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RESEARCH ARTICLE

Evaluation of hematological and plasma indices in grass carp, *Ctenopharyngodon idella*, with reference to age, sex, and hormonal treatment

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Abstract. The aim of this study was to assess the changes in hematological and plasma indices of *Ctenopharyngodon idella* (Val.) with regard to age, sex, and hormonal treatment. Sixty specimens of *C. idella,* comprising fifteen of each age (6, 12, 24, and 36 months) were used to evaluate the age factor. Additionally, 20 fish, both females and males, were designated to determine blood indices with regard to sex and hormonal treatment. The following biochemical indexes were determined in the blood plasma: glucose, cholesterol, triglycerides, and total protein (TP). Glucose and triglyceride levels differed significantly among ages, and significant age-related differences were noted in the values of red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). There were no significant differences in any parameters between males and females. In conclusion, our results showed that the plasma parameter profile could be affected by age (RBC, WBC, Hb, MCV, MCH, MCHC, glucose, triglycerides) and hormonal treatment (lymphocytes and eosinophils). The blood plasma biochemical and hematological profiles permitted assessing physiological state, which provided useful information for monitoring changes in the health status of the fish.

Keywords: grass carp, *Ctenopharyngodon idella,* hematological profile, plasma indexes

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Introduction

Hematological indexes are an important tool for assaying physiological and pathological changes, and they are used by fish biologists in many parts of the world (Gabriel et al. 2011). Since hematological analysis supplies valuable knowledge for effectively controlling the condition of both wild and cultured fishes, studies of blood parameters have been carried out to determine the systematic relationship among certain species and to obtain knowledge of their physiology and health status under adverse conditions (Pavlidis et al. 2007, Vázquez and Guerrero 2007). Great attention has been paid recently to blood biochemical parameters as indexes of the physiological state of the internal milieu (Luskova 1997, Bottcher 1998, Edsall 1999). The hematological and biochemical features of fish blood could be useful for monitoring and

assessing the physiological status and health condition of fish at a superlative level (Svetina et al. 2002). Evaluating blood parameters involves determining total erythrocyte count (RBC), total white blood cell count (WBC), hematocrit (PCV), hemoglobin concentration (Hb), and erythrocyte indexes (MCV, MCH, MCHC) (Campbell 2004). Numerous studies have documented that changes in hematological indexes depend on fish species, age, and sexual maturity cycle (Blaxhall 1972, Wedemeyer et al. 1983, Zhiteneva et al. 1989, Bielek and Strauss 1993, Hrubec et al. 2001, Vázquez and Guerrero 2007). Hence, fish sex might also influence blood parameters (Gabriel et al. 2004). Since blood tissue reflects physical and chemical changes in the body, accurate information can be obtained on the general metabolic and physiological status of fish in different age groups and from different habitats (Adeyemo 2007). Research on nile tilapia, *Oreochromis niloticus* (L.), rainbow trout, *Oncorhynchus mykiss* (Walbaum), and common carp, *Cyprinus carpio* L., revealed that hematological parameters were affected by sex (Ezzat et al. 1973, Van Vuren and Hatting 1978). Hormone injections are used to induce spawning in numerous fish species in aquaculture (Mylonas and Zohar 2001). Various anesthetics and bleeding methods affect hematological parameters in fish (Hoffman et al. 1982). Therefore, this study assessed the hematological profile and plasma indexes of *Ctenopharyngdon idella* in relation to age and sex. In addition, blood indexes were studied in males and females that were subjected to hormonal treatment.

Materials and Methods

Fish samples

This experiment was conducted in two trials. The fish were obtained from Kasmahi Cooperative Farm in Rasht, Iran. The farmed fish used in this study were reared in 0.5 ha earthen ponds at the Kasmahi farm. The fish used in the experiment were kept for 14 days in indoor holding tanks (1000 m^2, 1 m deep) to acclimate them and ensure that all the fish were obtained from the same conditions. Half of the water in the experimental tanks was exchanged daily. During the experiment, the water temperature was 22-23°C, and the dissolved oxygen content in the tanks was maintained at 6-7.5 mg l^{-1}. During the experiment, water quality tests indicated that all the parameters met the conditions set for the holding tanks. The values of the water quality parameters were as follows: pH 7.1 ± 0.1; total hardness 35.3 ± 1.6 mg l^{-1}; NH_3-N 0.05 ± 0.01 mg l^{-1}; NO_2 0.03 ± 0.002 mg l^{-1}; NO_3 0.54 ± 0.02 mg l^{-1}.

For the first trial (age study), a total of 60 *C. idella* comprising 15 fish each of age (6 months – mean length 16.40 ± 1.6 cm, mean weight 660 ± 11.93 g; 12 months – mean length 38.66 ± 2.27 cm, mean weight – 1016 ± 88.8 g; 24 months – mean length 46 ± 3.83 cm, mean weight 2578 ± 402.09 g; 36 months – mean length 57.3 ± 3.6 cm, mean weight 4398 ± 519.2 g) were tested. The study material in the second trial (the sex and hormonal treatment study) comprised 20 mature males and females (mean length 64.9 ± 1.47 cm, mean weight 6343.6 ± 618.3 g and mean length 64.7 ± 1.63 cm, mean weight 6336.9 ± 617.9 g, respectively). The females were administered intramuscularly with a double injection of 3 mg kg^{-1} of carp pituitary extract (CPE) homogenized in sterile saline (Akar et al. 2010). The first injection was 10% (0.3 mg kg body weight $^{-1}$) of CPE was given 12 h before the second injection (2.7 mg per kg body weight). The males were injected intramuscularly with CPE at dose of 0.5 mg kg^{-1}.

Blood sampling and analysis

At the termination of the experiment, the fish were captured and sampled after being anesthetized according to Horváth et al. (1984) (20 mgl^{-1}MS-222, Finquel®; Argent TR2905, Redmond, WA, USA). Then, approximately 2 ml of blood was collected from the caudal vein using a 2 ml heparinized syringe. Half of the blood was used for separating plasma (blood chemistry measurements) by centrifuging (3000 rpm for 5 min) and the remaining blood

Table 1
Hematological indexes of *C. idella* at different ages

Parameters	6-months	12-months	24-months	36-months
RBC ($\times 10^6$ µl)	1.25±0.28[a]	1.83 ± 0.25b	2.03 ± 0.20[d]	2.54 ± 0.28[c]
WBC ($\times 10^3$ µl)	13.6 ± 1.53[a]	14.40 ± 0.63[b]	14.57 ± 0.69[b]	14.72 ± 0.61[b]
Hematocrit (%)	31.3 ± 2.9[a]	36.6 ± 1.7[b]	38.8 ± 1.9[b]	39.5 ± 2.4[c]
Hemoglobin (g dl^{-1})	5.97 ± 0.35[a]	6.98 ± 0.73[b]	7.2 ± 1.03[b]	9.9 ± 1.6[c]
MCV (fl)	258.8 ± 41.32[d]	193.6 ± 13.03[c]	188.7 ± 13.6[b]	156.6 ± 17[a]
MCH (pg)	49 ± 8.5[b]	36.5 ± 1.6[a]	36.3 ± 1.7[a]	37.06 ± 1.5[a]
MCHC (g dl^{-1})	19.06 ± 0.79a	19.1 ± 10.05[a]	18.9 ± 2.2[a]	21.1 ± 3.8[b]
Lymphocyte (%)	70.9 ± 4.7	71 ± 4.6	70 ± 4.76	70 ± 4.77
Monocyte (%)	1.06 ± 0.79	1.13 ± 0.83	1.06 ± 0.7	1 ± 0.75
Neutrophil (%)	26.7 ± 4.5	26.6 ± 4.3	26.7 ± 4.7	26.7 ± 4.7
Eosinophil (%)	1.2 ± 0.56	1.2 ± 0.56	1.3 ± 0.61	1.4 ± 0.5
Glucose (mg dl^{-1})	84.2 ± 4.6[a]	118.9 ± 8.9[b]	154.9 ± 8.4[c]	164.5 ± 10[d]
Cholesterol (mg dl^{-1})	275.7 ± 70.8	275.8 ± 70.6	276 ± 68.3	287.2 ± 67.3
Triglycerides (mg dl^{-1})	249.8 ± 25.7[b]	250.7 ± 24.1[b]	265.6 ± 24.3[b]	209.6 ± 30.8[a]
Protein (mg dl^{-1})	3.62 ± 0.13	3.66 ± 0.12	3.62 ± 0.13	3.73 ± 0.15

Values with the different letters are significantly different (P<0.05)

was used for hematological analysis. The plasma indexes (glucose (mg dl^{-1}), cholesterol (mg dl^{-1}), triglyceride (mg dl^{-1}), and total protein (mg dl^{-1})) were measured spectrophotometrically (Technicon, RA-1000, USA) using standard kits (Man kit, Pars Azmoon Company, Iran). The numbers of white blood cells (WBC) ($\times 10^3$ µl), red blood cells (RBC) ($\times 10^6$ µl), mean corpuscular hemoglobin (MCH) (pg), mean corpuscular volume (MCV) (fl), and mean corpuscular hemoglobin concentration (MCHC) (g dl^{-1}) were calculated according to Ranzani-Paiva et al. (2004). The hematocrit value (%) was determined with the standard microhematocrit method and expressed in percentages. Hemoglobin (Hb) (g dl^{-1}) was measured with a spectrophotometer at 540 nm absorbance using the cyanmethemoglobin procedure with a commercial kit (Pars Azmoon) according to Hayatbakhsh et al. (2014). The differential leukocyte count was performed with blood smears stained with Giemsa solution. The smears were examined with light microscopy (Olympus, Tokyo, Japan) in oil immersion at 100 × magnification. All biochemical and hematological parameters were measured in triplicate.

Data analysis

In the age trial, the data were analyzed with one-way ANOVA, and differences among mean values were considered significant at 0.05 with Tukey's test. In the sex and hormonal treatment trial, the data were analyzed statistically with Student's t-test ($P < 0.05$). All statistical analyses were performed with SPSS statistical software (Version 14 for Windows XP). All data are presented as means with standard deviations (SD).

Results

The statistical comparison among different ages revealed significant differences in the values of RBC, WBC, Ht, Hb, MCV, MCH, and MCHC (Table 1, $P < 0.05$). Higher amounts of RBC, WBC, Ht, Hb, and MCHC were recorded in 36-month-old fish, while the highest MCV and MCH values were noted in 6-month-old fish ($P < 0.05$). As can be seen in Table 1, glucose levels increased with age, while a negative trend was observed in triglyceride levels ($P < 0.05$). No significant differences were noted in the hematological and biochemical indexes between the male

Table 2
Plasma indexes of *C. idella* of the two sexes. No significant differences were noted in the hematological and biochemical indexes between the male and female fish (P > 0.05)

Parameters	Males	Females
RBC ($\times 10^6$ µl)	1.9 ± 0.37	1.8 ± 0.34
WBC ($\times 10^3$ µl)	6.8 ±0 .47	7.8 ± 0.53
Hematocrit (%)	27.3 ± 3	24.5 ± 3
Hemoglobin (g dl^{-1})	7.53 ± 1.41	7.16 ± 1.37
MCV (fl)	144.6 ± 11.38	150.6 ± 14.05
MCH (pg)	38.35 ± 2.14	36.33 ± 2.58
MCHC (g dl^{-1})	26.65 ± 2.16	25.1 ± 1.27
Lymphocyte (%)	72.8 ± 2.61	72.1 ± 2.62
Monocyte (%)	1.1 ± 0.56	1.3 ± 0.48
Neutrophil (%)	25.5 ± 2.79	25.4 ± 2.87
Eosinophil (%)	0.36 ± 0.54	0.33 ± 0.37
Glucose (mg dl^{-1})	93.9 ± 8.08	93.4 ± 6.41
Cholesterol (mg dl^{-1})	190.4 ± 10.56	191.6 ± 8.5
Triglycerides (mg dl^{-1})	189.4 ± 17.75	190 ± 20.64
Protein (mg dl^{-1})	3.7 ± 0.14	3.64 ± 0.14

and female fish (Table 2, P > 0.05). The percentage of eosinophils increased after hormone treatment in males, and significant increases were noted in this index before the hormone injection in females (Table 3, P < 0.05). A reverse trend was observed for the percentage of lymphocytes in males and females (Table 3, P < 0.05). The hormone treatment did not affect the biochemical plasma indexes between the males and females (Table 3, P>0.05).

Discussion

Hematological values of cultured fish are determined for a variety of purposes: to establish the normal range of blood parameters (Etim et al. 1999, Baghizadeh and Khara 2015); to assess environmental conditions and to diagnose diseases and any changes in the physiological status of fish (Kori-Siakpere et al. 2005); to investigate conditions that might lead to changes in these values such as sampling methods, temperature, maturity, sex, size, age, and/or fish nutrition (Clarks et al. 1979, Barham et al. 1980, Ranzani-Paiva et al. 2004). Reproduction is a factor that affects the internal milieu of the fish organism; therefore, great attention is paid to the study of hematological and biochemical indexes during reproduction (Svoboda et al. 2001).

In the present study, many of the hematological indexes differed significantly among the fish of different ages with the exception of the percentages of lymphocytes, neutrophils, eosinophils, and monocytes. During the age study, RBC, WBC, Hb, and Ht were lower in 6-month-old fish than in fish of other ages. In caspian brown trout, *Salmo trutta caspius*, the levels of Hb, RBC, MCHC, monocytes, neutrophils, and eosinophils were higher in adult fish in comparison with fish of other ages (Jamalzadeh and Ghomi 2009). However, the levels of Ht, WBC, MCV, MCH, and lymphocytes were greater in smolt than in fish of other ages (Jamalzadeh and Ghomi 2009). That RBC and Hb levels tend to increase with the length and age of fish also corresponds with the results of our study (Clarks et al. 1979). In a study on Lebranche mullet, *Mugil lisa* Val., it was confirmed that the mean values of Ht, Hb, MCV, MCH, and MCHC showed a slight tendency to increase as individuals grew larger (Ranzani-Paiva 1995). Rhythms in Ht and Hb levels can also result from changes in the volumes of plasma or erythrocytes (Sandstrom 1989). According to Conroy (1972), age has a significant effect on the number of RBC. The effect of age-length-weight variables on blood parameters such as lymphocytes is reported to be an essential component of the immune system in the early stages of growth (Aldrin et al. 1982). An increase in age-length-weight independent variables caused increases in the percentages of Hb, RBC, MCHC, monocytes, neutrophils, and eosinophils in *Capoeta trutta* (Heckel) (Orun and Erdeml 2002). Ikechukwu and Obinnaya (2010) reported higher RBC and Hb when length and age of the fish increased. Svetina et al. (2002) report enhanced MCHC in carp, *C. carpio*, as the fish aged, which is in agreement with our findings. A similar increase of MCHC during carp growth from an average body weight of 2858 g to 5305 g was noted by Tran-Duy et al. (2008). It can be concluded that MCHC increases parallel with increasing body weight (Svetina et al. 2002). Blood glucose levels can vary according to season and water temperature, and

Table 3
Biochemical and hematological parameters of *C. idella* before and after hormonal treatment with pituitary gland hormone

	Males		Females	
Parameters	Before	After	Before	After
RBC ($\times 10^6$ µl)	1.94 ± 0.37	1.75 ± 0.64	1.85 ± 0.34	1.87 ± 0.36
WBC ($\times 10^3$ µl)	7.4 ± 0.47	7.4 ± 0.671	7.4 ± 0.48	7.47 ± 0.43
Hematocrit (%)	27 ± 3	28 ± 3	27 ± 3	29 ± 2
Hemoglobin (g dl^{-1})	7.53 ± 1.41	7.51 ± 1.39	7.16 ± 1.37	7.31 ± 1.43
MCV (fl)	144.6 ± 11.38	145.1 ± 11.92	150.6 ± 14.05	159.3 ± 22.73
MCH (pg)	38.3 ± 2.00	38 ± 1.69	38.3 ± 2.58	38.6 ± 3.83
MCHC (g dl^{-1})	26.65 ± 2.16	26.5 ± 2.22	25.7 ± 2.16	24.8 ± 3.35
Lymphocyte (%)	72.8 ± 2.61^b	60 ± 2.98^a	63.2 ± 3.19^a	77.4 ± 2.62^b
Monocyte (%)	1.1 ± 0.56	1.1 ± 0.73	1.3 ± 0.48	1 ± 0.66
Neutrophil (%)	25.5 ± 2.79	27.3 ± 2.98	25.4 ± 2.87	27.3 ± 3.05
Eosinophil (%)	0.6 ± 0.51a	1.6 ± 1.17b	1.5 ± 1.16b	0.6 ± 0.51a
Glucose (mg dl^{-1})	93.9 ± 8.08	91.2 ± 8.84	93.4 ± 6.41	95.6 ± 87
Cholesterol (mg dl^{-1})	190.4 ± 10.56	191.2 ± 9.35	190.6 ± 8.5	194.9 ± 10.92
Triglycerides (mg dl^{-1})	189.4 ± 17.75	190.9 ± 18.52	190 ± 20.64	192 ± 20.08

Values with the different letters are significantly different ($P<0.05$)

glucose levels in fish decrease with age and size (Bridges et al. 1976). Meanwhile, glucose concentrations increased with age in carp, *C. carpio*, in a study by Svetina et al. (2002). In the current age study, higher glucose and triglyceride levels were observed in fish aged 36 and 12 months, respectively. However, Hrubec et al. (2001) suggest that glucose levels in sea bass, *Dicentrarchus labrax* (L.), decreased with age and size. No significant changes in cholesterol or total protein levels were noted with age. Svetina et al. (2002) report similar results regarding cholesterol levels in carp, *C. carpio*. In contrast, data in the literature reveal that total protein and cholesterol levels increase with age and size (Jawad et al. 2004, Coz-Rakovac et al. 2005). Total protein levels are reported to increase with age in striped bass, *Morone saxatilis* (Walbaum) (Hrubec et al. 2001).

No sex-related differences were noted in hematological or biochemical values in the present study. Similar results were reported for plasma total protein in brood pike, *Esox lucius* L., (Pravda et al. 1989) and for plasma glucose in other fish species studied (Bhatnagar and Saksena 1989, Edsall 1999, Svoboda et al. 2001). Of course, high plasma glucose concentrations were reported in relation to gonad development (Robertson et al. 1961, Svobodova and Parova 1977, Zuim et al. 1988). A significant sex difference in total protein of brood tench, *Tinca tinca* (L.), was noted by Svobodova et al. (2001), who also report a sex-related decrease in concentrations of plasma cholesterol in tench immediately after reproduction. Fluctuations in serum cholesterol in male and female peral spot, *Etroplus suratensis* (Bloch), were linked with maturity (Diwan and Krishnan 1986). From these studies and our results, it can be concluded that sex-related changes in plasma indexes differ depending on species and maturity stage.

In our experiment the percentages of lymphocytes and eosinophils changed significantly in response to the hormonal treatment. Some studies indicated changes in plasma components after hormonal injections including increases in total proteins and glucose with Ovaprim and Pregnyl in male grass carp and total protein in female grass carp (Metwally and Fouad 2008), while increases in plasma glucose were noted in common carp after carp pituitary extract injections (Kime and Dolbern 1985). Similarly to our results, no differences in biochemical indexes were observed between two groups of tench females treated with GnRH analog and carp pituitary extract

(Kouril et al. 2007). It is reported that differences in plasma indexes in response to hormonal treatments could stem from species differences, the type of hormone administered, fish maturity, and also species sensitivity to injection stress (Luskova 1998, Hrubec et al. 2001, Öner et al. 2008, Balabanova et al. 2009). Balabanova et al. (2009) report increases in WBC in response to dexametazone phosphate (a synthetic hormone); thus, increases in the percentage of lymphocytes and eosinophils in our study could be related to injection stress.

The results of our study showed marked variability in hematological indexes in *C. idella* in all parameters related to age and hormonal treatment. In this regard, the hormonal-related parameters were the percentage of lymphocytes and eosinophils, while the age-related parameters were RBC, WBC, Hb, MCV, MCH, MCHC and glucose and triglyceride levels.

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