the laboratory. Sex and maturity stage of the samples were determined by necropsy. The females, had distinct eggs and males, had lobulated testes.

Analysis Methods: Before analysis, the frozen samples were left to stand at room temperature to thaw and then inverted several times to mix. The serum samples for each specimen were analyzed together in one batch, to avoid run-to-run variability, for the following analyses: total protein (TP), albumin(Alb), glucose (Glu), creatinine (CREA), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Creatine phosphokinase (CPK), Cholesterol (CHOL), Complement C3, Complement C4, Blood urea nitrogen (BUN), Immunoglobulin (IgM), Amylase, Calcium (Ca), Iron (Fe), phosphorus (P) and Magnesium (Mg). All analyses were performed using a blood chemistry Auto analysre (Model Eurolyser).

Statistical Analysis: Blood biochemical values of the Rainbow trout were statistically evaluated using an analysis of variance procedure using one-way ANOVA. Differences in $P<0.05$ were considered to be significant and all results in the text were stated as mean ± standard error (SE).

RESULTS

Male and female Rainbow trout's weight of 1.950±50 g were used in this experiment. Blood biochemical parameters of all groups are given and all parameter levels are compared with each other (Table 1). TOP, Alb, Glu, P and Mg were significantly higher in male than female rainbow trout ($P \leq 0.05$).

There were no significant differences but higher in male compared with female in case of the C3 (38.8±8.5, 37.2±9.5 mg/dl), C4(24.5±10.2, 35.7±17.7 mg/dl), Alkaline phosphatase (22.3±6.9, 19.5±5.8 IU/L), Amylase (157.3±44.3, 140.4±17.5 IU/L), Cholesterol (53.3±13.0, 49.4±9.5 mg/dl) and Creatine phosphokinase (54.3±12.6, 50.0±9.4 IU/L), The other biochemical parameter were higher in female than male however

Table 1: The biometrical parameters in brood male and female of Rainbow trout

Parameters	Male	Female
TP (g/dl)	7.7±3.1^a	4.3±1.4^b
Albumin (mg/dl)	0.5±0.1^a	0.34±0.9^b
Glucose (mg/dl)	5.6±0.7^a	4.01±0.8^b
Cratinin (mg/dl)	0.46±0.9	0.5±0.7
ALT (IU/L)	22.8±9.8	30.2±15.9
AST (IU/L)	67.6±25.6	89.9±23.6
C3 (mg/dl)	38.8±8.5	37.2±9.5
C4 (mg/dl)	24.5±10.2	35.7±17.7
IgM (mg/dl)	78.8±22.5	91.2±36.3
ALP (IU/ml)	22.31±6.9	19.5±5.8
CHOL (mg/dl)	53.3±13.0	49.4±9.5
CPK (IU/L)	54.3±12.6	50.0±9.4
BUN (mg/dl)	2.7±.0.6	2.8±0.9
Amylase (IU/L)	157.3±44.3	140.4±17.5
Ca (mg/dl)	6.8±0.6	6.7±0.9
Fe (µg/dl)	26.5±6.4	30.6±8.5
P (mg/dl)	1.6±0.2^a	1.3±0.1^b
Mg (mg/dl)	4.1±0.6^a	3.6±0.7^b

The difference alphabet, indicate the significant ($P<0.05$) in two sample groups

the differences were not significant, in case of; Aspartate aminotransferase (67.6±25.6, 89.9±23.6 IU/L), Alanine aminotransferase (22.8±9.8, 30.17±15.98 IU/L) IgM (78.8±22.5, 91.2±6.28 mg/dl), Iron (26.5±6.4, 30.6±8.5 µg/dl), Calcium (6.8±0.6, 6.7±0.9 mg/dl), Blood Urea Nitrogen (2.7±.6, 2.8±0.9 mg/dl), between male and female respectively. Cratinin (0.46±0.9, 0.5±0.7 mg/dl).

DISCUSSION

Biochemical analysis can provide valuable information for monitoring the health and condition of fishes. Biochemical indices changes depend on the fish species, age, the cycle of sexual maturity and health condition [6]. Moreover, analysis of serum constituents have showed useful information in detection and diagnosis of metabolic disturbances and disease in fishes [8]. Fish reproduction is one of the factors seriously affecting the internal milieu of the organism. Therefore, great attention is paid to the study of haematological and biochemical indices during the reproduction period [5].

Significant difference between total serum protein levels of male and female rainbow trout observed ($P \leq 0.05$). Total plasma protein concentration in fish range from 2 to 8 g/dl when determined by refractometry [9]. Plasma protein is mainly altered by changes in plasma volume, which in fish may be observed with prolonged starvation or stress [10]. The concentration of total protein in blood plasma is used as a basic index for the health status of brood fish [11] and as indicator of nutritional status [12]. In this study total protein for rainbow trout were consistent with those of previous study in Lake trout (*Salvelinus namaycush*) [13]. Lupi *et al.* [3] found lower total protein in immature rainbow trout in comparison with this study. This difference is mainly due to an increase in the globulin fraction and to some extend the albumin fraction [2, 6, 14]. In our previous study [6] we observed in Persian sturgeon (*Acipenser Persicus*) protein levels increased with age as it is in agreement with rainbow trout and hybrid striped bass [2, 14]. Sano[14] compared serum total protein in two sizes of rainbow trout and found serum total protein in fingerling fish is lower than bigger one and increase of serum total protein coincides with age.

Serum or plasma albumin measures as considerable diagnostic value in laboratory animals because it relates general nutritional status, the integrity of the vascular system and liver function. Hypoalbuminemia may result from impaired synthesis, loss through urine or feces, or increased catabolism [15]. Albumins in the fish organism participate in plastic metabolism and perform transport functions of substances necessary for life activities (first of all, lipids) [16]. Swain *et al.* [11] reported higher albumin concentration in *Labeo rohita* at the time of reproduction. In our study total Albumin levels of male and female rainbow trout were 0.54±0.1, 0.34±0.9 (mg/dl) respectively ($P \leq 0.5$). Similar to our finding Asadi *et al.* [17] found signinificantly higher Albumin in male Beluga (*Huso huso*) serum. Also in our previous study we found significantly higher Albumin in male Persian sturgeon than female fish [6]. Albumin concentration in rainbow trout serum with average weight of 240g was 1.38± 0.05 (g/dl) [18] and Velisek and Svobodova [19] fond albumin concentration 0.4 (g/dl) in rainbow trout serum, therefore our results were coincide with the latest once.

Determination of glucose concentration in blood serum is widely used as an indicator of stress in fish and increase with a fish stress response [20]. Generally, glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in the plasma [20]. Our reference interval for glucose concentration was much lower than that for some freshwater fish for example Lake trout [13], Rainbow trot and Tilapia [2], but was consistent with the mean value of other fish like Adriatic sturgeon [21], Tench [5], Persian sturgeon and starry sturgeon [22]. Glucose concentration also varies because of size, age and nutritional and reproductive status [9]. In our study glucose levels of male and female rainbow trout were 5.6±0.7, 4.0±0.8 (mg/dl) respectively. As we observed in rainbow trout, glucose concentration of male Bluga (*Huso hoso*) bluefin tuna (*Thunnus thynnus*) and Persian sturgeon (*Acipencer persicus*) was significantly higher than female [6, 17, 20]. Total protein and glucose in male were higher than female fish. These higher values for Glu and TP may reflect higher growth rates or higher conversion efficiency in males than females. Baker *et al.* [23], Giberson & Litvak [24] and Hardy and Litvak [25] compared two species of sturgeon in their studies and they attributed higher values for Glu and TP as reflection of higher growth rates or higher conversion efficiency in their comparisons.

Sterols are regarded as essential nutrients because of the role of cholesterol as a cell constituent and as a precursor of steroid and moulting hormones [26]. The liver plays a major role in cholesterol homeostasis by regulating plasma lipoprotein metabolism and lipid output in bile [27, 28]. The cholesterol concentration in rainbow trout in this study was inconsistent with data reported by Manera and Britti [18] and Rehulka *et al.* [29].

Cholesterol concentration varies both among and within fish species because of variations in diet, activity and sexual development [9].

Alkaline phosphatase also is a cell-membrane-associated glycoprotein found in all tissues. One of its functions is active transport of nutrients. This enzyme is often associated with transport mechanisms in the brush border and is used as a proxy for the development of enhanced nutrient uptake in fish larvae [30]. The level of this enzyme is an appropriate indicator for diagnosing hepatic and osteal problems. Plasma Alp activity is influenced by many factors including water chemistry, food intake, temperature and life stage [31]. Data obtained for ALP concentration were higher in male but not significantly. It is consistent with Tench [5], bluefin tuna [20].

Creatinine is derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Severe kidney damage can lead to increased creatinine levels [32]. The reference interval for creatinine concentration in rainbow trout in this study was 0.46±0.9 (mg/dl) for male and 0.5±0.7 (mg/dl) for female rainbow trout ($P>0.05$). They were consistent with data reported by other studies for example; Rehulka *et al.* [29] reported creatinine concentration 0.31 (mg/dl) for rainbow trout and also it is reported 0.41 and 0.46 (mg/dl) for brook and brown trout respectively. Also Manera and Britti, [18] reported serum creatinine concentration for rainbow trout 0.29 (mg/dl).

Little is known of the regulation or factors that affect plasma phosphate concentration. In the present study P levels of male rainbow trout were higher than those of female fish ($P<0.05$). Similar to our study the P levels of male Beluga and Persian sturgeon were significantly higher than females [6, 17].

Magnesium is an essential cofactor in many enzymatic reactions in intermediary metabolism. Magnesium is also required in skeletal tissue metabolism, osmoregulation and neuromuscular transmission. It plays an important role in the respiratory adaptation of freshwater fish, excess Mg is excreted via renal system [33]. In our study Magnesium concentration of male and female rainbow trout were (4.1±0.6, 3.6±0.7 mg/dl) respectively ($P\leq0.05$). Only data obtained from female fish is consistent with Manera and Britti [18] and male data is much higher. Also Hrubec & Smith [35, 39] found Magnesium concentration for rainbow trout 3.1 (mg/dl). In our previous study [6] we found male Persian sturgeon, had significantly ($P<0.05$) higher Magnesium concentration, in comparison with female fish, that it is consistent with this study. Also higher Magnesium concentration in males observed for Persian sturgeon and beluga [17, 34].

In our study calcium levels of male and female rainbow trout were 6.8±0.6, 6.7±0.9 mg/dl respectively ($P>0.5$), while calcium measured in other study were higher for rainbow trout like 9.92 mg/dl [35] and 12.52 mg/dl [18].

Neither stress nor circadian fluctuations have negligible effects on calcium levels [9]. Because about one-half of total plasma calcium is ionized and one-half is bound to plasma proteins [35- 37], a decline in plasma proteins in fasting fishes should also lower plasma calcium concentrations and also increased values can be seen with acute stress [2].

Concentrations of total magnesium are lower than for total calcium in freshwater species and are tightly regulated [9]. This is consistent with our study.

Fish are in close contact with their environment and, as a result, their physiology is influenced accordingly. It is evident that understanding the physiological indices of blood serum of rainbow trout in Haraz river condition is essential for aquaculture in Iran, because it reveals normal indices for propagation, rearing and stocking of this species. Therefore, ''normal'' values for a group of fish in one environment may be ''abnormal'' or deviate outside the reference interval for another population [38], hence evaluation of environment beside other factors seems necessary.

In conclusion, the current findings can provide a helpful reference for evaluating the health, nutritional status, physiological status of individuals and routine metabolic levels of rainbow trout in Haraz river aquaculture condition.

REFERENCES

1. Talas, Z.S. and M.F. Gulhan, 2009. Effects of various propolis concentrations on biochemical and hematological parameters of rainbow trout (Oncorhynchus *mykiss*) Ecotoxicology and Environmental Safety, 72: 1994-1998.
2. Hrubec, T.C., J.L. Robertson and S.A. Smith, 1997. Effects of temperature on hematologic and serum biochemical profiles of hybrid striped bass (*Morone chrysops & Morone saxatilis*). American J. Veterinary Res., 58: 126-130.
3. Lupi, P., V. Vigiani and M. Mecatti, 2006, Contribution to the definition of the metabolic profile of farmed rainbow trout *(Oncorhynchus mykiss)*, Italian J. Animal Sci., 5: 63-71.

4. Melotti, P., A. Roncarati, L. Angellotti, A. Dees, G.E. Magi, C. Mazzini, C. Bianchi and R. Casciano, 2004. Effects of rearing density on rainbow trout welfare, determined by plasmatic and tissue parameters. Italian J. Animal Sci., 3: 393-400.
5. Svoboda, M., J. Kouil, J. Hamackova, P. Kalab, L. Savina, Z. Svobodova and B. Vykusova, 2001. Biochemical profile of blood plasma of tench (*Tinca tinca* L.) during pre- and postspawning period, Acta Vet. Brno., 70: 259-268.
6. Yousefisn, M., M. Sheikholeslami Amiri and D. Kor, 2010. Serum biochemical parameter of male, immature and female Persian sturgeon (Acipencer persicus). In press. Australian J. Basic and Applied Research.
7. Bahmani, M., R. Kazemi and P. Donskaya, P. 2001. A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). Fish Physiology and Biochemistry, 24: 135-140.
8. Jamalzadeh, H.R., A. Keyvan, M.R. Ghomi and F. Gherardi, 2009. Comparison of blood indices in healthy and fungal infected Caspian salmon (*Salmo trutta caspius*), African J. Biotechnol., 8: 319-322.
9. McDonald, D.G. and C.L. Milligan, 1992. Chemical properties of the blood. In: W.S. Hoar, D.J. Randall and A.P. Farrell, (eds). Fish Physiology, San Diego, CA: Academic Press Inc., 12B: 55-133.
10. Knowles, S., T.C. Hrubec, S.A. Smith and R.S. Bakal, 2006. Hematology and plasma chemistry reference intervals for cultured shortnose sturgeon (*Acipenser brevirostrum*), Veterinary Clinical Pathol., 35: 434-440.
11. Swain, P., S. Dash, P.K. Sahoo, P. Routray, S.K. Sahoo, S.D. Gupta, P.K. Meher and N. Sarangi, 2007. Non-specific immune parameters of brood Indian major carp *Labeo rohita* and their seasonal variations, Fish & Shellfish Immunol., 22: 38-43.
12. McCarthy, D.H., J.P. Stevenson and M.S. Roberts, 1973. Some blood parameters of the rainbow trout. *(Salmo gairdneri* Richardson). J. Fish Biol., 5: 1-8.
13. Edsall, C.C., 1999. A Blood Chemistry Profile for Lake Trout, J. Aquatic Animal Health, 11: 81-86.
14. Sano, T., 1960. Hematological studies of the culture fishes in Japan. 3. Changes in blood constituents with growth of rainbow trout. Journal Tokyo University of Fish, 46: 77-87.
15. Nguyen, H.T., 1999. Transport Proteins. In: W.F. Loeb and F.W. Quimby, (Eds.), The Clinical Chemistry of Laboratory Animals, Second Edition. Taylor & Francis, Philadelphia, PA, USA, pp: 309-335.
16. Andreeva, A.M., 1999. Structural and Functional Organization of the Blood Albumin System in Fish, Vopr. Ikhtiol., 39: 825-832.
17. Asadi, F., A. Hallajian, M. Pourkabir, P. Asadian and F. Jadidizadeh, 2006a, Serum biochemical parameters of *Huso huso*. Comparative Clinical Pathol., 15: 245-248.
18. Manera, M. and D. Britti, 2006. Assessment of blood chemistry normal ranges in rainbow trout. J. Fish Biol., 69: 1427-1434.
19. Velisek, J. and Z. Svobodova, 2004. Anaesthesia of rainbow trout (*Oncorhynchus mykiss*) with 2-phenoxyethanol: acute toxicity and biochemical blood profile. Acta Veterinary, 73: 379-384.
20. Percin, P. and S. Konyalioglu, 2008. Serum biochemical profiles of captive and wild northern bluefin tuna (*Thunnus thynnus* L. 1758) in the Eastern Mediterranean, Aquaculture Res., 39: 945-953.
21. Cataldi, E., P. DiMarco, A. Mandich and S. Cataudella, 1998. Serum parameters of Adriatic sturgeon *Acipenser naccarii* (Pisces: Acipenseriformes): effects of temperature and stress. Comparative Biochemistry and Physiol., 121: 351-354.
22. Asadi, F., A. Hallajian, A. Asadian, A. Shahriari and M. Pourkabir, 2009. Serum lipid, free fatty acid and proteins in juvenile sturgeons: *Acipenser persicus* and *Acipenser stellatus*, Comparative Clinical Pathol., 18: 287-289.
23. Baker, D.W., A.M. Wood, M.K. Litvak and J.D. Kieffer, 2005. Hematology of juvenile *Acipenser oxyrinchus* and *Acipenser brevirostrum* at rest following forced activity. J. Fish Biol., 66: 208-221.
24. Giberson, A.V. and M.K. Litvak, 2003. Effects of feeding frequency on growth, food conversion efficiency and meal size of juvenile Atlantic sturgeon and shortnose sturgeon. North American J. Aquaculture, 65: 99-105.
25. Hardy, R.S. and M.K. Litvak, 2004. Effects of temperature on the early development, growth and survival of shortnose sturgeon, *Acipenser brevirostrum* and Atlantic sturgeon, *A. oxyrhinchus*, yolk sac. Environmental Biol. of Fishes, 70: 145-154.
26. Teshima, S.I., 1972. Sterol metabolism. Mem. Fac. Fish., Kagoshima Univ., 21: 69-147.
27. Marzolo, M.P., A. Rigotti and F. Nervi, 1990. Secretion of biliary lipids from the hepatocyte. Hepatol., 12: 134S-142S.
28. Dietschy, J.M., S.D. Turley and D.K. Spady, 1993. Role of the liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J. Lipid Res., 34: 1637-1659.

29. Rehulka, J., B. Minarik and E. Rehulkova, 2004. Red blood cell indices of rainbow trout *oncorhynchus mykiss* (Walbaum) in aquaculture. Aquaculture Res., 35: 529-546.
30. Rust, M.B., 2002. Nutritional Physiology. In: Fish Nutrition (ed. by J.E. Halver and R.W. Hardy), 3rd edn, pp: 367-452. Elsevier Academic Press, San Diego, CA, USA.
31. Sknoberg, D.I., L. Yogev, R.W. Hardy and F.M. Dong, 1997. Metabolic response to dietary phosphorus intake in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 157: 11-24.
32. Aengwanich, W. and A. Tanomtong, 2004. Hematological and serum biochemical values of white ibis (*Threskiornis melanocephalus*), Songklanakarin J. Sci. and Technol., 26: 823-828.
33. Lall, S.P., 2002. The Minerals. In: Fish Nutrition (ed. by J.E. Halver and R.W. Hardy), 3rd edn, pp: 181-257. Elsevier Academic Press, San Diego, CA, USA.
34. Asadi, F.M., A. Masoudifard, K. Vajhi, M. Lee, Pourkabir and Khazraeinia, P. 2006b. Serum biochemical parameters of *Acipenser persicus*. Fish Physiology and Biochemistry, 32: 43-47.
35. Hrubec, T.C. and S.A. Smith, 1999. Differences between plasma and serum samples for the evaluation of blood chemistry values in rainbow trout, channel catfish, hybrid tilapias and hybrid striped bass, J. Aquatic Animal Health, 11: 116-122.
36. Andreasen, P., 1985. Free and total calcium concentrations in the blood of rainbow trout, *Salmo gairdneri*, during 'stress' conditions. J. Experimental Biol., 118: 111-120.
37. Bjornsson, B.T., G. Young, R.J. Lin, L.J. Deftos and H.A. Bern, 1989. Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma calcium regulation, osmoregulation and calcitonin. General and Comparative Endocrinol., 74: 346-354.
38. Hunn, J.B. and L.M. Christenson, 1977. Chemical composition of blood and bile of the shovelnose sturgeon. Progressive Fish Culture, 39: 59-61.
39. Hrubec, T.C. and S.A. Smith, 2000. Hematology of fish. In: B.F. Feldman, J.G. Zinkl and N.C. Jain, (eds). Schalm's Veterinary Hematology. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins, pp: 1120-1125.