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

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### 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), (Orun and Erdeml, 2002: age 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 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 physiological natural 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 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<sup>2</sup>), 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°C, dissolved oxygen in the tanks was maintained at 67.5. Also, during the experiment water quality investigations indicated that all determined parameters fulfill conditions set for holding tanks. Therefore, it may be assumed that the physicochemical constituents of tanks' water did not have a harmful effect on 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 (9months 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  $73.8 \pm 3.1$ 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<sup>-1</sup> 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 by light examined 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 cyanmethemoglobin procedure. hematological indices including MCV, MCH and MCHC were calculated according to Haney et al., (1992). Plasma indices (glucose, cholesterol, triglyceride, protein, calcium phosphorus) were measured spectrophotometrically (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 revealed significant different ages 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 male treatment in and female, respectively (Table 5). After hormone injection blood triglycerides increased in male fish, while a negative pattern was observed in phosphorus level.

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

		Age		
Parameters	9- months	1-year	2-years	3-years
RBC (×10 <sup>6</sup> μl)	1.46 ± 0.10 <sup>d</sup>	1.61 ± 0.22 °	1.87 ± 0.13 <sup>a</sup>	1.75 ± 0.85 <sup>b</sup>
WBC (× $10^3 \mu l$ )	$15.6 \pm 3.7$ cd	$17.8 \pm 1.5$ °	$24.1 \pm 5.1$ $^{\rm a}$	$23.1 \pm 4.4$ ab
Hematocrit (%)	$30\pm3.2$	$34.5 \pm 3.52$	$33.7 \pm 4.71$	$33.2 \pm 5.72$
Hemoglobin (gr/dl)	$5.9 \pm 0.43$ d	$7.5\pm1.4$ bc	$9.09 \pm 0.66^{a}$	$8.2\pm0.8$ b
MCV (fl)	$206.54 \pm 10.36$ b	215.22 ± 17.18 a	183.79 ± 13.74 °	$185.43 \pm 13.62$ °
MCH (pg)	$40.78\pm7~^{\rm c}$	$46.66 \pm 3.81~^{ab}$	$48.48 \pm 3.44$ a	$48.03 \pm 2.85$ a
MCHC (gr/dl)	$19.51 \pm 2.93$ cd	$21.76 \pm 1.9$ °	$26.19\pm0.58~^{a}$	$25.89 \pm 0.78~^{ab}$
Lymphocyte (%)	$68.5 \pm 18.3$	$59.5 \pm 16.2$	$57.2 \pm 5.7$	$58.3 \pm 8.15$
Monocyte (%)	$0.5\pm0.71^{\rm \ d}$	$1.1 \pm 1.10^{\circ}$	$3.6\pm1.27$ a	$3.3 \pm 2.41$ b
Neutrophil (%)	$30.4\pm18.8$	$39.4 \pm 16.5$	$38.8 \pm 4.9$	$38.9 \pm 5.8$

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)	$8.9 \pm 0.16^{d}$	$11.8 \pm 0.98^{c}$	$16.04 \pm 0.65^{ab}$	16.8±1.26 <sup>a</sup>
Glucose (mg/dl)	$84.5 \pm 29.7^{b}$	$78.3 \pm 14.9^{bc}$	$151.4 \pm 21.6^{a}$	$150.2 \pm 8.2^a$
Cholesterol (mg/dl)	$122\pm34.5^{\rm d}$	$176.7 \pm 34.6^{a}$	$169\pm29.2^{bc}$	173.9±23.8ab
Triglycerides (mg/dl)	$38.1\pm17.4^d$	$77.4 \pm 28.8^{c}$	151.8± 31.4 <sup>a</sup>	$149.9 \pm 37.4^{ab}$
Protein (mg/dl)	$1.86\pm0.29^{c}$	$3.10\pm0.37^{ab}$	$3.82\pm0.45^a$	$3.88{\pm}0.42^{a}$
Phosphorus (mg/dl)	$12.6 \pm 1.44^{a}$	$12.3 \pm 2.95^{ab}$	8.9±2.58°	8.6±2.46 <sup>cd</sup>

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 (×10 <sup>6</sup> μl)	$1.8 \pm 0.93$	$1.8 \pm 0.15$
WBC ( $\times 10^3 \mu l$ )	25.5 ± 1.6 a	$21.7 \pm 1.8$ b
Hematocrit (%)	$36\pm2.4~^{\rm a}$	$30.93 \pm 2.6^{\ b}$
Hemoglobin (gr/dl)	$9.11 \pm 0.77$	$8.2 \pm 0.67$
MCV (fl)	197.3 ± 1.7 a	171.9 ± 3.54 b
MCH ( pg )	50.9 ± 1.54 <sup>a</sup>	45.5 ± 1.52 b
MCHC (gr/dl)	$25.7 \pm 0.76$	$26.3 \pm 0.39$
Lymphocyte (%)	$58.2 \pm 6.05$	$57.3 \pm 7.09$
Monocyte (%)	$2.8\pm1.81$	$4.1 \pm 1.79$
Neutrophil (%)	$40.1 \pm 4.8$	$37.6 \pm 5.5$
Calcium (mg/dl)	$12.25 \pm 1.53$ b	$20.59 \pm 0.31$ a
Glucose (mg/dl)	$145.8 \pm 12.16$	$155.8 \pm 18.15$
Cholesterol (mg/dl)	$187 \pm 22.7~^{\rm a}$	155.9 ± 19.4 <sup>a</sup>
Triglycerides (mg/dl)	$119.5 \pm 9.3$ b	$182.2 \pm 10.8$ b
Protein (mg/dl)	$3.7 \pm 0.45$	$3.9 \pm 0.38$
Phosphorus (mg/dl)	$8.7 \pm 0.59$	$8.8 \pm 3.52$

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 (×10 <sup>6</sup> μl)	$1.82 \pm 0.09$	$1.79 \pm 0.10$	$1.88 \pm 0.15$	$1.75 \pm 0.12$
WBC (× $10^3 \mu l$ )	$25.5 \pm 4.4~^{\rm a}$	$19.2\pm7.7$ b	$21.7 \pm 4.3$	$25.4 \pm 6.1$
Hematocrit (%)	$36\pm2.4$	$35.4 \pm 2.4$	$30.9 \pm 2.6$	$29\pm2.58$
Hemoglobin (gr/dl)	$9.1 \pm 0.77$	$8.8 \pm 0.58$	$8.2 \pm 0.67$	$7.6 \pm 0.64$
MCV (fl)	$197.3 \pm 1.72$	$196.4 \pm 6.63$	$172 \pm 3.54$	$160.8 \pm 12$
MCH ( pg )	$50.96 \pm 2.17$	$48.02 \pm 2.63$	$45.55 \pm 1.52$	$42.74 \pm 3.11$
MCHC (gr/dl)	$25.7 \pm 0.76$	24.3 ±1.04	$26.3 \pm 0.39$	$27.3 \pm 1.02$
Lymphocyte (%)	$58.2 \pm 6.05$	$60.5 \pm 5.72$	$57.3 \pm 7.92$	$52.9 \pm 5.78$
Monocyte (%)	$2.8 \pm 1.81$	$3.3 \pm 1.89$	$4.1 \pm 1.79$	$3.1 \pm 1.73$
Neutrophil (%)	40.1 ± 4.89	$36.6 \pm 5.56$	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)	$12.2 \pm 0.67$	$11.6 \pm 0.87$	$20.5 \pm 2.3$	$15.9 \pm 2.7$
Glucose (mg/dl)	$145.8 \pm 12.16$ b	195.2 ± 16.73 a	$155.8 \pm 18.51$ b	$278.1 \pm 40.04~^{a}$
Cholesterol (mg/dl)	$187 \pm 22.7$	$193.4 \pm 23.1$	155.9 ± 19.47 <sup>a</sup>	$133.2 \pm 29.4$ b
Triglycerides (mg/dl)	$119.5 \pm 9.3$ b	$132.2\pm8.8~^{\rm a}$	$18.2\pm10.8$	$186.3 \pm 30.6$
Protein (mg/dl)	$3.71 \pm 0.45$	$3.83 \pm 0.64$	$3.99 \pm 0.38$	$3.45 \pm 0.64$
Phosphorus (mg/dl)	$8.7\pm0.59$ a	$5.8 \pm 0.84$ b	$8.8 \pm 3.56$	$9.6 \pm 1.6$

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 exist that could parameters 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 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 erythrocyte or 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 saxatilis, striped bass Moron 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 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 physiological natural 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 characteristics of hematological 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.

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