

Original Article

Age and sex specific variation in hematological and serum biochemical parameters of Beluga (*Huso huso* Linnaeus, 1758)

Reza Akrami¹, Ahmad Gharaei*², Roghayeh Karami²

¹Department of Fisheries, Islamic Azad University, Azadshahr branch, P.O. Box: 30, Iran.

²Department of Fisheries, International Hamoun Wetland Research Institute, University of Zabol, P.O. Box: 98615-538, Iran.

Abstract: In the present study, the age- and sex-specific changes of various haematological and blood serum biochemical parameters of Beluga (*Huso huso*) were investigated. Blood samples were collected from 4-, 6-, 7-, and 8-year-old beluga (n = 7 for each sex and age). The specimens were fed at a rate of 0.5-3% body weight per day. AST and LDH levels in 7- and 8-year-old fish of both sexes were significantly higher ($P<0.05$) than those in 4- and 6-year-old individuals. The mean ALT were significantly different ($P<0.05$) in both sexes of 4-, 6-, and 7-year-old sturgeon. However, the 6-, 7-, and 8-year-old female sturgeon had higher ALP levels ($P<0.05$). Additionally, mean RBC, PCV, and Hb values were significantly higher ($P<0.05$) in 7- and 8-year-old females and males than the others. Two-tailed Pearson's correlation between the biochemical and haematological parameters obtained for beluga sturgeon indicated significant positive correlations between AST and ALP, AST and LDH, ALP and LDH, RBC and Hb, RBC and PCV, Hb and PCV, MCH and MCHC, and MCV and MCH. However, significant negative correlations were found between RBC and MCV and MCH. These results suggest that the blood parameters of beluga are influenced by age- and sex-specific factors.

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Introduction

Analysis of serological and hematological parameters is one of the most informative methods to monitor physiological status because these parameters are specific among different species and sexes (Celik, 2004; Asadi et al., 2006). The physiological parameter provides a diagnostic tool to assess possible dysfunction and detect the metabolic disturbances, infectious and non-infectious etiologies (Aldrin et al., 1982).

There are many studies concerning the hematological and serum enzyme activities of different sturgeon species (Slynko, 1976; Shahsavani et al., 1999, 2001, 2010; Falahatkar et al., 2005; Asadi et al., 2006; Gharaei et al., 2010; Rajabipour et al., 2006, 2010; Ahmadifar et al.,

2011). However, fluctuations in serum component levels and hematological indices during the artificial conditions have not been investigated yet.

Sturgeons, the producers of valuable black caviar, live exclusively in the northern hemisphere. The Caspian Sea is the habitat of four commercial species of sturgeon (Keyvanshokoo et al., 2009). The giant sturgeon (*Huso huso*) is the largest species of Acipenseriformes and one of the most important species of sturgeon in the Caspian Sea (Jalali et al., 2008). This species is suitable for aquaculture because of its fast growth, ease of reproduction, high tolerance to adverse environmental conditions (Keyvanshokoo et al., 2009).

The aim of this study was to (1) determine the normal fluctuations of the serum biochemical properties of

* Corresponding author: Ahmad Gharaei
E-mail address: agharaei551@gmail.com
Tel: +985422232600

Table 1. The age, mean weight and total length of female and male (n = 7 for each sex and age) Beluga.

Age (year)	Sex	Weight (kg) (Mean±SD)	Length (cm) (Mean±SD)
4+	Female	7.60 ± 0.84	101.20 ± 8.30
	Male	7.80 ± 0.59	102.20 ± 8.90
6+	Female	12.43 ± 0.97	111.14 ± 12.86
	Male	13.12 ± 1.23	114.37 ± 15.92
7+	Female	19 ± 1.11	139.75 ± 8.12
	Male	23.25 ± 2.21	140.05 ± 16.17
8+	Female	25 ± 2.41	142.04 ± 12.65
	Male	26.50 ± 2.36	145.25 ± 9.71

Beluga reared in ponds until maturity, (2) compare the parameters between sexes, and (3) provide a basis for future comparative investigations.

Materials and methods

This experiment was carried out on 4-, 6-, 7-, and 8-years old Beluga. The fish were reared in the earthen ponds supplied with ground water ($19 \pm 3^\circ\text{C}$) from the larval stage at the Shahid Marjani Breeding and Rearing Centre, Iran, from May 2005 to May 2009. They were fed a commercial feed containing 39% protein, 12% lipid, 8% ash, 3% fiber, 0.8% phosphorus and 11% moisture (AbizanTM, Iran). Following sampling from each age group, total length and body weight were measured and recorded (Table 1).

To measure serum biochemical factors during the culture period, blood samples (n = 7 for each sex and age) were taken on 4-, 6-, 7-, and 8-years old fish. All blood samples were collected from behind the anal fin using a 5 mL plastic syringes with 23 gauge needles. Prior to sampling, the fish were anesthetized with 200 mg L⁻¹ MS 222. Blood sera were obtained by centrifuging the sample at 3,000 rpm for 10 min (using a Heraeus Labofuge 400) and the sera were removed with a disposable transfer pipette (Shakoori et al., 1996; Wood et al., 1996). The sera samples were analyzed using an auto-analyzer (Technicon RA1000, USA) and diagnostic kits (Pars Azmoon Co., Tehran, Iran).

To study the hematological parameters, blood samples (n = 7 for each sex and age) were collected in heparinized tube prior to anaesthesia of the fish to prevent haemolysis. The following parameters were subsequently determined according to Akrami et al.

(2009): red blood cell (RBC), white blood cell (WBC), hematocrit (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Statistical analysis: Data were tested for normality and homogeneity of variance before the analysis. The data were examined using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (Duncan, 1995) to rank the groups using SPSS (Version 10). The Pearson's correlation coefficient was used to examine the correlation between each hematological index and serum biochemical parameters.

Results

The fluctuations in serum enzyme activity (i.e. AST, ALP, ALT, and LDH) in female and male Beluga are listed in Table 2. AST levels were significantly higher in 7- and 8-years old males and females than in 4- and 6-years old individuals ($P < 0.05$). Mean ALT levels were significantly different ($P < 0.05$) in both sexes of 4-, 6-, and 7-year-old fish. 6-8 years old females had higher ALP levels than 4 years old females ($P < 0.05$). However, 7- and 8-year-old males and females exhibited significantly higher LDH levels than the others ($P < 0.05$). There were significant positive correlations between AST, ALP, and LDH (Table 3).

The hematological parameters of both sexes and all ages of the fish are listed in Table 4. The mean values of RBC, PCV, and Hb in 7- and 8-year-old females and males different significantly from those of the other age group.

Table 2. The values of serum enzymes activity of female (n = 7) and male (n = 7) Beluga in difference ages.

Parameter	4 ⁺ years		6 ⁺ years		7 ⁺ years		8 ⁺ years	
	Male	Female	Male	Female	Male	Female	Male	Female
AST	297.4 ± 20 ^a	315.7 ± 76.2 ^a	280.8 ± 51.23 ^a	274.5 ± 40.5 ^a	666.5 ± 15.5 ^b	656.2 ± 113 ^b	565.6 ± 81.6 ^b	570.2 ± 124 ^b
ALT	3.4 ± 1.14 ^b	3.8 ± 0.92 ^b	1.1 ± 0.69 ^a	1.6 ± 0.55 ^a	10.3 ± 3.46 ^c	10.3 ± 4.1 ^c	6.1 ± 1.51 ^{bc}	6.3 ± 1.1 ^{bc}
ALP	184.8 ± 28 ^a	184 ± 41 ^a	165.8 ± 39.4 ^a	266 ± 46.5 ^b	184.7 ± 51.6 ^a	208 ± 32.6 ^b	157 ± 37.4 ^a	204.3 ± 33.5 ^b
LDH	487.4 ± 103 ^a	628.3 ± 204 ^a	600 ± 214 ^a	717.5 ± 176.4 ^a	3392 ± 673 ^c	4094 ± 712 ^c	1852 ± 580.2 ^b	2219 ± 634 ^b

Values followed by different superscript letters in each row are significantly different ($P < 0.05$).

Table 3. Pearson correlation and p value between serum enzymes activity of Beluga.

Parameter	AST	ALT	ALP	LDH
AST	1	-0.06	*0.911	*0.865
p value	0	0.939	<0.001	<0.001
ALT		1	-0.033	-0.053
p value		0	0.842	0.753
ALP			1	*0.836
p value			0	<0.001
LDH				1
p value				0

* showed significantly correlation between parameters.

There were significant positive correlation between RBC and Hb, RBC and PCV, Hb and PCV, MCH and MCHC, and MCV and MCH. There were significant negative correlations between RBC, and MCV and MCH (Table 5).

Discussion

Many studies have documented changes in hematological and biochemical parameters in fish according to age or size (Satheeshkumar et al., 2011). However, these investigations are limited to the comparisons between juvenile and adult fish or examination of only wild-caught fish, in which other factors may influence blood variables (Terry et al., 2001). Few studies have investigated blood values from sturgeon of known ages (Gharai et al., 2012; Terry et al., 2001), which makes comparison of our results with those on other fish species difficult.

The mean levels of AST and LDH in the sera of 7- and 8-years old Beluga were significantly higher than those of 4- and 6-year old. The activity of serum enzymes can be increased in two ways; leakage from damage, or induction that increases the amount of the enzyme and hence the amount that is also likely to leak. It is also important to mention that such an

induction may occur when the organism mobilizes its energy sources, including amino acids, to manage stress and repair damage caused by metals (Masola et al., 2008). However, Asadi et al. (2006) reported that mature female *A. persicus* exhibited higher AST activity than immature females. LDH is very nonspecific and is located in the cytoplasm of the most cells. LDH catalysis the conversion of lactate to pyruvate and is therefore an important enzyme for generating energy in cells (Shahsavani et al., 2010). In this study, the higher AST and LDH levels in the older Beluga may have been due to the beginning of the sexual maturation. It should also be noted that LDH levels are directly correlated with growth rate (Rajabipour et al., 2010).

ALT and AST are non-plasma-specific enzymes located within the cells of the liver, heart, gills, kidneys, muscles, and other organs (Gharai et al., 2010). In Beluga, simultaneous increases of ALT and AST enzyme activity with age may be related to changes in physiological metabolites due to sexual development, feeding management and differences in size (Trivedi et al., 2001; Cech et al., 2000). Shahsavand et al. (2010) measured the activity of AST in the sera of mature Beluga and stated that the

Table 4. Hematological parameters of female (n = 7) and male (n = 7) Beluga in difference ages.

Parameters	4 ⁺ years		6 ⁺ years		7 ⁺ years		8 ⁺ years	
	Male	Female	Male	Female	Male	Female	Male	Female
(fl) MCV	314.3 ±8.6	317.9±10.4	288.6 ±9.65	295.7 ±11.32	283.2 ±12.9	300.8 ±27	294 ±19.14	293.8 ±16.11
MCH(pg)	103.8 ±4.5	101.3±11.17	92.2 ±8.1	94.8 ±12	92.3 ±8.5	98.5 ±13.7	95.9 ±14.3	94.56 ±8.3
MCHC (%)	33 ±1.21	32±1.4	32 ±0.96	32.1±1.3	32.6 ±0.95	32.5 ±1.44	32.6 ±0.91	32.1 ±1.63
RBC (10 ⁶ mm)	0.82 ±0.1 ^a	0.79±0.14 ^a	0.85 ± 0.2 ^a	0.84 ± 0.13 ^a	1.13 ± 0.19 ^b	0.97 ± 0.13 ^b	1.02 ± 0.17 ^b	0.99 ± 0.03 ^b
PCV (%)	22.51 ± 2.9 ^a	21.96± 4.4 ^a	24.1 ± 3.4 ^a	24.8 ± 2.1 ^a	28.9 ± 4.3 ^b	27.6 ± 3.1 ^b	29.6 ± 3.4 ^b	29.2 ± 2.2 ^b
Hb (g dl ⁻¹)	7.3 ± 0.87 ^a	7.52± 1.24 ^a	7.74 ± 1.2 ^a	7.62 ± 0.67 ^a	10.1 ± 1.19 ^b	9.93 ± 1.2 ^b	9.7 ± 1.12 ^b	10.4 ± 1.01 ^b
Leukocyte (mm ³)	24.01 ±1.8	24.3±1.5	23.6 ±2.3	24.2 ±1.88	25.7 ±1.12	23 ±1.19	25.4 ±0.84	24.3 ±1.84
Lymphocyte (%)	68.2 ±2.94	72.5±3.4	71 ±3.5	69.6 ±3.14	70.3 ±3.8	71 ±3.33	70.25 ±3.69	70.2 ±3.43
Neutrophil (%)	18.2 ±1.8	19.1±1.16	19.4 ±2.14	20.5 ±1.87	20.4 ±2.21	19.2 ±2.9	19 ±2.45	20.3 ±2.1
Eosinophil (%)	5.8 ±1.2	5.4±1.3	6 ±1.58	6.3 ±1.4	6 ±1.16	6.2 ±1.87	6.5 ±1.12	5.5 ±1.11
Monocyte (%)	4.2 ±0.86	3.41±0.77	3.6 ±0.62	3.3 ±0.46	3.75 ±0.57	3.66 ±1.01	4.01 ±0.94	4.00 ±0.34

Values followed by different superscript letters in each row are significantly different ($P < 0.05$).

Table 5. Pearson correlation and p value between hematological parameters of Beluga.

parameter	Leukocyte (mm ³)	RBC (10 ⁶ mm)	Hb (g dl ⁻¹)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Leukocyte (mm ³)	1	0.224	0.214	0.259	0.011	-0.059	-0.200
p value	0	0.144	0.164	0.090	0.944	0.702	0.194
RBC (10 ⁶ mm)		1	0.832*	0.873*	-0.489*	-0.465*	-0.77
p value		0	<0.001	<0.001	0.002	<0.001	0.619
Hb (g dl ⁻¹)			1	0.982*	0.026	0.096	0.183
P value			0	<0.001	0.869	0.535	0.236
PCV (%)				1	-0.017	0.000	0.003
P value				0	0.915	0.998	0.978
MCV (fl)					1	0.949*	0.064
P value					0	<0.001	0.681
MCH (pg)						1	0.373*
P value						0	0.013
MCHC (%)							1
P value							0

* showed significantly correlation between parameters.

significant increase in AST levels may be related to sexual maturation.

ALP exhibited increased serum activity in 6-, 7-, and 8-years old female Beluga. ALP induces activity transfer and is normally secreted in bile from the liver. This enzyme mainly leaks from the lining of the bile canaliculi and sinusoidal surfaces of hepatocytes (Gottelli et al., 1985). Based on our results, the increase of ALP activity may be due to an increase in biliary canal or osteoblast activity, or feeding conditions (Moraes et al., 2005; Cech et al., 2000). The activities of enzymes that play roles in amino acid catabolism differ based on sex and stage of maturity. Direct significant correlations were obtained between AST, ALP, and LDH, suggesting

that individuals with higher AST levels also tend to have higher ALP and LDH levels.

RBC, PCV, and Hb values were significantly higher in both sexes of 7- and 8-years old Beluga, suggesting that these parameters are age-specific (Table 4). The direct significant correlations found among some hematological parameters suggest that individuals with higher RBC and Hb levels tended to have the parameters that have greater correlations with each other. RBC and HB concentration tended to increase with length and age of the fishes (Satheeshkumar et al., 2011). However, the significant negative correlations between RBC, MCV and MCH could be argumentative.

In this study, we determined the age- and sex-specific changes in biochemical and hematological parameters of the serum in cultured Beluga. A better understanding of relationships between changes in these parameters could have beneficial impact on commercial rearing of this species. These findings can assist environmental and aquaculture officials responsible for making decisions on the management and rearing of this fish.

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