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Full Length Research Paper

Comparison of blood indices in healthy and fungal infected Caspian salmon (*Salmo trutta caspius*)

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Hematological parameters were measured for 27 healthy and 20 fungal infected Caspian salmon. White blood cells (WBC), neutrophile, and eosinophile have higher values (P < 0.05) in fungal infected fishes than healthy Caspian salmon. The other parameters like hemoglobin (Hb), hematocrit (HCT), red blood cells (RBC), lymphocyte, and monocyte were greater in healthy Caspian salmon. Weight of healthy fishes had positive significant correlation with WBC. However, a negative correlation was seen between length and weight of fungal infected samples and WBC.

Key words: Blood indices, Caspian salmon, fungal infected and healthy *Salmo trutta caspius*.

INTRODUCTION

Hematological analysis can provide valuable knowledge for monitoring the health and condition of both wild and cultured fishes. Hematological indices changes depend on the fish species, age, the cycle of sexual maturity and health condition (Blaxhall, 1972; Hrubec et al., 2000). Moreover, hematological tests and analysis of serum constituents have showed useful information in detection and diagnosis of metabolic disturbances and disease in fishes (Aldrin et al., 1982).

One of the main types of fungal diseases in farmed salmonid fish is saprolegniasis, caused by species of the genus *Saprolegnia*. It causes considerable economic problems in the fish farming industry (Pottinger and Day, 1999), infecting both fish and fish eggs (Bruno and Wood, 1999). Handling, poor water quality such as water with low circulation, low dissolved oxygen, and high ammonia content, crowding, stress, and decreasing temperatures all help the fungus to establish. The source of fish (wild or cultured) may influence its state of health. This could be revealed by changes in the hematological parameters due to variations in the physico-chemical parameters of habitats, exposure to an etiological agents and environmenttal pollution.

The Caspian salmon *Salmo trutta caspius* is one of the nine subspecies of brown trout *Salmo trutta* (Quillet et al., 1992) that live in Caspian sea and can be considered as an endangered species. They are very rare and can be found mainly in the southwest part of the Caspian Sea, in Iranian waters, where they were harvested commercially (Kiabi et al., 1999). Despite the importance of Caspian salmon as an endangered species, however, little is known about the blood hematology of this species in fungal infected condition. This study was aimed to compare the blood parameters of Caspian salmon in healthy and fungal infected conditions. The study of correlations between hematological parameters with weight and total length of Caspian salmon was the second scope in the present work.

MATERIALS AND METHODS

Hematological parameters were measured for 27 healthy and 20 fungal infected Caspian salmon *Salmo trutta caspius* at Dr. Bahonar salmonid farm, Kelardasht, Iran. The studied fishes belonged to a matured group that were continuously captured from Caspian sea and adapted in this salmonid farm for about 1 - 3 months with a small number of them infected by *Saprolegnia*. They were held at two separate flowing water channels and all rearing conditions and

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Table 1. Hematology of healthy (n = 27) and infected (n = 20) Caspian salmon *Salmo trutta caspius.*

Factor	Healthy	Infected	*t*	*P*-value
Hb (g/dL)	11.38±1.2	9.53±1.6	4.41	0.000
HCT (%)	45.25±4.3	36.45±7.2	5.18	0.000
RBC (× 10^6/µL)	1.34±0.2	1.05±0.2	4.07	0.000
WBC (/µL)	5207±1660	10565±3946	-6.35	0.000
MCV (fL)	347.39±65.3	348.25±62.1	-0.04	0.964
MCH (pg)	86.98±17.2	92.26±17.8	-1.02	0.312
MCHC (g/dL)	25.18±2.1	26.52±2.4	-1.99	0.052
Lymphocyte (%)	73.37±5.0	61.35±7.7	6.43	0.000
Monocyte (%)	4.37±1.3	1.65±0.8	7.80	0.000
Neutrophile (%)	16.59±4.7	27.35±9.9	-4.91	0.000
Eosinophile (%)	5.29±1.4	9.75±3.3	-6.19	0.000

Data are expressed as means ± SD.

photoperiod were held stable for them. Studied fishes were anaesthetized with MS-222 (Sigma Chemical Co., MO, USA) and blood samples (2 mL) for hematological analysis were taken from the caudal vein and collected in a heparinized tube and then stored in a polystyrene cool bag until used. Blood sampling was performed immediately after reproduction and fish were placed in a separate tank for recovery.

Hematocrit (HCT) was determined by spinning blood samples in heparinized capillary tubes in micro-hematocrit centrifuge (13,500 g, 5 min). Hemoglobin (Hb) was measured with spectrophotometer at 540 nm absorbance using cyanmethemoglobin method. For counting red blood cells (RBC) and white blood cells (WBC) a neubauer chamber following the method of Blaxhall and Daisley (1973) with Dacies' solution as a diluting fluid were used. The erythrocyte indices including mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) was calculated according to Haney et al. (1992). Hematological data's distribution for determination of normality has been tested by the Kolmogorov–Smirnov test. All data were analyzed using independent sample t-test at ($P < 0.05$) by SPSS 15. Correlations between variables were established by Pearson's coefficient for linear correlation (r).

RESULTS AND DISCUSSION

To investigate the fish blood factors and their changes, the normal rate of these factors must be initially measured in healthy fish. WBC, neutrophile, and eosinophile have been measured in higher values ($P < 0.05$) in fungal infected fishes than healthy Caspian salmon (Table 1). Quality and quantity of leukocyte cells are generally used in the determination of immune reactions and diseases (Cagirgan, 1990). Changes in WBC and differential counts have been reported to play important roles in the assessment of the state of health of *Clarias gariepinus* (Gabriel et al., 2004). It is known that leukocyte cells are normally lower in healthy fishes and could be used as a significant indicator for infectious diseases. In this study, WBC count (mean ± SD) in healthy fishes was 5207 ± 1660 and in fungal infected ones was 10565 ± 3946. In the present study, the increases in WBC and neutrophile quantities in infected samples were accepted as a response of cellular immune system to fungal infection. Palikova and Navratil (2001) concluded that immune system of fish displays similar responses to unfavorable conditions. Sahan et al. (2007) reported an increase in leukocyte cells of fish infected with the parasite in European eel, *Anguilla anguila.*

The other parameters including Hb, HCT, RBC, lymphocyte, and monocyte were greater in healthy Caspian salmon (Table 1, $P < 0.05$). The lower value of RBC in fungal infected Caspian salmon was in accordance with European eel *A. anguilla*. Genc et al. (2005) and Boon et al. (1989) pointed out the erythrocyte amount of fish infected with parasite was significantly lower in compareson to those in non-infected. Boon et al. (1989) reported there was no significant difference between the fish infected with the parasite and non-infected ones in terms of HCT level, but it was reported that after 7 weeks following infection, HCT and plasma protein levels decreased, WBC quantities reached the highest level in fishes infected with parasite, and there was an adverse relation between the percentages of lymphocyte and granulocyte (neutrophile and eosinophile). Similar to this work, a decrease in the percentage of lymphocyte and an increase in the percentage of granulocyte (neutrophil and eosinophil) were seen in European eel *A. anguilla* infected with the parasite (Sahan et al., 2007). Van Der Heijden et al. (1996) determined an increase in the numbers of lymphocyte and granulocyte cells of fish infected with *A. crassus* and they claimed that a cellular response to this parasite from specific antibodies in eels infected could be produced in time, and certain fish were less affected, thanks to their resistance mechanism derived from genetic variation as respect physiology.

Mean weight of healthy and fungal infected Caspian salmon was 2695.0 g and ranged from 1300.0 to 4200.0

Table 2. Pearson's correlation (r) for body weight and total length of fish samples and hematology factors of healthy (n = 27) and infected (n = 20) Caspian salmon *Salmo trutta caspius.*

Factors	Healthy fish		Infected fish	
	Weight	Length	Weight	Length
Hb	-0.08	-0.13	0.27	0.13
HCT	0.12	0.23	-0.08	0.06
RBC	0.21	0.12	-0.14	-0.04
WBC	0.39*	0.31	-0.36	-0.63**
MCV	-0.14	-0.01	0.05	0.13
MCH	-0.22	-0.18	0.06	0.18
MCHC	-0.23	-0.43*	0.00	0.09
Lymphocyte	-0.12	-0.03	0.19	-0.08
Monocyte	0.25	0.14	0.10	0.01
Neutrophile	-0.01	-0.09	-0.36	-0.10
Eosinophile	0.09	0.00	0.56**	0.47*
Length	0.88**	1	0.75**	1

*Significant at (P < 0.05); ** Significant at (P < 0.01).

g in this work. Mean total length of healthy and fungal infected Caspian salmon was 626.0 cm (in the range of 480.0 to 810.0 cm). According to Table 2, weight of healthy fishes had positive significant correlation with WBC (r = 0.39, P < 0.05) which were similar to *Capoeta trutta* (Orun and Erdeml, 2002). A negative significant relationship (r = -0.43, P < 0.05) can be seen between length of healthy fishes and MCHC (Table 2). In fungal infected samples, a negative correlation was seen between length and weight of fishes and WBC as the correlation was significant for length (r = -0.63, P < 0.01, Table 2). Also weight (r = 0.56, P < 0.01) and length (r = 0.47, P < 0.05) of Caspian salmon was influenced on elevation of eosinophile in fungal infected samples (Table 2). These results were not in agreement with Houston and Wilde (1972), Vuren and Hattingh (1978) and Sahan et al. (2007) who reported that length and weight discrepancies of various fish species had no significant effect on hematological parameters.

The effect of age-length-weight variables on blood parameters such as enhanced formation of lymphocytes is an essential component of immune system in the early stages of growth period (Aldrin et al., 1982). In a research for Nile tilapia *Oreochromis niloticus*, there was an effect of fish size (between small and big fishes) on all the parameters (P < 0.05) except for MCV (Tran-Duy et al., 2008). In a study on *Mugil platanus* it was verified that mean values for HCT, Hb, MCV, MCH and MCHC showed a slight increasing tendency as individuals got larger (Ranzani-Paiva, 1995). Also, larger individuals present higher mean values for MCV, MCH and MCHC for dourado *Salminus maxillosus* (Ranzani-Paiva et al., 2001).

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