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Sexual Differentiation in Blood Biochemistry of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract The sexual differentiation in blood biochemical values were assessed in rainbow trout (*Oncorhynchus mykiss*) cultured in Laribal Trout Culture Farm, Dachigam National Park, Srinagar, India. The blood from the healthy rainbow trout brood was collected for serum and analyzed & expressed as mean±SD in male and female fish. The present investigation revealed some significantly higher in males than female fish (P≤0.05), including total protein (6.8±2.1, 4.4±0.8 g/dl), albumin (0.52±0.1, 0.35±0.2 mg/dl), glucose (5.8±0.3, 4.14±0.5 mg/dl), phosphorous (1.5±0.1, 1.4±0.1 mg/dl), magnesium (4.2±0.3, 3.7±0.2 mg/dl). There were no significant differences with higher values in male than female of the C3 (39.2±3.7, 37.3±3.6 mg/dl), C4 (26.3±2.5, 36.1±3.4 mg/dl), alkaline phosphatase (23.2±2.5, 19.8±2.3 IU/L), amylase (161.4±22.4, 149.2±11.8 IU/L), cholesterol (54.2±2.8, 48.5±2.8 mg/dl) and creatinine phosphokinase (55.2±3.4, 49.8±2.8 IU/L). The other biochemical parameters were higher in female and the differences were not significant, in case of aspartate aminotransferase (70.3±7.2, 88.3±4.8 IU/L), alanine aminotransferase (21.9±3.6, 29.6±6.2 IU/L), IgM (80.1±8.2, 92.1±8.4 mg/dl), Iron (25.4±2.1, 29.2±0.5 µg/dl), calcium (6.9±0.2, 6.8±0.3 mg/dl). The findings would be helpful to establish a baseline to draw any conclusive remarks against the health status of rainbow trout, showing deviation from the normal parameters.
Keywords *Rainbow Trout, Biochemical Parameters, Serum, Trout Farm, Laribal*

1. Introduction

The Laribal trout culture farm is located in the picturesque surroundings of the world famous Dachigam National Park, the only abode of the highly endangered deer species, Hangul (*Cervus elaphus hanglu*). The farm is one of the largest trout breeding and rearing facilities in Asia producing about 6 to 7 tonnes of table size fish annually in addition to about 3.0 lacs of rainbow and brown trout fry. The farm is spread over an area of 40 kanals with water spread area of 5 kanals.

Trout are the most commonly cultured fish in the world, and are a food staple in many parts of Africa, Asia and South America [Talas and Gulhan, 2009]. Aquaculture of trout, as with other species of

finfish, is adversely affected by production related disorders and infectious diseases. Unfortunately, there are few diagnostic tools available to veterinarians and fish health professionals to evaluate disease in fish. Many of the clinical tools used to evaluate mammalian health are not developed for use in fishes. As the aquaculture industry expands, there is an increasing need for improved diagnostic methods. Hematology and clinical chemistry analysis, although not used regularly in fish medicine, can provide substantial diagnostic information once reference values are established. In this study, we determined reference intervals for hematologic and plasma chemistry analytes in cultured trout.

We also evaluated clinical chemistry results from a small group of trout raised under different culture conditions. To our knowledge, this is the first study to determine complete hematologic and clinical chemistry results for trout from Kashmir province of Jammu and Kashmir State, India and to report the values as reference intervals suitable for diagnostic use. Rainbow trout *(Onchorhynchus mykiss)* are an important aquaculture species yet there are few diagnostic tools available to assess their health.

Hrubec (1970) envisaged the need for new diagnostic tools corresponding to increase in aquaculture industry. To understand the species-specific response to various factors including nutritional and farm management practices, the knowledge of haematology is of utmost importance [3]. The concept of 'metabolic profile' in this regard was given by Payne *et al*. (1970). By this method, the productive and reproductive performances of the haemothermic animals could be protected by ensuring the deficiencies which may occur from time to time [3]. The practical utility of this diagnostic technique is this 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 an index of the state of internal milieu [5].

2. Materials and Methods

Healthy rainbow trout brooders (Length 24±2.0 cm and Weight 1.95±50 g) were collected from brood ponds of Laribal fish farm, Srinagar. Water temperature, oxygen and pH ranged from 9-10°C, 8-9 mg/l and 6.8-7.5 respectively. Fish were not fed up to sampling to reduce any dietary influences on metabolic status. Clinical and pathological testing was performed on apparently looking healthy brood fishes only.

Individual rainbow trout was rapidly netted and carefully placed in a circular tank and was anesthetized with MS 222. Ten male and ten female with an average weight of 1.950±50 g were bled 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 were maintained at 20°C until processed in the laboratory. Sex and maturity stage of the samples were determined by necroscopy. The females had distinct eggs and males had lobulated testes.

2.1. Analysis Methods

The blood of the test fishes was collected by cardiac puncture. The blood as frozen before further use. The serum of the blood was collected for the estimation of total protein (TP), albumin (Alb), glucose (Glu), creatinine (CREA), aspartate aminitransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine phosphokinase (CPK), cholestrol (CHOL), complement C3, complement C4, blood urea nitrogen (BUN), immunoglobulin (IgM), amylase, calcium (Ca), iron (Fe), phosphorous (P) and magnesium (Mg).

2.2. Statistical Analysis

One way ANOVA was used to evaluate the biochemical values of rainbow trout. The results were presented as mean±SE.

3. Results

During the present experimental work, male and female rainbow trout were used to analyze the changes in blood values depending upon the sex (Table 1). Significantly higher values (P≤0.05) in males were observed for total protein, albumin, globulin, phosphorous and magnesium. The results were higher but not significant in males in case of C3 (38.3±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 (55.3±13.0, 49.4±9.5 mg/dl) and creatinine phosphokinase (54.3±12.6, 50.0±9.4 IU/L). In contrast to higher values in males, some of the biochemical values showed higher values, though non-significant, in females which include aspartate aminotransferase (67.6±325.6, 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), and blood urea nitrogen (2.7±0.6, 2.8±0.9 mg/dl). The results of the present investigation set up a baseline data for future use in fisheries science to assess the stressors by analyzing any changes in normal haematological/biochemical values in male and female rainbow trout.

Table 1: *The Blood Biochemistry of Brood Male and Female Rainbow Trout (Oncorhynchus mykiss)*

Parameters	Male	Female
TP (g/dl)	6.8±2.1 a	4.4±0.8 b
Albumin (mg/dl)	0.52±0.1 a	0.35±0.2 b
Glucose (mg/dl)	5.8±0.3 a	4.14±0.5 b
Creatinine (mg/dl)	0.46±0.9	0.51±0.3
ALT (IU/L)	21.9±3.6	29.6±6.2
AST (IU/L)	70.3±7.2	88.3±4.8
C3 (mg/dl)	39.2±3.7	37.3±3.6
C4 (mg/dl)	26.3±2.5	36.1±3.4
IgM (mg/dl)	80.1±8.2	92.1±8.4
ALP (IU/ml)	23.2±2.5	19.8±2.3
CHOL (mg/dl)	54.2±2.8	48.5±2.8
CPK (IU/L)	55.2±3.4	49.8±2.8
BUN (mg/dl)	2.6±0.2	2.8±0.4
Amylase (IU/L)	161.4±22.4	149.2±11.8
Ca (mg/dl)	6.9±0.2	6.8±0.3
Fe (µg/dl)	25.4±2.1	29.2±0.5
P (mg/dl)	1.5±0.1a	1.4±0.1 b
Mg (mg/dl)	4.2±0.3a	3.7±0.2 b

The difference alphabet, indicate the significance (P<0.05) in two sample.

4. Discussion

Blood biochemistry is the most economical and authentic tool to assess the health status of fishes. The blood profile of any fish can change with the fish species, age, the cycle of sexual maturity and health conditions [6]. This is through biochemical constituents of the fish blood that the metabolic disturbances of fishes could easily be assessed [8]. Sex of a fish is a great differentiation in various components of the blood of fish, depending upon the quantum of metabolic activities taking place in the organism [5].

The present study revealed significant differences (P≤0.05) in serum protein levels in male and female trout. Plasma protein is the protein component of the blood and is vulnerable to increase with starvation or any other stress [10]. In the present case, plasma protein concentration in rainbow trout ranged from 2 to 8 g/l, as determined through refractometry [9]. Plasma protein gives an index of the health status of the 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 nemaycush*) [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 extent the albumiun fraction [2, 6, 14]. Zorriehzahra *et al*. [40] observed in Persian sturgeon (*Acipensor percius*) protein levels increased with age as it is in agreement with rainbow trout and hybrid stripped bass [2, 14]. Sano [14] compared serum total protein in two sized 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.

Albumin in fish blood performs the transportation of lipids [16] and helps in the general metabolism of fish. The rise in albumin concentration in animals due to loss through urine or faeces or through break down may result in impaired synthesis [15]. Swain *et al*. [11] reported higher albumin concentration in *Lebeo 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≤0.5). Similar to our finding Asadi *et al*. [17] found significantly higher albumin in male Beluga (*Huso huso*) serum. Studies on Persian sturgeon [40] revealed higher albumin values in males than female fish [6]. Manera and Britti (2006) reported a value of 1.38±0.05 (g/dl) of albumin in rainbow trout with an average weight of 240 g [18]. In contrast, Valisek and Svobodova [19] reported a value of 0.4 g/dl, which supports our finding.

Percin and Konyalioglu (2008) reported that glucose in blood serum is the best indicator of stress in fish [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 trout 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. Total protein and glucose in male were higher than female fish. The higher concentration of glucose and total protein in males than female sturgeons is attributed to higher growth rate and higher food conversion efficiency [23, 24, 25].

The liver plays a major role in cholesterol homoestatis [26] 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 is important for active transport of nutrients, and is a cell membrane associated glycoprotein [30]. Sknoberg (1991) documented various factors including age as a factor influencing the ALP concentration [31]. Our results showed non-significant but higher values in males than females. More or less similar results were obtained for Tench [5] and bluefin tuna [20].

Creatinine levels in fish blood may show a rise after severe kidney damage [31]. The reference interval for creatinine concentration in rainbow trout in this study was 0.46±0.9 (mg/dl) for male rainbow trout (P>0.05). They were consistent with data reported by other studies foe examples; 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 trout and brown trout respectively. Also Manera and Britti [18] reported serum creatinine concentration for rainbow trout 0.29 (mg/dl).

Similarly, the P levels of male rainbow trout were higher than those of female fish rainbow trout ($P<0.05$). Our results are supported by the work of [6, 17] who worked on Beluga and Persian sturgeon. 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 and Smith [35, 39] found magnesium concentration for rainbow trout 3.1 mg/dl. Zorriehzahra *et al*. [17, 34, 40] found male Persian sturgeon had significantly ($P<0.05$) higher magnesium concentration, in comparison with female fish that is consistent with this study.

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 circardian 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 a consistent with our study.

Fishes are the best indicators of environment and best experimental animals to assess the impact of various stressors which may arise in any ecosystem. A baseline data of the hematology and biochemistry of the fish are mandatory to get an idea of the impact of various stressors [38], hence evaluation of environment besides 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 aquaculture condition.

5. Conclusion

The present research revealed significant differences in blood profile of the rainbow trout, owing to the sexuality of the fish. The findings of the present research make a baseline for further research in fish haematology and may act as a baseline for the normal hematological indices of this commercially important fish.

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