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Full Length Research Paper

Hematological characteristics and correlations of diploid and triploid Caspian salmon *Salmo trutta caspius* in juvenile stage

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Hematological analysis can provide valuable knowledge for monitoring the health and conditions of both wild and cultured fishes. The objective of this work was to measure and compare of blood parameters and studying their correlations for Caspian salmon *Salmo trutta caspius.* The values for Hb and HCT were higher for diploid than triploid Caspian salmon (P < 0.05). Triploidisation in Caspian salmon led to a decrease of erythrocyte number about 16%. Diploid Caspian salmon showed the higher level in WBC count than triploid and also eosinophile, neutrophile, and large-lymphocyte revealed the same results (P < 0.05) and only monocyte resulted no significant difference (P > 0.05) between two ploidies. Lymphocyte individually showed more values for triploid than diploid (P < 0.05). There was not a significant difference (P > 0.05) in MCV, MCH, and MCHC among diploid and triploid fishes and we found their proportions with greater values for triploid Caspian salmon. The highest and lowest correlation between hematological parameters for diploid Caspian salmon has been found for RBC-MCH (r = -0.81, n = 30, P < 0.05) and RBC-MCHC (r = -0.02, n = 30, P > 0.05), respectively. The greatest and lowest correlation for triploid Caspian salmon were found for MCV-MCH (r = 0.92, n = 30, P < 0.05) and Hb-RBC (r = -0.01, n = 30, P > 0.05), respectively.

Key words: Caspian salmon, *Salmo trutta caspius*, Correlation, Diploid, Hematological parameters, Triploid.

INTRODUCTION

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). In hematological analyses measurement of blood indices in the liquid part of the blood and the morphological composition of the blood is important. To investigate the fish blood factors and their changes, the normal rate of these factors must be initially measured in healthy fish. Hematological determination can provide substantial diagnostic information once reference values are established under standardized conditions. Hematological studies in animal research and in human diseases are well accepted and considered to be routine procedure in diagnoses (Ranzani-Paiva et al., 2001).

Triploid fish show impaired gametogenesis and subsequent energy investment in somatic growth is not hindered by the metabolic cost of sexual maturation (Peruzzi et al., 2005). Additionally, sterility makes triploids of interest as a means to prevent declines in flesh quality associated with sexual maturation and to address concerns regarding the environmental impact of domestic escapees (Peruzzi et al., 2004). The interest in sterility lies in the possibility that this may lead to increased growth which will normally be directed for gonadal growth (gonad formation) and reproduction is used for somatic growth

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Table 1. Hematology in diploid (n = 30) and triploid (n = 30) Caspian salmon *Salmo trutta caspius.* Data are expressed as means ± SD.

Factors	Diploid	Triploid	*t*	*P*-value
Hb ($g\ dl^{-1}$)	9.16±0.6	7.84±0.7	7.47	0.000**
HCT (%)	45.13±3.2	37.96±4.2	7.26	0.000**
RBC ($\times 10^{6}\ l^{-1}$)	0.88±0.1	0.74±0.1	5.67	0.000**
WBC (/µl)	10531±1665	9124±697	4.27	0.000**
MCV (fl)	495.05±109.3	519.14±80.9	-0.97	0.336
MCH (pg)	104.60±13.7	107.80±17.9	-0.77	0.440
MCHC ($g\ dl^{-1}$)	20.35±1.5	20.74±1.3	-1.03	0.307
Lymphocyte (%)	88.00±3.7	92.30±1.6	-5.71	0.000**
Large-Lymphocyte (%)	1.73±0.7	1.23±0.4	3.06	0.003**
Monocyte (%)	2.03±0.8	1.73±0.7	1.49	0.139
Neutrophile (%)	6.56±2.9	3.60±1.2	5.03	0.000**
Eosinophile (%)	1.7±0.9	1.13±0.3	3.06	0.003**
Weight (g)	38.20±6.0	36.10±5.1	1.45	0.151
Total length (mm)	155.60±9.9	146.9±6.3	4.02	0.000**

** Significant at ($P < 0.01$).

(Pandian and Koteeswaran, 1998). Also Boulanger (1991) believed that growth rate of triploid fish and food conversion rate were better as compared with diploids because of sterility in triploids. Triploidy can be induced in fish by inhibiting the second meiotic division and the extrusion of the second polar body by shocking eggs shortly after fertilization (Malison et al., 1993). A variety of treatments have proven to be effective in inducing polar body retention, including thermal (cold or heat), chemical (colchicine or cytochalasin B) or hydrostatic pressure shocks (Malison et al., 1993).

The Caspian salmon *Salmo trutta caspius* is one of commercial fishes in Caspian sea and prefers to live in the south, west and southwest of the Caspian sea which is much deeper (Berg, 1962). This species adapts easily to captivity and shows acceptable reproductive features and survival rate in cultural conditions, but has a slower growth rate than rainbow trout in rearing condition. Despite of the commercial importance of Caspian salmon as a highly-rare species, relatively little information is available concerning the blood hematology of both diploid and triploid of that species. The objective of this study was to measuring the blood parameters for diploid and triploid and studying their correlations for Caspian salmon.

MATERIALS AND METHODS

Fish samples and experimental condition

Measurements of hematological factors were applied for 30 diploid (2n) and 30 triploid (3n) Caspian salmon *Salmo trutta caspius* at Dr. Bahonar salmonid farm, Kelardasht, Iran. The mean weights of fishes for blood experiments after 2 years-old were 38.20 g and 36.10 g for diploid and triploid, respectively (Table 1) and there were not significant differences (t = 1.45, n = 30, $P > 0.05$) between them.

The diploid fishes received no thermal shock and routinely reproduced from the broodstocks harvested from the Caspian sea and adapted in this salmonid farm. The triploid fingerlings purchased from a triploid producer with application of thermal shock for induction of triploids. Ploidy level was confirmed by the same triploid producer for triploid fish by measuring erythrocyte dimensions from blood smears described by Benfey et al. (1984). The both fingerling groups were held at the similar flowing water channels during experiments and all rearing conditions and photoperiod were relatively held stable for both diploid and triploid fishes. Dissolved oxygen in throughout the culture period was more than 7 ppm and water temperature during blood sampling was about 12 - 13°C. Also the fishes in both groups showed normal behavior, and no apparent signs of infectious or nutritional diseases were observed.

Blood sampling

Studied fishes were anaesthetized with MS-222 (Sigma Chemical Co., MO, USA) and blood samples (1 mL) for hematological analysis for diploid and triploid were taken from the caudal vein and collected in a heparinized tube and then stored in a polystyrene cool bag until used. Blood analysis was performed immediately after sampling 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) by the following formula:

$$MCV\ (fl) = Hct / RBC\ (10^{6}\ l^{-1})$$

$$MCH\ (pg) = [Hb\ (g\ dl^{-1}) \times 10] / RBC\ (10^{6}\ l^{-1})$$

$$MCHC\ (g\ dl^{-1}) = [Hb\ (g\ dl^{-1})] / Hct$$

Table 2. Correlations among blood indices for diploid fishes (n = 30).

	Hb	HCT	RBC	MCV	MCH	MCHC
Hb	1	0.35	0.08	0.29	0.48	0.53
HCT		1	0.09	0.08	0.12	-0.59
RBC			1	-0.29	-0.81	-0.02
MCV				1	0.44*	0.17
MCH					1	0.30
MCHC						1

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

Statistical analysis

Hematological data has been analyzed using independent sample *t*-test at (P < 0.05) by SPSS 14. The correlations between hematological variables for Caspian salmon was analyzed by Pearson's coefficient for linear correlation (r) at P < 0.05.

RESULTS AND DISCUSSION

The mean value for hematological parameters for diploid and triploid Caspian salmon *Salmo trutta caspius* has been summarized in Table 1. There was not a significant difference (P > 0.05) in MCV, MCH, MCHC, and monocyte among diploid and triploid fishes (Table 1). We found MCV, MCH and MCHC with greater values for triploid Caspian salmon. In the literature, values for total Hb and MCHC concentrations in diploid and triploid fish are not consistent and vary among species, whereas the mean cellular hemoglobin content (MCH) is commonly reported to be higher in polyploids (Benfey, 1999). Dorafshan et al. (2007) have been similarly presented that MCV, MCH (P < 0.05), and MCHC (P > 0.05) had greater values in triploid Caspian salmon. Peruzzi et al. (2005) showed a similar result in MCV and MCH in greater amount in triploid than diploid sea bass *Dicentrarchus labrax* (P < 0.05) but there was an adverse result for MCHC with higher value for diploid and their difference was no significant (P > 0.05). As well, Sadler et al. (2000) resulted that triploid Atlantic salmon *Salmo salar* had greater values in MCV and MCH (P < 0.05) but had less value for MCHC (P > 0.05) than diploid.

The values for Hb, HCT, and WBC were higher for diploid than triploid Caspian salmon and their differences were significant (P < 0.05) (Table 1). This is in agreement with the findings reported by Dorafshan et al. (2007) for Caspian salmon. Atlantic salmon triploids have lower Hb concentration (Benfey and Sutterlin, 1984), although this is not always the case with triploids of other species, including some salmonids (Benfey, 1999). Hematocrit values for diploid sea bass was greater than triploid but were not significantly different (P > 0.05) between ploidies (Peruzzi et al., 2005). Also, HCT percentage for diploid of rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis* was slightly higher than their triploids without a significant difference (P > 0.05) (Benfey and Biron, 2000). By contrast, Virtanen et al. (1990) reported a markedly greater increase in HCT, and reduction in MCHC, from triploid rainbow trout forced to swim in a flume. Triploidisation in sea bass *Dicentrarchus labrax* led to a decrease of erythrocyte number (34%) (Peruzzi et al., 2005) and decrease in triploid Caspian salmon in this work was about 16%. The hematological profile of fewer, and larger, erythrocytes in the triploid salmon is also consistent with findings for other triploid species (Benfey and Sutterlin, 1984; Graham et al., 1985; Biron and Benfey, 1994). However, cell size was not measured in this study, decreased blood cell numbers in triploids Caspian salmon could be implied that cell numbers were reduced in proportion to their increase in size. In triploid tench *Tinca tinca* (Svobodova et al., 1998) and shortnose sturgeon *Acipenser brevirostrum* (Beyea et al., 2005) both erythrocyte count and Hb content were decreased.

Diploid Caspian salmon showed the higher level in WBC count than triploid and also its indices including eosinophile, neutrophile, and large-lymphocyte revealed the same results (P < 0.05) and only monocyte resulted no significant difference (P > 0.05) between two ploidies (Table 1). Lymphocyte individually showed more values for triploid than diploid (P < 0.05) . Dorafshan et al. (2007) has been reported no significant difference (P > 0.05) for lymphocytes and neutrophils between diploid and triploid Caspian salmon.

The correlations for the hematological variables have been shown to be a valuable tool in diagnosis procedures. The correlations between hematological parameters regarded to diploid and triploid Caspian salmon has been illustrated in Tables 2 and 3. The highest positive and negative correlation for diploid Caspian salmon (Table 2) was for Hb-MCHC (r = 0.53, n = 30, P < 0.05) and RBC-MCH (r = -0.81, n = 30, P < 0.05), respectively. The greatest positive and negative correlation for triploid Caspian salmon (Table 3) was occurred for MCV-MCH (r = 0.92, n = 30, P < 0.05) and RBC-MCH (r = -0.79, n = 30, P < 0.05), respectively.

The highest and lowest correlation between hematological parameters for diploid Caspian salmon has been found for RBC-MCH (r = -0.81, n = 30, P < 0.05) and

Table 3 Correlations among blood indices for triploid fishes *(n= 30).*

	Hb	HCT	RBC	MCV	MCH	MCHC
Hb	1	0.82	-0.01	0.65	0.61	-0.02
HCT		1	0.20	0.57	0.32	-0.57
RBC			1	-0.68	-0.79	-0.40
MCV				1	0.92**	-0.05
MCH					1	0.33
MCHC						1

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

RBC-MCHC (r = -0.02, n = 30, $P > 0.05$), respectively (Table 2). The greatest and lowest correlation for triploid Caspian salmon were found for MCV-MCH (r = 0.92, n = 30, $P < 0.05$) and Hb-RBC (r = -0.01, n = 30, $P > 0.05$), respectively (Table 3).

A negative correlation (-0.60) was found for MCV and RBC of dourado *Salminus maxillosus* (Ranzani-Paiva et al., 2001). Similar result has been found in our study for Caspian salmon (-0.29 for diploid and -0.68 for triploid). The same pattern occurred in correlation between MCH and RBC (Tables 2 and 3) for both ploidies.

Regression analysis revealed a high correlation between MCV and MCH (r = 0.81, $P < 0.05$) for *Prochilodus lineatus* (Parma De Croux, 1994) that was in accordance with Caspian salmon (r= 0.44 and r= 0.92, for diploid and triploid, respectively, Tables 2 and 3). We found a positive linear relationship between HCT and Hb concentration for triploid Caspian salmon (r = 0.82, $P < 0.05$, Table 3) that was similar to *P. lineatus* (r = 0.88; $P < 0.05$) (Parma De Croux, 1994). There was a poor correla-tion between RBC and HCT for Caspian salmon (r = 0.09 and r = 0.20, for diploid and triploid, respectively, Tables 2 and 3). In contrast, there was a high correlation between RBC and HCT for *P. lineatus* (r = 0.74) (Parma De Croux, 1994).

As a final conclusion, triploidisation in Caspian salmon caused a remarkable decrease in most of hematological parameters such as Hb, HCT, RBC, WBC, large-lymphocyte, neutrophile, and eosinophile. Other parameters includ-ing MCV, MCH, MCHC, and monocyte did not show a significant difference between ploidies. The highest and lowest correlation between hematological parameters for both diploid and triploid fish in this study has been occurred for MCV-MCH and Hb-RBC, respect-tively.

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REFERENCES

Aldrin JF, Messager JL, Laurencin FB (1982). La Biochimie Clinique en Aquaculture. Interet et Perspective. CNEXO, Actes Colloq 14: 291-326.

Benfey TJ (1999). The physiology and behavior of triploid fishes. Rev. Fish. Sci. 7(1): 39-67.

Benfey TJ, Biron M (2000). Acute stress response in triploid rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Aquaculture 184: 167-176.

Benfey TJ, Sutterlin AM (1984). The haematology of triploid landlocked Atlantic salmon, *Salmo salar* L. J. Fish Biol. 24: 333-338.

Benfey TJ, Sutterlin AM, Thompson RJ (1984). Use of erythrocyte measurements to identify triploid salmonids. Can. J. Fish Aquat. Sci. 41: 980–984.

Berg LS (1962). Freshwater fishes of the U.S.S.R. and adjacent countries. Vol. 1, National Science Foundation, Washington D.C.

Beyea MM, Benfey TG, Kieffer JD (2005). Hematology and stress physiology of juvenile diploid and triploid shortnose sturgeon (*Acipenser brevirostrum*). Fish Physiol. Biochem. 31: 303-313.

Biron M, Benfey TJ (1994). Cortisol, glucose and hematocrit changes during acute stress, cohort sampling, and the diel cycle in diploid and triploid brook trout (*Salvelinus fontinalis* Mitchill). Fish Physiol. Biochem. 13: 153–160.

Blaxhall PC, Daisley KW (1973). Routine Haematological Methods for Use Fish with Blood. J. Fish Biol. 5: 771-781.

Boulanger Y (1991). Performance comparison of all-female, diploid and triploid brook trout. In: Pepper VA (Ed.): Proceedings of the Atlantic Canada workshop on methods for the production of non-maturing salmonids, Can. Tech. Rep. Fish Aquat. Sci. 1789: 111-119.

Dorafshan S, Kalbassi MR, Pourkazemi M, Amiri BM, Soltan Karimi S (2007). Effects of triploidy on the Caspian salmon *Salmo trutta caspius* haematology. Fish Physiol. Biochem. Doi: 10.1007/S10695-007-9176-Z.

Graham MS, Fletcher GL, Benfey TJ (1985). Effect of triploidy on blood oxygen content of Atlantic
salmon. Aquaculture 50: 133–139.

Haney DC, Hursh DA, Mix MC, Winton JR (1992). Physiological and hematological changes in chum salmon artificially infected with erythrocytic necrosis virus. J. Aqua. Anim. Health 4: 48–57.

Malison JA, Kayes TB, Held JA, Barry TP, Amundson CH (1993). Manipulation of ploidy in yellow perch (*Perca flavescens*) by heat shock, hydrostatic pressure shock and spermatozoa inactivation. Aquaculture 110: 229–242.

Pandian TJ, Koteeswaran R (1998). Ploidy induction and sex control in fish. Hydrobiologia 384: 167-243.

Parma De Croux MJ (1994). Some haematological parameters in *Prochilodus lineatus*. Rev. Hqdrobiol. Trop. 27(2): 113-119.

Peruzzi S, Varsamos S, Chatain B, Fauvel C, Menu B, Falguière J-C, Severe A, Flik G (2005). Haematological and physiological characteristics of diploid and triploid sea bass, *Dicentrarchus labrax* L. Aquaculture 244: 359-367.

Peruzzi S, Chatain B, Saillant E, Haffray P, Menu B, Falguière J-C (2004). Production of meiotic gynogenetic and triploid sea bass, *Dicentrarchus labrax* L. 1. Performances, maturation and carcass quality. Aquaculture 230: 41-64.

Ranzani-Paiva MJT, Rodrigues EDL, Veiga MLD, Eiras ACD (2001). Association between the hematological characteristics and the biology of the “dourado” *Salminus maxillosus* Valenciennes, 1840 from Mogi-Guaçu River, state of São Paulo, Brazil. Maringa 23(2):

527-533.
Sadler J, Pankhurst NW, Pankhurst PM, King H (2000). Physiological stress responses to confinement in diploid and triploid Atlantic salmon. J. Fish Biol. 56: 506-518.
Svobodova Z, Kola Ova J, Flaj Hans M (1998). The first finding of the differences in complete blood count between diploid and triploid tench, *Tinca tinca* L. Acta vet. Brno 67: 243-248
Virtanen E, Forsman L, Sundby A (1990). Triploidy decreases the aerobic swimming capacity of rainbow trout (*Salmo gairdneri)*. Comp. Biochem. Physiol. 96: 117–121.