Variability in hematology and plasma indices of common carp *Cyprinus carpio*, associated with age, sex and hormonal treatment

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Received: October 2012 Accepted: June 2014

Abstract

The purpose of this study was to determine changes in hematological and plasma indices of Common Carp *Cyprinus carpio* with regard to age, sex and hormonal treatment. Sixty fish were used to assess the age factor. Also, 20 fish including female and male were assigned to determine blood indices with regard to sex and hormonal treatment. The pituitary gland extract was used as hormone for males and females. Red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), calcium, glucose, cholesterol, triglyceride, protein and phosphorus showed significant differences among ages. However, hematocrit (HCT), lymphocyte and neutrophil did not show significant differences. Statistical analysis revealed that males had higher values of RBC, Ht, Hb, MCV, MCH, lymphocyte, neutrophil and cholesterol compared to females. Some hematology and plasma indices influenced both sexes before and after hormonal treatment. These results provide useful information for monitoring changes in the health status of fish.

Keywords: Hematology, Blood indices, Serum biochemistry, *Cyprinus carpio*

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Introduction

Hematology parameters provide understanding of physiological status of fish at wild and cultured conditions (Wedemeyer and Yasutake, 1977). Changes in these parameters depend on several factors such as species (Ranzani-Paiva *et al*., 2003; Anthony *et al*., 2010), age (Orun and Erdeml, 2002; Jamalzadeh and Ghomi, 2009), sex (Gabriel *et al*., 2001; Orun and Erdeml, 2002) season (Dawson, 1990; Jamalzadeh and Ghomi, 2009), cycle of sexual maturity and health condition (Luskova, 1988; Hrubec *et al*., 2001). Blood parameters are useful criteria for showing physiological disturbances in intensively farmed fishes and can provide important information for prognosis and diagnosis of diseases. Hematological indices have also been used to detect physiological changes in fish (Satheeshkumar *et al*., 2001), as well as indirectly assisting in monitoring aquatic ecosystems (Kori-Siakpere *et al*., 2005). Changes in temperature are known to affect oxygen metabolism and hematology (Powers, 1980). Variations in blood indices may be related to natural physiological cycles, environmental stimuli, or both (Luskova, 1998). Knowledge about erythrocytes is the major and reliable indicators of various sources of stress (Ranzani-Paiva *et al*., 2003). Red blood cells can be used to identify and assess conditions that cause stress to the fish, and consequently ailment in the fish (Pavlidis *et al*., 2007; Tavares-Dias and Moraes, 2007). Their changes may be affected by natural variations in environmental (Lane, 1979), season (Hardig and Hoglund, 1983; Orun and Erdeml, 2002) and feeding behavior (Satheeshkumar *et al*., 2001). Blood plasma is influenced by other factors such as management (Svobodová *et al*., 2006), diseases (Chen *et al*., 2005) and stress (Cnaani *et al*., 2004). Blood serum biochemistry parameters are often used to assess the health status and stress indicators in fishes (Francesco *et al*., 2012). Hematological studies have been performed in *Ctenopharyngodon idella* (Flajšhans, 1997), *Tinca tinca* (Folmar *et al*., 1992) and silver carp, *Hypophthalmichthys molitrix* (Svetina *et al*., 2002). The aim of the present work was to analyze hematological and plasma indices in relation to age and sex. Also, blood indices were investigated when hormonal treatment was used for male and female fish.

Materials and methods

Fish samples

This experiment was conducted in two trials. The fish were provided from Kasmahi Cooperative, Rasht, Iran. Farmed fish used in this study were reared in earthen ponds of 0.5 ha of the Kasmahi company farms. For acclimatization and obtaining the same condition to all fish, the experimental fish were kept for 14 days in indoor holding tanks (1000 m^2) , 1 m deep each. Half of the water in the experimental tanks was exchanged on daily basis. During the experiment, water temperature was 22–3^{0}C, dissolved oxygen in the tanks was maintained at 6–

7.5. Also, during the experiment water quality investigations indicated that all determined parameters fulfill the conditions set for holding tanks. Therefore, it may be assumed that the physicochemical constituents of tanks' water did not have a harmful effect on the investigated hematological and blood chemical indices of *C. carpio*. For the first trial (age study), a total of 40 *C. carpio* comprising 10 fish each (9-months old: mean length 17.1±1.46 cm; mean weight: 870.9 g±22.2, 1-year: mean length 30.4±4.1 cm; mean weight: 1455g±215.5, 2-years: mean length 65.2 ±5.5 cm; mean weight: 4650 g±705.9 and 3-years: mean length 69.1±5.9 cm; mean weight: 5210 g±338.1) were tested. In the second trial (sex and hormonal treatment studies), 20 mature males and females (mean length 73.8±3.1 cm; mean weight: 6585g±318.9. mean length 79.5±7.3 cm; mean weight: 10283g ± 1269.6) were used. Female fish were injected intramuscularly with 3 mg kg^{-1} body weight of pituitary gland at two doses separated by 12 hr interval. Male fish were also injected the same way at the second injection time of female fish.

Blood collection

In both studies for hematological analysis, individual blood samples were taken from caudal vessels of anaesthetized specimens (MS-$_{222}$) using syringes containing EDTA (10%) and then stored in a polystyrene cool bag until used. Blood collection was completed within 1-2 minutes to minimize stress on the fish. After blood samples were taken for hematological analyses, the rest of samples were centrifuged at 3000 rpm for 5 min and plasma was separated for blood chemistry measurements.

Blood analysis

RBC and white blood cells WBC was performed with Neubauer chambers, using Rees diluting solution (1g Brilliant cresyl blue, 31.3g sodium citrate, 10mL formalin (37%) and 1000mL distilled water). Differential leukocyte count was performed with blood smears stained with Giemsa solution. The smears were examined by light microscope (Olympus, Tokyo, Japan) under oil immersion at 100×magnification. Ht was determined using micro hematocrit capillaries filled with blood, centrifuged at 3000 rpm for 5 min, and expressed as percentage of total blood volume. Hb was measured with a spectrophotometer at 540 nm absorbance using the cyan-methemoglobin procedure. The hematological indices including MCV, MCH and MCHC were calculated according to Haney *et al*., (1992). Plasma indices (glucose, cholesterol, triglyceride, protein, calcium and phosphorus) were measured spectro-photometrically (Technicon, RA-1000, America) using standard kits (Man kit, Pars Azmoon Company, Iran).

Data analysis

For the first trial (age study) data were analyzed using one-way ANOVA and differences between mean values were found significant by Turkey's test at 0.05% probability. For the second trial,

data were statistically analyzed using the Student's t-test (p<0.05). All these statistical analyses were performed using the SPSS statistical software (Version 14 for Windows XP). All data are presented as the mean and standard deviation (SD).

Results

The statistical comparison among different ages revealed significant differences in values of RBC, WBC, MCV, MCH, MCHC, Hb and monocyte (Table 1). Higher amounts of RBC, WBC, Ht, Hb, MCHC and monocyte were recorded in 2-year old fish. All biochemical parameters were changed in relation to different ages (Table 2). As can be seen in table 2, with the increase the age, calcium, glucose, cholesterol, triglycerides and protein levels were increased, while a negative trend was observed in phosphorus level. Some hematological and biochemical indices such as WBC, MCV, MCH, Ht, calcium, cholesterol and triglycerides showed significant differences between male and female fish (Table 3). WBC and neutrophil values were increased after hormone treatment in male and female, respectively (Table 4). Some plasma indices such as glucose and cholesterol were increased markedly after hormone treatment in male and female, respectively (Table 5). After hormone injection blood triglycerides was increased in male fish, while a negative pattern was observed in phosphorus level.

Table 1: Hematological parameters of *C. carpio* at different ages.

Parameters	Age			
	9- months	**1-year**	**2-years**	**3-years**
RBC ($\times 10^6$ µl)	1.46 ± 0.10 ^{d}	1.61 ± 0.22 ^{c}	1.87 ± 0.13 ^{a}	1.75 ± 0.85 ^{b}
WBC ($\times 10^3$ µl)	15.6 ± 3.7 ^{cd}	17.8 ± 1.5 ^{c}	24.1 ± 5.1 ^{a}	23.1 ± 4.4 ^{ab}
Hematocrit (%)	30 ± 3.2	34.5 ± 3.52	33.7 ± 4.71	33.2 ± 5.72
Hemoglobin (gr/dl)	5.9 ± 0.43 ^{d}	7.5 ± 1.4 ^{bc}	9.09 ± 0.66 ^{a}	8.2 ± 0.8 ^{b}
MCV (fl)	206.54 ± 10.36 ^{b}	215.22 ± 17.18 ^{a}	183.79 ± 13.74 ^{c}	185.43 ± 13.62 ^{c}
MCH (pg)	40.78 ± 7 ^{c}	46.66 ± 3.81 ^{ab}	48.48 ± 3.44 ^{a}	48.03 ± 2.85 ^{a}
MCHC (gr/dl)	19.51 ± 2.93 ^{cd}	21.76 ± 1.9 ^{c}	26.19 ± 0.58 ^{a}	25.89 ± 0.78 ^{ab}
Lymphocyte (%)	68.5 ± 18.3	59.5 ± 16.2	57.2 ± 5.7	58.3 ± 8.15
Monocyte (%)	0.5 ± 0.71 ^{d}	1.1 ± 1.10 ^{c}	3.6 ± 1.27 ^{a}	3.3 ± 2.41 ^{b}
Neutrophil (%)	30.4 ± 18.8	39.4 ± 16.5	38.8 ± 4.9	38.9 ± 5.8

Values with the different alphabetic letters are significantly different (p<0.05)

Table 2: Plasma indices of *C. carpio* at different ages.

	Age			
Parameters	**9- months**	**1-year**	**2-years**	**3-years**
Calcium (mg/dl)	8.9 ± 0.16^{d}	11.8 ± 0.98^{c}	16.04 ± 0.65^{ab}	16.8 ± 1.26^{a}
Glucose (mg/dl)	84.5 ± 29.7^{b}	78.3 ± 14.9^{bc}	151.4 ± 21.6^{a}	150.2 ± 8.2^{a}
Cholesterol (mg/dl)	122 ± 34.5^{d}	176.7 ± 34.6^{a}	169 ± 29.2^{bc}	173.9 ± 23.8^{ab}
Triglycerides (mg/dl)	38.1 ± 17.4^{d}	77.4 ± 28.8^{c}	151.8 ± 31.4^{a}	149.9 ± 37.4^{ab}
Protein (mg/dl)	1.86 ± 0.29^{c}	3.10 ± 0.37^{ab}	3.82 ± 0.45^{a}	3.88 ± 0.42^{a}
Phosphorus (mg/dl)	12.6 ± 1.44^{a}	12.3 ± 2.95^{ab}	8.9 ± 2.58^{c}	8.6 ± 2.46^{cd}

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

Table 3: Comparison of hematological and plasma indices between males and females of *C. carpio*.

Parameters	**Males**	**Females**
RBC ($\times 10^6$ µl)	1.8 ± 0.93	1.8 ± 0.15
WBC ($\times 10^3$ µl)	25.5 ± 1.6^{a}	21.7 ± 1.8^{b}
Hematocrit (%)	36 ± 2.4^{a}	30.93 ± 2.6^{b}
Hemoglobin (gr/dl)	9.11 ± 0.77	8.2 ± 0.67
MCV (fl)	197.3 ± 1.7^{a}	171.9 ± 3.54^{b}
MCH (pg)	50.9 ± 1.54^{a}	45.5 ± 1.52^{b}
MCHC (gr/dl)	25.7 ± 0.76	26.3 ± 0.39
Lymphocyte (%)	58.2 ± 6.05	57.3 ± 7.09
Monocyte (%)	2.8 ± 1.81	4.1 ± 1.79
Neutrophil (%)	40.1 ± 4.8	37.6 ± 5.5
Calcium (mg/dl)	12.25 ± 1.53^{b}	20.59 ± 0.31^{a}
Glucose (mg/dl)	145.8 ± 12.16	155.8 ± 18.15
Cholesterol (mg/dl)	187 ± 22.7^{a}	155.9 ± 19.4^{a}
Triglycerides (mg/dl)	119.5 ± 9.3^{b}	182.2 ± 10.8^{b}
Protein (mg/dl)	3.7 ± 0.45	3.9 ± 0.38
Phosphorus (mg/dl)	8.7 ± 0.59	8.8 ± 3.52

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

Table 4: Blood characteristics of *C. carpio* before and after hormonal treatment with pituitary gland hormone.

parameters	Male		Female	
	Before	**After**	**Before**	**After**
RBC ($\times 10^6$ µl)	1.82 ± 0.09	1.79 ± 0.10	1.88 ± 0.15	1.75 ± 0.12
WBC ($\times 10^3$ µl)	25.5 ± 4.4 [a]	19.2 ± 7.7 [b]	21.7 ± 4.3	25.4 ± 6.1
Hematocrit (%)	36 ± 2.4	35.4 ± 2.4	30.9 ± 2.6	29 ± 2.58
Hemoglobin (gr/dl)	9.1 ± 0.77	8.8 ± 0.58	8.2 ± 0.67	7.6 ± 0.64
MCV (fl)	197.3 ± 1.72	196.4 ± 6.63	172 ± 3.54	160.8 ± 12
MCH (pg)	50.96 ±2.17	48.02 ± 2.63	45.55 ± 1.52	42.74 ± 3.11
MCHC (gr/dl)	25.7 ± 0.76	24.3 ±1.04	26.3 ± 0.39	27.3 ± 1.02
Lymphocyte (%)	58.2 ± 6.05	60.5 ± 5.72	57.3 ± 7.92	52.9 ± 5.78
Monocyte (%)	2.8 ± 1.81	3.3 ± 1.89	4.1 ± 1.79	3.1 ± 1.73
Neutrophil (%)	40.1 ± 4.89	36.6 ± 5.56	37.6 ± 5.52 [b]	43.2 ± 4.21 [a]

Values with the different alphabetic letters are significantly different (p<0.05)

Table 5: Blood biochemical parameters of *C. carpio* before and after hormonal treatment with pituitary gland hormone.

parameters	Male		Female	
	Before	**After**	**Before**	**After**
Calcium (mg/dl)	12.2 ± 0.67	11.6 ± 0.87	20.5 ± 2.3	15.9 ± 2.7
Glucose (mg/dl)	145.8 ± 12.16 [b]	195.2 ± 16.73 [a]	155.8 ± 18.51 [b]	278.1 ± 40.04 [a]
Cholesterol (mg/dl)	187 ± 22.7	193.4 ± 23.1	155.9 ± 19.47 [a]	133.2 ± 29.4 [b]
Triglycerides (mg/dl)	119.5 ± 9.3 [b]	132.2 ± 8.8 [a]	18.2 ± 10.8	186.3 ± 30.6
Protein (mg/dl)	3.71 ± 0.45	3.83 ± 0.64	3.99 ± 0.38	3.45 ± 0.64
Phosphorus (mg/dl)	8.7 ± 0.59 [a]	5.8 ± 0.84 [b]	8.8 ± 3.56	9.6 ± 1.6

Values with the different alphabetic letters are significantly different (p<0.05)

Discussion

The hematology parameters of a number of cultured fish species have been studied with the aim of establishing normal value ranges, and deviations indicating disturbance in the physiological processes (O'Neal *et al*., 2001). Several of these studies were attempts to determine if significant variations from normal values of these parameters exist that could be attributable to some internal or external factors (Munkittrick and Leatherland, 1983; Gabriel *et al*., 2001). The general trend in the relationship between blood hematocrit and body length is that the longer the fish, the higher the hematocrit in *C. carpio*. For example, Murachi, (1959) found that hematocrit increased as the fish length increased. Similar results were obtained for *Clarius batrachus* (Joshi and Tandon, 1977). Hematological parameters in the present study shows significant differences in RBC, WBC, MCV, MCH, MCHC, Hb and monocyte among different ages. As regards the age, our study showed that RBC, WBC, Ht, Hb, MCHC and monocyte were higher in 2 year old fish than those measured for other ages. In carp *C. carpio*, hematocrit and hemoglobin increased markedly as the fish size increased. This means that the rapid increases of mean body weight, as well as the increase of blood volume, are accompanied by adequate erythropoiesis (Svetina *et al*., 2002). Rhythms in hematocrit and hemoglobin levels may also result from changes in plasma volume or erythrocyte volume (Sandstrom, 1989). RBC and HB levels tend to increase with length and age of the fish (Clark *et al*., 1976). Anthony *et al*., (2010) reported that RBC and Hb levels are higher when length and age of the fish are increased. In agreement with our findings, in carp *C. carpio*, MCHC increased as the fish age increased (Svetina *et al*., 2002). A similar increase of MCHC during the growth of carp from an average body weight of 2858 g to 5305 g was found (Tran-Duy *et al*., 2008). It can be concluded that MCHC increases parallel with the increase of body weight (Svetina *et al*., 2002). In a study on *Mugil platanus* it was verified that mean values for Ht, Hb, MCV, MCH and MCHC showed a slight increase as individuals became larger (Hilge, 1980). Also, larger individuals present higher mean values for MCV, MCH and MCHC for dourado *Salminus maxillosus* (Ranzani-Paiva, 1995). These fluctuations may be due to adaptation strategies in different life stages. Data in the literature revealed that protein and cholesterol levels increased with age and size (Jawad *et al*., 2004; Coz-Rakovac *et al*., 2005), as we showed in our experiment. Protein level in striped bass *Moron saxatilis*, increased with age (Hrubec *et al*., 2001). In sea bass *Dicentrarchus labrox*, reported glucose level decreased with age and size (Hrubec *et al*. 2001). Also, in carp *C. carpio*, no significant changes in cholesterol level with regards to age were observed (Svetina *et al*., 2002). Blood glucose level may vary according to season and water temperature, and glucose level in fish decreased with age and size (Bridges *et al*., 1976). Sex

dependent changes in hematological parameters revealed that some parameters like hemoglobin, hematocrit, erythrocyte number and lymphocyte for male fish were significantly higher than female fish (Orun and Erdem, 2002). Higher hematocrit and hemoglobin levels in male versus female rainbow trout *Onchorhynchus mykiss* were reported (Hardig and Hoglund, 1983). Male *Tenualosa ilisha* showed higher blood hematocrit values than females (Jawad *et al*., 2004). This is in agreement with our results and other fish species such as *Telapia zilli*, Ezzat *et al*., (1973), *C. carpio* Fourie and Hattingh (1976), *Cyprinion macrostomus* Al–Mehdi and Khan, (1984), *Amphiprous cuchia* Banerjee, (1986). Summerfelt, (1967) observed that males consistently had significantly higher hematocrit values than the females. This observation is consistent with the results obtained from our study. Fourie and Hattingh, (1976) suggested that the differences in hematocrit between the two sexes are genetically determined, although Raizada *et al*., (1983) considered that the differences might be due to the higher metabolic rate of males compared to females. Our results support this suggestion, which has been related to an increase in fish activity with an increase in size (Chaudhuri *et al*., 1986). Parma and Croux, (1994) demonstrated that variation in hematological indices with regard to male and female might due to differential oxygen demand by sex. No sex-related difference in glucose level was found for tench, *T. tinca* (Svoboda *et al*., 2001). Changes of blood plasma protein of tench, *Tinca tinca* and grayling, *Thymallus thymallus* were observed between male and female fish (Hlavova, 1989; Svoboda *et al*., 2001). Sex-related differences in cholesterol level were found, with values for females being higher. The differences of blood plasma indices in different life stages and sexes reflect status of many biochemical processes in the metabolism (Oner *et al*., 2008). Changes in plasma indices may be related to natural physiological cycles, environmental stimuli, or both (Luskova, 1998; Hrubec *et al*., 2001). No differences in plasma indices were recorded between the two groups of females tench, *T. tinca* treated with GnRH analogue, or carp pituitary (Kouril *et al*., 2007). In our experiment, glucose and cholesterol levels were increased in both sexes after administration of carp pituitary hormone. The results of this study provided the knowledge on the characteristics of hematological and biochemical parameters of *C.carpio* from three aspects and show that many biological factors influence the ranges of hematology and of serum biochemistry of fish within the same species suggesting that blood parameters may therefore be a value in monitoring the effects of age and sex changes on fish biology and fish culture practices.

Acknowledgments

This research was carried out at Lahijan Islamic Azad University, Iran. The authors wish to thank the manager and

staff of laboratory center for their valuable contribution.

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