Rainbow trout (*Oncorhynchus mykiss*) blood profile alterations

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Abstract

There has been a very meager study on the effect of age on the haemo-biochemical performance of rainbow trout at different ages. The present investigation focused on the age specific changes in the hematological and biochemical parameters in rainbow trout, *Oncorhynchus mykiss*. Blood samples collected from 6, 9, 12, 15 and 18 month-old trout (n = 30 for each age) revealed varying results. Values for PCV and RBC indices were significantly lower, and plasma protein concentration was significantly higher in younger fish. Total WBC and lymphocyte counts were significantly higher in fish at 6 and 9 months of age, while neutrophil and monocytes counts were higher at 6, 9, 12 and 15 months. Eosinophil counts were significantly higher in 9 month old fish. Values for most biochemical analytes were significantly different among age groups except for creatinine and potassium concentration.

Introduction

Rainbow trout *(Oncorhynchus mykiss)* are an important aquaculture species yet there are few diagnostic investigations on the health status based on the age. Hematology and clinical chemistry analyses are not used extensively in fish medicine due to the lack of reference intervals for various fish species, and because of the factors such as age which affect blood values. Analysis of serological and hematological parameters is one of the most informative methods to monitor physiological status because these parameters are specific among different species (Celik, 2004; Asadi *et al*., 2006). There is little published information regarding age related changes in blood values of juvenile fish. It is important to evaluate juvenile fish, as this is the time they are raised in aquaculture conditions for commercial purpose. Determining age-related changes in the blood values of fishes would further develop clinical profiles as a diagnostic tool, enhancing both the science of fish medicine and the aquaculture.

Rainbow trout *(Oncorhynchus mykiss)* are an important aquaculture species raised for food and sports, yet little is known about their haemato-biochemical response based on age. There are few tools available to monitor health status in rainbow trout and other species of fish. One such tool, the analysis of blood and plasma, can detect acute and chronic patho-physiologic changes attributable to nutrition, water quality, toxicants, and disease. However, routine diagnostic hematology and clinical chemistry analyses are not used extensively in fish medicine due to the lack of reference intervals for various fish species. Also, external factors such as water quality, diet, culture conditions, and age or sex of the fish can affect some blood values (Hille, 1982; Hrubec *et al*., 1996 a,b; 1997 a,b; Lane 1979; McDonald & Milligan 1992; Ram & Srinivasa, 1989).

There are many studies concerning the hematological and serum enzyme activities of different fish species (Slynko, 1976; Shahsavani *et al*., 2001, 2010; Falahatkar *et al*., 2005; Asadi *et al*., 2006; Gharaei *et al*., 2010, 2012; Rajabipour *et al*., 2006, 2010; and Ahmadifar *et al*., 2011). However, fluctuations in serum component levels and hematological indices during the artificial conditions have not been investigated yet. Owing to the little literature on the normal physiology of trout and their response to different factors particularly age, our study determined the results of complete hematologic (n = 30) and plasma biochemical profiles (n = 30) in rainbow trout. The fish were collected from Trout Culture Farm, Laribal. Blood was analyzed using standard techniques, and reference intervals were determined using nonparametric methods. The aim of this study was to determine the normal fluctuations of the serum biochemical properties of Rainbow trout reared in ponds and provide a basis for future comparative investigations.

Materials and methods

This experiment was carried out on 6-, 9-, 12-, 15 and 18-months old rainbow trout. The fish were reared in the cemented raceways with continuously flowing stream water (10 ± 1.3°C) in Laribal Trout Culture Farm, Dachigam National Park, Srinagar. All fish were fed a pelleted diet (prepared at Kokernag feed mill) during the tenure of the study, at approximately 2 % of body weight per day. The fishes were fed with the commercial trout diet containing fishmeal = 67%, soyabeen meal = 9%, whole wheat =8%, minerals =1%, yeast = 4%, D.L. methionine = 1%, choline chloride = 1%, vitamins = 2%, fish oil = 6%, algenate = 1%. By the start of the study, the fish were accustomed to routine procedures such as daily cleaning, water changes, water sampling, and periodic netting in order to minimize any hematologic or biochemical changes associated with routine handling. The fishes from each age group were evaluated for total length and body weight, measured and recorded (Table 1).

Table 1: The age, mean weight and total length of juvenile rainbow trout (n = 30)

Age (Months)	Weight (g) (Mean±SD)	Length (cm) (Mean±SD)
6	40.00±2.00	9.00±1.12
9	120.00±2.40	12.5±1.5
12	160.00±3.22	15.00±2.12
15	220.00±4.22	22.12±3.00
18	260.00±3.56	25.20±2.5

Blood collection

Fish were bled at the following ages: 6, 9, 12, 15 and 18 months. All fish were fasted 24 h prior to sampling and appeared clinically healthy and in good body condition. During sampling, care was taken to minimize stress in the netted fish and in fish remaining in the tank. All fish were netted within 20 sec and were immediately anesthetized with aerated and buffered tricaine methane sulfonate (150 mg/L MS-222, Sigma Chemical Co, Saint Louis, Mo, USA). Blood samples were collected from the caudal tail vessels with 21 or 23 gauge needles and 1 or 3 cc syringes as soon as the fish lost equilibrium and did not respond to handling, but before ventilatory response was noticeably depressed (−after ~30 seconds of anesthesia). To prevent repeated sampling, fish were not returned to the original tanks after blood collection.

Hematologic analysis
Collected blood was placed into individual EDTA-containing tubes. For each age group, 30 fish were sampled for hematologic analysis. Packed cell volume (PCV) was determined in microhematocrit tubes after centrifugation for 5 min. Plasma protein concentration was determined on the plasma in the microhematocrit tube with a clinical refractometer (Reichert-Jung, Buffalo, NY, USA). Total RBC and WBC counts were determined manually with a Neubauer hemacytometer using Natt-Herrick's solution as diluents (Natt and Herrick, 1952). Cell counts were determined manually because nucleated RBCs prevent accurate enumeration of cell counts on automated analyzers (Huffman *et al*., 1997). Since thrombocytes could not be distinguished from WBC on the hemacytometer, both were counted together; thrombocytes were enumerated and subtracted from the WBC count during the differential count. Blood smears were made using EDTA anticoagulated blood and stained with Wright's-Giemsa for differential counts. Leukocytes and thrombocytes were counted until 200 WBC were enumerated. Hemoglobin was determined using the cyanomethemoglobin method (Sigma Chemical Co); samples were centrifuged prior to reading the absorbance to remove nuclear debris. The MCV, MCH and MCHC were calculated by standard formulas.

Biochemical analysis
Blood was collected as described for hematologic analysis and placed into individual heparinized tubes held on ice. The tubes were centrifuged immediately at 2,750 g and the plasma promptly removed and frozen at –10 °C. Plasma collected from 6 and 9 month old fish was of insufficient volume, such that samples from 2 fish of the same age were pooled to provide composite samples of sufficient volume for analysis. For each age group, 20 samples (composite or individual) were analyzed. Samples were analyzed within 10 days of collection and thawed immediately prior to analysis. Samples were analyzed using an automated dry chemistry system (Kodak Ektachem 700, Rochester, NY, USA) for the following analytes: total protein, albumin, creatinine, and total bilirubin concentrations, alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities, and glucose, cholesterol, ammonia, sodium, chloride, potassium, calcium, magnesium, and phosphorus concentrations. The concentration of globulins was calculated (total protein minus albumin concentrations).

Statistical analysis
Statistical analysis was conducted using a commercial statistical program (Statistix, version 4, Tallahassee, FL, USA). Differences in results among the 5 age groups were determined with a Kruskal-Wallis one-way nonparametric ANOVA. When significant differences ($P < 0.05$) between groups were observed, a Tukey's means comparison test was used to identify which groups were different.

Results

Blood profile of rainbow trout at different ages is presented in table 2. The hemoglobin concentration, MCH, and MCHC were significantly lower in 6 month old fish than in fish at other ages. Leukocytes showed a parabolic trend with smaller values in 6 month old fish (36.07±4.3), peaking up in 12 month old fish (72.0±3.14) and declining back in 18 month old fish (38.4±3.24). More or less similar trend was shown by the lymphocyte count. RBC count was significantly lower in 6 month old fish as compared to other age groups. Erythrocyte counts were observed to be significantly higher at 12 and 15 months of age. Total WBC and lymphocyte were significantly higher in 12 month old fish, while neutrophil were higher in 15 month old fish. Eosinophil counts were significantly higher in 9 month old fish, while as 12 month old fish recorded the highest monocyte count.

Table 2: Blood profile of juvenile rainbow trout (n = 30)

Parameter	6	9	12	15	18
MCV (fl)	83.9±5.8	87.9 ±9.65	83.2 ±12.9	84.0 ±12	81.8 ±13.6
MCH (pg)	18.8±3.5	23.3 ±4.3	20.81 ±3.6	19.09 ±4.5	19.10 ±3.9
MCHC (%)	22.41±4.02	26.8 ±3.40	25.0 ±3.4	22.7 ±3.29	23.3 ±2.6
RBC (10^6 mm)	3.35±0.2 ^{a}	3.68 ±0.15^a	4.18	4.19	4.03
PCV (%)	28.11±1.8^a	32.1 ±1.2^a	±0.20^b	±0.21^b	±0.14^b
Hb (g dl^{-1})	6.3±0.52 ^{a}	8.6 ±0.88^a	34.8 ±1.8^b	35.2 ±2.2^b	33.0 ±1.3 ^{b}
Leukocyte	36.07±4.3	58.3 ±5.2	8.7 ±0.90^b	8.00 ±0.8^b	7.7 ±0.85 ^{b}
(mm^3)	31.6±2.80	46.7 ±3.2	72.0 ±3.14	42.3±4.04	38.4 ±3.24
Lymphocyte	0.6±0.2	1.2 ±0.05	52.3 ±4.2	28.3 ±3.13	22.1 ±3.4
(%)	0.7±0.02	1.0 ±0.02	1.6 ±0.09	2.2 ±0.24	0.8±0.18
Neutrophil (%)	0.2±0.02	1.4 ±0.01	0.4 ±0.03	0.6 ±0.02	0.6 ±0.02
Eosinophil (%)			1.8 ±0.01	1.6 ±0.02	1.05 ±0.02
Monocyte (%)					

Table 3: Pearson correlation and *p* value between hematological parameters of Rainbow trout

Parameter	RBC (10^6 mm)	Hb (g dl^{-1})	PCV (%)	Leukocyte (mm^3)	MCV (fl)	MCH (pg)	MCHC (%)	Lymph (%)	Neutro (%)	Eosino (%)	Mono (%)
RBC (10^6	1	0.67	0.97	0.36	-0.39	-0.07	0.02	0.03	0.73	-0.61	0.84
mm)	0	<0.00	<0.00	0.002	<0.00	<0.00	<0.001	0.004	<0.001	<0.001	<0.00
P value		1	1	0.79	1	1	0.74	0.60	0.59	-0.03	1
Hb (g dl^{-1})		1	0.80	0.003	0.31	0.68	<0.001	0.006	<0.001	<0.001	0.94
P value		0	<0.00	0.47	<0.00	<0.00	0.20	0.17	0.82	-0.44	<0.00
PCV (%)			1	0.05	1	1	0.003	0.561	<0.001	<0.001	1
P value			1	1	-0.16	0.12	0.79	0.93	0.36	-0.17	0.93
Leukocyte			0	0	<0.00	<0.00	0.015*	0.006	0.002	0.002	<0.00
(mm^3)					1	1	0.70	0.51	0.09	0.83	1
P value					0.30	0.68	<0.001	<0.001	<0.001	<0.001	0.71
MCV (fl)					0.006	0.009*	0.98	0.75	0.08	0.58	0.001
P value					1	0.82	<0.001	0.035	<0.001	<0.001	0.12
MCH (pg)					0	<0.00	1	0.77	0.06	0.46	<0.00
P value						1	0	0.075*	<0.001	<0.001	1
MCHC (%)						1		1	0.21	0.04	0.44
P value						0		0	0.003	0.003	<0.00
Lymphocyte (%)									1	-0.29	1
									0	0.132	0.50
P value										1	<0.00
Neutrophil										0	1
(%)											0.47
P value											0.003
Eosinophil											0.80
(%)											0.715
P value											-0.25
Monocyte											0.161
(%)											1
P value											0

* Showed significantly correlation between parameters

The Pearsons correlation matrix and *p* values between various haematological values of rainbow trout are presented in table 3. Significant (p<0.001) differences were observed between RBC, Hb and PCV in contrast to lymphocytes, leukocytes, neutrophils, eosinophils and monocytes. The biochemical variation in fishes of different ages is presented in table 4. Out of all the components, concentrations of total protein, globulin, creatinine, aspartate aminotransferase, phosphorous and glucose were significantly low in 12 month old. Whereas albumin, total bilrubin, sodium, chlorine, calcium, and magnesium were significantly lower in 6 month old, but gradually showed an increase with the increase in age. The Pearsons correlation matrix and *p* values between biochemical parameters of different age groups are presented in table 5.

Table 4: Blood profile of juvenile rainbow trout (n = 30)

Parameter	6	9	12	15	18
Total proteins (g/dl)	3.3	3.2	3.1	3.5	3.6
Albumin (g/dl)	1.2	1.2	1.3	1.3	1.4
Globulin (g/dl)	2.2	1.8	1.8	2.1	2.2
Creatinine (mg/dl)	0.2	0.2	0.1	0.2	0.2
Ammonia (µg/dl)	468	385	346	283	249
Total bilrubin (mg/dl)	0.1	0	0.2	0.1	0.2
Alkaline phosphatase (mU/ml)	73	68	73	59	57
Aspartate aminotransferase (mU/dl)	195	84	28	27	29
Sodium (mEq/L)	134	144	149	154	156
Potassium (mEq/L)	3.40	3.19	3.22	3.22	3.25
Chloride (mEq/L)	143	145	152	150	147
Calcium (mEq/L)	9.98	11.0	10.77	11.26	11.38
Magnesium (mEq/L)	2.0	2.0	2.2	2.3	2.3
Phosphorous (mg/dl)	8.9	6.5	6.4	8.9	9.2
Glucose (mg/dl)	168	141	86	109	110
Cholestrol (mg/dl)	148	159	162	179	184

Discussion

Age related blood and plasma analysis are important to set a baseline regarding the health status of fish at different ages. The parameters can be used as on ground data to assess any impact of stress on the fish by comparing the blood and plasma chemical references with the normal fish. There are certain natural changes in the blood profile of fish after the maturity is attained, so in order to understand the basic haematological profile of fishes, the stages of fish before attainment of sexual maturity are important and worthwhile (Salman, 2010; Lone, 2012). Changes in blood values can be expected as the hematopoietic tissues, the kidney and spleen, continue to develop and mature in juvenile fish (O'Neill, 1989). Additionally, a fish's immune system continues to develop until adulthood (Rijkers and Van Muiswinkel, 1977).

Although there are various studies documenting age related changes in different fishes, but it lacks the comparative analysis between the adult and juvenile fish. There are a few controlled studies comparing blood values from fishes of known ages (Sano, 1960). In rainbow trout, PCV, hemoglobin concentration, MCV, MCH, and MCHC were all lower in juvenile fish compared to mature adults (McCarthy *et al*., 1975). In the present study, although hematologic values were not determined in mature adults, higher values tended to occur in the older juvenile fish. The decrease in WBC and lymphocyte counts in older rainbow trout in this study was similar to the difference described by others between young and adult striped bass (Blaxhall, 1972; Mc Carthy*et al*. 1975).

Remarkable changes in biochemical values between juveniles and adults, and between fish of different sizes have been found previously. As we observed in rainbow trout, protein levels in striped bass increased with age, both in juvenile and adult fish (Sano, 1960). This increase was mainly due to an increase in the globulin fraction and to some extent the albumin fraction (Sano, 1960). Glucose concentration in goldfish decreased with age (Chauin and Young, 1970). In rainbow trout, both glucose and cholesterol concentrations increased as fish size increased (Hille, 1982). No changes in creatinine, bilirubin and magnesium values were detected with age in rainbow trout (Hille, 1982).

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