

[Research]

## Effects of starvation and re-feeding on some hematological and plasma biochemical parameters of juvenile Persian sturgeon *Acipenser persicus* Borodin, 1897

M.Yarmohammadi<sup>1\*</sup>, M.Pourkazemi<sup>2</sup>, R.Kazemi<sup>1</sup>, M.Pourdehghani<sup>1</sup>, M.Hassanzadeh Sabet<sup>1</sup>, L.Azizzadeh<sup>1</sup>

1. International Sturgeon Research Institute, Agricultural Research Education and Extension Organization (AREEO), Rasht, Iran

2. Iranian Fisheries Research Organization, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran

\* Corresponding author: Mahtabyarmohammadi@gmail.com

(Received: Oct. 06.2014 Accepted: April. 06.2015)

### ABSTRACT

The effect of starvation and re-feeding was investigated on growth, hematology and biochemical parameters in juvenile Persian sturgeon (*Acipenser persicus*). Three hundred and seventyfive fish ( $108 \pm 0.63$  g) were divided into five feeding groups. The control group (C) was fed to satiation three times a day during the experiment. The four groups were starved for 1 (W1), 2 (W2), 3 (W3), and 4 (W4) weeks respectively, and then fed to satiation during a 4 week re-feeding period. The results indicated that some parameters including final weight, specific growth rate, body weight increase, plasma enzymes (ALT, Alanine aminotransferase, AST, Aspartat aminotransferase and ALP, Alkaline phosphatase), hematological parameters [Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH)] were significantly affected by feeding regimes. The plasma cortisol, hematocrit, lymphocytes, neutrophils, eosinophils, and monocytes were not affected by starvation and subsequent re-feeding. These findings showed that short term starvations had no significant negative effects on growth performance, most biochemical and hematological parameters in Persian sturgeon could recover when re-feeding resumed.

**Keywords:** *Acipenser persicus*, Starvation, Physiology, Biochemistry, Hematology, Compensatory growth

### INTRODUCTION

The feed restriction for fish occur both in nature and captive condition aquaculture. Wild populations, undergo feed restriction due to food limitation, migration, weather events, and during the reproductive cycles (Liu *et al.*, 2011). In captive condition, fish species undergo periods of starvation because of solving water quality problems, reduce handling stress and mortality due to disease outbreaks, temperature changes, and also save feed to increase farm profit (Gaylord and Gatlin III,

2000, Caruso *et al.*, 2011, Wang *et al.*, 2000). During starvation, fish may employ various behavioral, physiological, and structural responses to cover their metabolic needs (Navarro & Gutiérrez, 1995). However, the metabolic and endocrine responses depend on the species and the duration of the deprivation period (Pottinger *et al.*, 2003, Mommsen *et al.*, 1999, Navarro & Gutiérrez, 1995). There is growing information on physiological responses following feed deprivation and re-feeding in fish species due to interest in the

compensatory growth phenomena. Compensatory growth followed by different feeding regimes, has been reported in several fish species (Abolfathi *et al.*, 2012, N Montserrat *et al.*, 2007, NÚria Montserrat *et al.*, 2007, Zhu *et al.*, 2005, Tian and Qin, 2003, Hayward *et al.*, 1997, Nicieza & Metcalfe, 1997). However, there are few studies on the sturgeons, that have shown the morpho-physiological responses following starvation and re-feeding (Falahatkar, 2012, Furné *et al.*, 2012, Yarmohammadi *et al.*, 2012, Liu *et al.*, 2011, Gillis and Ballantyne, 1996, Hung *et al.*, 1997). The physiological responses depending on mobilization of energy reserves are different in fish species. The assessment of hematological and biochemical parameters of blood provides valuable information for evaluating the physiological responses followed by different feeding regimes in fish (Bani & Vayghan, 2011, Coles, 1986). However, the effects of feed deprivation and re-feeding on blood parameters of Persian sturgeon are not understood and therefore further investigation is needed. Regarding the physiological responses under different feeding regimes, changes in hematological factors such as hemoglobin (Hb), hematocrit (Hct), white and red blood cells (WBC and RBC), and corpuscle indices (Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) have been reported in fish species such as Siberian sturgeon, *A. Baerii* (Morshedi *et al.*, 2011) and Nile tilapia, *Oreochromis niloticus* (Abdel-Tawwab, *et al.* 2006). Changes in plasma cortisol levels are different in fish species, and it has been shown from decrease to no consistent changes, or increase in blood cortisol concentration in feed deprived specimens (Mommssen *et al.*, 1999, Pottinger *et al.*, 2003, Polakof *et al.*, 2007). The liver enzymes including alkaline phosphatase (ALP), Aspartat amino transfers (AST) and alanine aminotransferase are localized within the tissue cells of liver and other organs. However, ALP is mainly produced in the liver compare to other organs. Measurement of the these

enzymes in the blood, is one of the most valuable diagnostic tools because they reflect the physiological conditions of fish (Shahsavani *et al.* 2010, Anver 2004). Elevation of liver enzyme activity appears to reflect liver disorders. The stock of the Persian sturgeon, *Acipenser persicus* (Borodin, 1897) has decreased over the past 20 years (Pourkazemi *et al.* 1999). Due to unique biological characteristics of sturgeons such as very long life span and late sexual maturation, make it a suitable species for studies of repetitive feeding regimes or compensatory growth. The Persian sturgeon as a native fish with a fast growth rate and delicious meat, it has become a candidate species for Iranian aquaculture in recent years (Mohseni *et al.*, 2007). Persian sturgeon, like many other fish species, experience periods of starvation in nature and captive condition (Falahatkar, 2012). Despite several studies in teleost fishes regarding starvation effects on physiological functions, there is still much to be understood about the physiology of sturgeons species. The aim of the present study is to assess the metabolic strategies of Persian sturgeon exposed to different periods of starvation and followed by re-feeding or compensatory growth. In this study, the effect of starvation and re-feeding was monitored on some hematological and plasma biochemical parameters. Knowledge of how fish respond to starvation periods in this study could provide a basis for improved nutrition and rearing and thereby help to optimize *Acipenser persicus* culture.

## MATERIALS AND METHODS

### Test specimens and experimental conditions

Juveniles (7-month-old) Persian sturgeon (*A. persicus*) were provided by Shahid Beheshti Sturgeon Propagation and Rearing Complex (Rasht, Iran) between October and December 2009. Prior to the experiment, fish were acclimated to experiment conditions for 2 weeks while they fed three times a day (08:00, 13:00 and 18:00) on a commercial food (BIOMAR, INICIO Plus 868Nersac, France) containing 48% crude protein, 22% crude fat.

Fish were maintained under a free-stress condition during the experimental period. Water quality parameters, such as dissolved oxygen (6–8 mg.L<sup>-1</sup>), pH (7.5) and temperature (16.5–18.5 C°) were monitored daily and did not change significantly between treatments during the experiment.

After acclimation, 375 fish with a mean initial weight  $108 \pm 0.63$  g were randomly assigned to five feeding groups. Three replicates were assigned for each group, consisting of 25 fish per tank in a flow-through system with water supplied from the Sefidrood River and aerated well. The experiment lasted for 8 weeks and was divided into two periods, starvation periods (weeks 1 to 4) and a re-feeding period (weeks 4 to 8). The control fish (C) were fed on the commercial diet to satiation three times a day throughout the experiment.

Fish in other four groups were subjected to the following four periods of starvation: 1 week (W1), 2 weeks (W2), 3 weeks (W3), and 4 weeks (W4) and then fed to satiation three times a day during the 4-week re-feeding period. Starvation was timed so that the end of the starvation period occurred in all groups at week 4. The W1 fish were starved only in week 4; the W2 fish were starved from week 3 to week 4; the W3 fish were starved from week 2 to week 4, and W4 fish were starved from week 1 to week 4. Fish from each group were fed by hand until apparent satiety throughout the experiment. The tanks were siphoned weekly to exonerate excessive food and feces. Fish in all 15 tanks were weighed (g) and measured for total length (mm) individually at the beginning of the experiment and at the end of starvation and re-feeding periods.

Growth indices including final weight, body weight increase (BWI), and specific growth rate (SGR) were calculated according to the formulae below:

$$\text{BWI} = 100 \times (\text{FW} - \text{IW}) / \text{IW}$$

$$\text{SGR} = 100 \times [\ln (\text{FW} - \text{IW})] / t$$

Where FW stands for final body weight, IW refers to initial body weight, and t is days of experiment (Eroldogan et al., 2008).

### Blood sampling and biochemical analysis

Five fish were randomly sampled from each tank at the end of each period of experiment for blood sampling. The fish were killed quickly with blow to the head and blood was taken from the caudal vessels using heparinized syringes. Blood sampling took less than 1 min for each fish and then the blood was transferred to heparinized 1.5 ml Eppendorf tubes, and kept on ice. The blood was divided into two parts. In one of them, the whole blood was suspended in the diluent (Natt & Herrick, 1952) for red and white blood cell levels using a hemacytometer. Hemoglobin concentration (Hb) was measured using a commercial kit by photometric assay of cyanomethemoglobin method. Hematocrit (Hct%) was determined by centrifuging whole blood in heparinized microhematocrit capillary tubes at 3500×g for 10 min. (Osterode, Germany). Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) were calculated using the following formulae (Houston, 1990):

$$\text{MCV (fl)} = (\text{Hct} / \text{RBC}) \times 10$$

$$\text{MCHC (\%)} = (\text{Hb} / \text{Hct}) \times 100$$

$$\text{MCH (fl)} = (\text{Hb} / \text{RBC}) \times 10$$

Differential WBC counts (lymphocytes, neutrophils, eosinophils and monocytes) were conducted on Giemsa stained blood smears. Cells were identified on the basis of morphology and cell ultrastructure, as documented in previous fish leukocyte studies (Afonos et al., 1997, Rowley, 1990, Ellis, 1977).

For biochemical assays, blood samples were immediately centrifuged (5 min at 5,000 rpm, Hettich D7200, Germany) then plasma was separated and stored in 1.5 ml Ependorf tubes at -20 °C for subsequent analysis of biochemical parameters. Plasma Cortisol was determined by radioimmunoassay (RIA) using the Coat-A-Count Cortisol kit (#TKCO2, Diagnostics Products Corporation, Los Angeles, CA, USA). The concentration of ALT (Alanine aminotransferase), AST (Aspartate aminotransferase) and ALP (Alkaline phosphatase) of blood plasma samples were analyzed by auto-analyzer (Technicon,

RA1000, USA) and diagnostic kits (Pars Azmoon Co., Tehran, Iran) based on the protocols described by Shahsavani *et al.*, (2010).

#### Data analysis

Data was presented as mean  $\pm$  SEM (standard error of mean). All statistical analyses were conducted using SPSS statistical software, version 17 (IBM, Chicago, IL, USA). Normality of data and homogeneity of variance were checked by using Kolmogorov-Smirnov and Leven's test, respectively. The starvation/re-feeding effects were analyzed with One-Way ANOVA followed by Duncan's test where variances were homogeneous. Differences between fed and food deprived groups at each sampling time were estimated using a t-test.  $P < 0.05$  was the accepted significance level.

## RESULTS

### Growth performance

Means of fish initial weight did not significantly differ among treatments at the start of experiment ( $P > 0.05$ ). Throughout the experiment, fish survival was 100%. The mean final body weight of juveniles was significantly impacted by deprivation (Table 1). Mean body weight of W2, W3, and W4 treatments were significantly lower than those of control fish ( $P < 0.05$ ). After re-feeding for four weeks, the W1, W2, and W4 treatments were not significantly different compared to the final weight of control fish (Table 1). After re-feeding, body weight increased (BWI) and specific growth rate (SGR) in all four starvation treatments were significantly higher than in the control fish ( $P < 0.05$ ).

### Hematological and biochemical indices

The effect of different starvation treatments and subsequent re-feeding on Persian sturgeon blood indices are displayed in Table 2. Starvation and re-feeding had no significant effect on some hematological parameters including Hematocrit, lymphocytes, neutrophils, eosinophils, and monocytes; while Hb, RBC, MCHC, MCV, and MCH significantly changed during starvation and showed the

least value for W2 group compared to control. After a 4-week re-feeding, the hematological and biochemical values of all food deprived groups recovered to the normal level of full-fed control fish. Red blood cell (RBCs) levels significantly increased during starvation periods in W1, W3, and W4 treatments. After fish re-feeding, Hb and RBCs values returned to the normal level of full-fed control fish showing that the fish had recovered and became healthy.

Starvation did not considerably affect plasma cortisol levels. But after one month re-feeding period, cortisol concentration in W groups increased compared to control fish, so that in W2 group, its level reached maximum ( $P < 0.05$ ) (Table 2). Plasma alkaline phosphatase (ALP), alanine aminotransferase and aspartate aminotransferase (AST) increased in different W groups during starvation period ( $P < 0.05$ ). But after re-feeding period, their levels decreased in W groups and reached to the control level.

## DISCUSSION

In this study, we investigated the effects of nutritional restriction and subsequent re-feeding on compensatory growth and physiological responses of Persian sturgeon. Starvation for 2, 3, and 4 weeks resulted in significant reduction in body weight.

After re-feeding for one month, the final weight of fish starved for 1, 2 and 4 weeks did not differ from the control, while those starved for 3 weeks (W3) were significantly lower than the control groups (C). During re-feeding period, BWI and SGR were significantly higher in the starved fish than that in the control group, indicating that the experiment was ended during a phase when the fish were still undergoing GC. Findings showed that the juvenile Persian sturgeon starved for 1, 2, and 4 weeks showed complete compensatory growth while fish starved for 3 weeks showed partial compensatory growth. Thus compensatory growth exists in the juveniles of Persian sturgeon, and the compensatory response depends on the length of feed deprivation.

**Table 1:** Changes in final body weight, body weight increase (BWI), specific growth rate (SGR), and survival in Persian sturgeon reared under different feeding regimes. Values are mean±SEM. Different superscripts in the same rows indicate significant differences ( $P<0.05$ ) between feeding strategies by One - Way ANOVA.

	C	W1	W2	W3	W4
Initial weight (g)	108.65±1.35	109.18±0.37	108.94±1.16	106.96±0.34	107.944±2.88
Weight after deprivation (g)	139.08±2.27 <sup>a</sup>	125.02±5.06 <sup>a</sup>	108.15±1.18 <sup>b</sup>	100.36±1.80 <sup>b</sup>	99.68±2.30 <sup>b</sup>
Final weight (g)	218.56±8.11 <sup>a</sup>	219.33±2.76 <sup>a</sup>	190.23±3.01 <sup>a, b</sup>	179.6±5.2 <sup>b</sup>	191.01±2.01 <sup>a, b</sup>
BWI (%)	57.16±5.56 <sup>c</sup>	75.84±5.09 <sup>b</sup>	75.86±1.14 <sup>b</sup>	78.87±2-21 <sup>b</sup>	91.81±4.04 <sup>a</sup>
SGR(%day)	1.61±0.12 <sup>c</sup>	2.01±0.10 <sup>b</sup>	2.01±0.02 <sup>b</sup>	2.07±0.04 <sup>a, b</sup>	2.32±0.07 <sup>a</sup>
Survival (%)	100	100	100	100	100

C: Control (fed three times daily to apparent satiation); W1: Treatment 1 (one week starvation and 4 weeks re-feeding), W2 (two weeks starvation and 4 weeks re-feeding), W3 (three weeks starvation and 4 weeks re-feeding), W4 (four weeks starvation and 4 weeks re-feeding)

**Table 2:** Hematological indices of juvenile Persian sturgeon under different starvation periods and subsequent re-feeding. MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration. Values are mean±S.E. Different superscripts in the same rows indicate significant differences ( $P<0.05$ ) between feeding strategies by One - Way ANOVA.

Blood parameters	Starvation					Re-feeding				
	C	W1	W2	W3	W4	C	W1	W2	W3	W4
Hematocrit (%)	19.67 ± 0.67	19.07 ± 0.40	17.67 ± 0.73	18.53 ± 0.65	17.67 ± 0.69	21.73 ± 0.50	21.60 ± 0.89	20.33 ± 0.56	20.87 ± 0.50	20.73 ± 0.59
Hemoglobin (gdl <sup>-1</sup> )	3.22 ± 0.16 <sup>b</sup>	3.65 ± 0.13 <sup>b</sup>	2.40 ± 0.47 <sup>b</sup>	4.16 ± 0.18 <sup>a</sup>	3.35 ± 0.16 <sup>a</sup>	5.31 ± 1.73	3.31 ± 0.25	2.95 ± 0.12	3.04 ± 0.26	3.52 ± 0.20
106μ <sup>-</sup> ×RBC (1)	439.33 ± 34.36 <sup>b</sup>	540 ± 28.33 <sup>a</sup>	432.67 ± 27.68 <sup>b</sup>	556 ± 27.36 <sup>a</sup>	559.33 ± 31.84 <sup>a</sup>	453.33 ± 81.21	464.67±89.35	522 ± 188.33	434.67±59.63	494.67±186.08
WBC 103μ <sup>-1</sup> ×(	24.07 ± 8.10	19.43 ± 7.85	19.03 ± 11.95	21.13 ± 8.11	20.83 ± 12.05	14.40 ± 5.69	9.50 ± 4.16	14.20 ± 5.63	14.27 ± 8.35	12.30 ± 5.30
MCHC (gdl <sup>-1</sup> )	16.44 ± 0.68 <sup>c</sup>	19.24 ± 0.68 <sup>b</sup>	14 ± 2.88 <sup>a, b</sup>	22.52 ± 0.79 <sup>a</sup>	19.25 ± 1.04 <sup>b</sup>	24.38 ± 7.83	15.47 ± 1.09	14.59 ± 0.65	14.59 ± 1.15	16.99 ± 0.85
MCH (pg)	79.30 ± 7.02 <sup>a</sup>	69.79 ± 3.70 <sup>a, b</sup>	52.26 ± 10.34 <sup>a</sup>	75.73 ± 2.70 <sup>a, b</sup>	62.78 ± 4.86 <sup>b</sup>	24.38 ± 7.83	15.47 ± 1.09	14.61 ± 0.65	14.59 ± 1.15	16.99 ± 0.85
MCV (fl)	479.50±35.54 <sup>a</sup>	363.06±16.25 <sup>b, c</sup>	426.13±25.87 <sup>c</sup>	339018±12.57 <sup>c</sup>	326.66±18.70 <sup>c</sup>	488.71±17.45	478.41±27.93	433.43±39.24	488.02±19.14	455.53±32.90
Lymphocyte (%)	75.67 ± 5.73	75.50±1.48	81.89±1.52	73.67±4.32	78.43±5.20	84.22±1.50	82.33±2.52	83.38±2.88	79.67±2.53	74.57±3.41
Neutrophil (%)	11.67±2.40	9.67±1.09	5.33±0.85	10.89±2.22	10.57±2.72	8.56±1.08	11.67±1.52	8.13±1.22	12.56±2.14	13±3.09
Eosinophil (%)	3.67±0.33	3.17±0.70	3.22±0.46	3.11±0.26	2.86±0.70	3.44±0.77	3.78±1.12	4.50±1.10	3.89±0.59	4.57±0.65
Monocyte (%)	11.3± 0.21	9.67±0.61	9.56±1.07	14.22±2.64	8.14±2.22	4.00±0.96	2.22±1.06	4.00±1.41	3.89±1.15	7.86±1.68
AST (IU.L <sup>-1</sup> )	311.33±18.05 <sup>b</sup>	420.47±23.23 <sup>a</sup>	402.47±38.06 <sup>a</sup>	389.6±16.71 <sup>a, b</sup>	357±31.34 <sup>a, b</sup>	448.47±56.64	344.47±29.04	427.40±56.40	371.93±34.97	362.93±27
ALT (IU.L <sup>-1</sup> )	7 ± 1.44 <sup>a, b</sup>	8.27±1.37 <sup>a</sup>	6±0.96 <sup>a, b</sup>	4.27±0.67 <sup>b</sup>	4.93±0.77 <sup>a, b</sup>	6.13±1.64	4.00±0.74	6.60±1.12	4.40±0.83	3.67±0.73
ALP (IU.L <sup>-1</sup> )	301±11.22 <sup>b</sup>	425.33±12.54 <sup>a</sup>	446 ± 30.78 <sup>a</sup>	422.93±25.13 <sup>a</sup>	461.13±28.01 <sup>a</sup>	336.27±17.05	309.20±15.51	298.87±10.40	305.73±18.24	295.47±9.99
Cortisol (g.dl <sup>-1</sup> )	3.30±0.68	5.45±1.46	3.59±0.97	5.72±1.30	5.77±0.95	0.93±0.20 <sup>b</sup>	1.93±0.49 <sup>a, b</sup>	3.62±1.16 <sup>a</sup>	2.49±0.61 <sup>a, b</sup>	2.77±0.66 <sup>a, b</sup>

Control (fed three times daily to apparent satiation); W1: Treatment 1 (one week starvation and 4 weeks re-feeding), W2 (two weeks starvation and 4 weeks re-feeding), W3 (three weeks starvation and 4 weeks re-feeding), W4 (four weeks starvation and 4 weeks re-feeding).

The findings of the current experiment were similar to that shown in trout (*Onchorhynchus mykiss*) whereby full compensation after 1 week and partial compensation in 2 and 3 weeks were observed after fasting (N Montserrat *et al.*, 2007). Similar findings have also been observed in barramundi (*Lates calcalifer*) (Tian and Qin, 2003) and hybrid tilapia, (*Oreochromis mossambicus* × *O. niloticus*) (Wang *et al.*, 2000). However, in Atlantic cod (*Gadus morhua*) after 3 weeks fasting, complete compensation was observed (Jobling *et al.*, 1994). It is known that starvation may induce different responses on blood hematological parameters. The responses are dependent on the starvation term and are also species-specific character which are related to the metabolism and its regulation (Caruso *et al.*, 2010). In this study the effects of starvation/re-feeding on hematological parameters were different. In the Persian sturgeon juveniles, the hematocrit (%) did not seem to be affected by starvation, whereas hemoglobin (Hb) and blood indices (MCV, MCH and MCHC) were affected following changes in red blood cell number in food deprived groups. Different responses were previously reported concerning the effects of starvation on hematological parameters. The increase in the hematocrite value in response to different starvation periods have reported in Japanese eel (*Anguilla japonica*), European eel (*A. anguilla*), Atlantic cod (*Gadus morhua*), and beluga (*Huso huso*) (Sano 1962, Johansson-Sjöbeck *et al.* 1975, Kamra 1966, Falahatkar 2012). On the other hand, the decrease in the hematocrite and haemoglobin contents have reported in starved carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), Lake sturgeon (*A. fulvescens*), catfish (*Ictalurus punctatus*) (Gillis and Ballantyne, 1996, Lim & Klesius, 2003, Murachi, 1959). Additionally, no effect on the hematocrite values were found in food deprived, European sea bass (*Dicentrarchus labrax*), blackspot sea bream (*Pagellus bogaraveo*), and red porgy (*Pagrus pagrus*) (Caruso *et al.*, 2012, Caruso *et al.*, 2011). No significant differences in Hct and Hb concentrations were observed in European

eels which were starved for 150 and 5 days respectively (Caruso *et al.*, 2010, Larsson & Lewander, 1973). In the present study the highest level of Hb was observed in W3, which caused high value of MCH and MCHC.

It could be as a result of the high amount of RBC in starved groups and reflected the increase of oxygen carrying capacity of blood and then increased oxygen requirement of the sturgeon during starvation periods. The observed decrease in Hb and MCHC in some treatments including control fish is probably due to decrease in oxygen requirement (Falahatkar, 2012). Re-feeding of fish after starvation periods recovered the blood indices.

Furthermore, starvation and subsequent re-feeding had no significant effect on the WBC count and therefore the percentage of lymphocytes, neutrophils, eosinophils and monocytes in the Persian sturgeon juveniles. Leukocytes were found to be most sensitive to starvation (Mahajan & Dheer, 1983). While lymphocytes are recognized as immunocompetent cells and can affect immune responses in fish (Ellis, 1977). The increase in total leukocyte values was observed in an air-breathing fish *Channa punctatus* Bloch (Mahajan and Dheer, 1983) and traíra (*Hoplias malabaricus* Bloch) (Rios *et al.*, 2005) during starvation. In contrast, a successive decrease in the white blood cells (WBC) count during the starvation period was observed in the European eel, *Anguilla anguilla* L. (Johansson-Sjöbeck *et al.*, 1975).

In the present study, plasma cortisol levels were not different between treatments. Due to rapid decrease in liver size, it was expected that cortisol in unfed fish would increase because of the need for energy made available by cortisol role for gluconeogenesis, but it did not occur (Davis & Gaylord, 2011). However at the end of the re-feeding period, cortisol concentration increased. It seems that increased cortisol levels in fed fish, might be a physiological result of feeding itself or possibly a result of stress associated with hand feeding activity, but not a result of feeding regimes. These data, therefore, suggest that even under severe conditions of

feed restriction, cortisol does not appear to play a functional role in mobilizing energy in the Persian sturgeon. Similar findings in that feed deprivation which did not affect plasma cortisol concentration were observed in rainbow trout and channel catfish (Farbridge & Leatherland 1992, Pottinger *et al.* 2003, Weber & Bosworth 2005). In contrast, other studies in a range of species showed that feed deprivation either suppress (Barton *et al.*, 1988, Vijayan and Moon, 1994) or increase plasma cortisol level (Sumpter *et al.*, 1991, Kelley *et al.*, 2001, Blom *et al.*, 2000, Barcellos *et al.*, 2010). Furthermore, it has been clearly shown that cortisol possesses atabolic properties in teleost fish (Mommensen *et al.*, 1999). Therefore, considering the present study, results provide no evidence that energy mobilization in un-fed Persian sturgeon is cortisol-dependent which can be considered as species-specific character.

The liver tissue of fish species appears to be rich in ALP (Alkaline phosphatase) (Shahsavani *et al.*, 2010) and possible AST (Aspartat aminotransferase) (Evans, 1996). According to previous investigations (Zammit & News-holme, 1979), there are positive correlations between AST and ALT levels in the liver and their respective levels in the blood plasma . Therefore, the plasma activity of these enzymes may be elevated with several hepatocellular injuries in some fish species (Thrall *et al.*, 2012). There is lack of information regarding the influence of starvation on the plasma hepatic enzymes activity of sturgeons. In the present study, plasma hepatic enzymes concentration (AST and ALP) in feed deprived fish differed significantly from fed fishes. Plasma ALP and AST concentrations increased in W groups compared to control fish, and rapidly recovered when feeding of un-fed groups were resumed. The effect of starvation on ALT and AST activities in other fish species is variable, as either increases or no changes after starvation have been reported (Moon & Johnston, 1981, Cowey & Walton, 1989). As aminotransferase plays a significant role in linking carbohydrate and protein, the increase in AST after starvation appears to indicate their

more prominent roles in protein mobilization as a substrate for gluconeogenesis in Persian sturgeon. In contrast to the present study, Johnston *et al.* (1994) indicated that plasma ALP activities in feeding fish were enhanced by increasing the processing of energy substances in the liver. As a consequence, hepatic ALP is transferred into the blood as plasma metabolite. In conclusion, results of the present study demonstrated that physiological parameters were affected by feeding strategies following different starvation periods and subsequent re-feeding and also changes in blood biochemical and hematological indices. Results showed that most hematological parameters were recovered upon re-feeding. With regard to the obtained results, starvation for 1, 2, and 3 weeks and subsequent re-feeding for at least 4 weeks has no significant negative effects on most growth and physiological parameters. Results of the present study would be interesting for sturgeon farming when under certain circumstances fish should be starved.

#### ACKNOWLEDGMENT

This work was supported by the Iranian Fisheries organization. We would like to thank the staff at the Shahid Beheshti Sturgeon Fish Propagation and rearing Complex for providing fish and rearing facilities.

#### REFERENCES

- Abdel-Tawwab, M. Khattab, Y. A. Ahmad, M. H. & Shalaby, A. M. (2006) 'Compensatory growth, feed utilization, whole-body composition, and hematological changes in starved juvenile Nile Tilapia, *Oreochromis niloticus* (L.)', *Journal of Applied Aquaculture*, 18(3):17-36.
- Abolfathi, A. A. Mohajeri, D. Rezaie, A. & Nazeri, M. (2012) 'Protective effects of green tea extract against hepatic tissue injury in streptozotocin-induced diabetic rats', *Evidence-Based Complementary and Alternative Medicine*, 20(12): 1-10.
- Afonos, A. Ellis, A. E. Silva, M. T. (1997) 'The leucocyte population of the unstimulated peritoneal cavity of rainbow trout (

- Oncorhynchus mykiss*'), *Fish & Shellfish Immunology*, 7(5): 335-348.
- Anver, C. (2004) 'Blood chemistry (electrolytes, lipoprotein and enzymes) values of black scorpion fish (*Scorpaena porcus*, 1758) in the Dardanelles', *Turkey J Biol Sci*, 4: 716-719.
- Bani, A. & Vayghan, A. H. (2011) 'Temporal variations in haematological and biochemical indices of the Caspian kutum, *Rutilus frisii kutum*', *Ichthyological research*, 58(2): 126-133.
- Barcellos, L. Marqueze, A. Trapp, M. Quevedo, R. & Ferreira, D. (2010) 'The effects of fasting on cortisol, blood glucose and liver and muscle glycogen in adult jundiá *Rhamdia quelen*', *Aquaculture*, 300(1): 231-236.
- Barton, B. A. Schreck, C. B. & Fowler, L. G. (1988) 'Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile chinook salmon', *The Progressive Fish-Culturist*, 50(1): 16-22.
- Blom, S. Andersson, T. B. & Förlin, L. (2000) 'Effects of food deprivation and handling stress on head kidney 17 $\alpha$ -hydroxyprogesterone 21-hydroxylase activity, plasma cortisol and the activities of liver detoxification enzymes in rainbow trout', *Aquatic toxicology*, 48(2): 265-274.
- Caruso, G. Denaro, M. G. Caruso, R. Genovese, L. Mancari, F. & Maricchiolo, G. (2012) 'Short fasting and refeeding in red porgy (*Pagrus pagrus*, Linnaeus 1758): response of some haematological, biochemical and non specific immune parameters', *Marine environmental research*, 81:18-25
- Caruso, G. Denaro, M. G. Caruso, R. Mancari, F. Genovese, L. & Maricchiolo, G. (2011) 'Response to short term starvation of growth, haematological, biochemical and non-specific immune parameters in European sea bass (*Dicentrarchus labrax*) and blackspot sea bream (*Pagellus bogaraveo*)', *Marine environmental research*, 72(1): 46-52.
- Caruso, G. Maricchiolo, G. Micale, V. Genovese, L. Caruso, R. & Denaro, M. (2010) 'Physiological responses to starvation in the European eel (*Anguilla anguilla*): effects on haematological, biochemical, non-specific immune parameters and skin structures', *Fish Physiology and Biochemistry*, 36(1): 71-83.
- Coles, E. (1986) 'Erythrocytes, leukocytes and bone marrow', *Veterinary Clinical Pathology*, 4th. edn. Philadelphia: Saunders, 10-97.
- Cowey, C. B. & Walton, M. J. (1989) 'Intermediary metabolism', *Fish nutrition*, 2: 259-329.
- Davis, K. B. & Gaylord, T. G. (2011) 'Effect of fasting on body composition and responses to stress in sunshine bass', *Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology*, 158(1): 30-36.
- Ellis, A. (1977) 'The leucocytes of fish: a review', *Journal of fish biology*, 11(5): 453-491.
- Eroldoğan, O. T. Taşbozan, O. & Tabakoğlu, S. (2008) 'Effects of restricted feeding regimes on growth and feed utilization of juvenile gilthead sea bream, *Sparus aurata*', *Journal of the World Aquaculture Society*, 39(2): 267-274.
- Evans, G. (1996) 'General enzymology', *Animal Clinical Chemistry*, 54.
- Falahatkar, B. (2012) 'The metabolic effects of feeding and fasting in beluga *Huso huso*', *Marine environmental research*, 82:69-75.
- Farbridge, K. & Leatherland, J. (1992) 'Plasma growth hormone levels in fed and fasted rainbow trout (*Oncorhynchus mykiss*) are decreased following handling stress', *Fish Physiology and Biochemistry*, 10(1): 67-73.
- Furné, M. Morales, A. E. Trenzado, C. E. García-Gallego, M. Carmen Hidalgo, M. Domezain, A. & Sanz Rus, A. (2012) 'The metabolic effects of prolonged starvation and refeeding in sturgeon and rainbow trout', *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 182(1): 63-76.
- Gaylord, I. G. & Gatlin III, D. M. (2000) 'Assessment of compensatory growth in channel catfish *Ictalurus punctatus* R. and associated changes in body condition



- indices', *Journal of the World Aquaculture Society*, 31(3): 326-336.
- Gillis, T. & Ballantyne, J. (1996) 'The effects of starvation on plasma free amino acid and glucose concentrations in lake sturgeon', *Journal of fish biology*, 49(6): 1306-1316.
- Hayward, R. S. Noltie, D. B. & Wang, N. (1997) 'Use of compensatory growth to double hybrid sunfish growth rates', *Transactions of the American Fisheries Society*, 126(2): 316-322.
- Houston, A. (1990) 'Blood and circulation', *Methods for fish biology*, 1990: 273-334.
- Hung, S. S. Liu, W. Li, H. Storebakken, T. & Cui, Y. (1997) 'Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*', *Aquaculture*, 151(1): 357-363.
- Jobling, M. Meløy, O. Dos Santos, J. & Christiansen, B. (1994) 'The compensatory growth response of the Atlantic cod: effects of nutritional history', *Aquaculture International*, 2(2): 75-90.
- Johansson-Sjöbeck, M.-L. Dave, G. Larsson, Å. Lewander, K. & Lidman, U. (1975) 'Metabolic and hematological effects of starvation in the European eel, *Anguilla anguilla* L.—II. Hematology', *Comparative Biochemistry and Physiology Part A: Physiology*, 52(3): 431-434.
- Johnston, C. Horney, B. Deluca, S. MacKenzie, A. Eales, J. & Angus, R. (1994) 'Changes in alkaline phosphatase isoenzyme activity in tissues and plasma of Atlantic salmon (*Salmo salar*) before and during smoltification and gonadal maturation', *Fish Physiology and Biochemistry*, 12(6): 485-497.
- Kamra, S. (1966) 'Effect of starvation and refeeding on some liver and blood constituents of Atlantic cod (*Gadus morhua* L.)', *Journal of the Fisheries Board of Canada*, 23(7): 975-982.
- Kelley, K. Haigwood, J. Perez, M. & Galima, M. (2001) 'Serum insulin-like growth factor binding proteins (IGFBPs) as markers for anabolic/catabolic condition in fishes', *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 129(2): 229-236.
- Larsson, Å. & Lewander, K. (1973) Metabolic effects of starvation in the eel, *Anguilla anguilla* L', *Comparative Biochemistry and Physiology Part A: Physiology*, 44(2): 367-374.
- Lim, C. & Klesius, P. H. (2003) 'Influence of dietary levels of magnesium on growth, tissue mineral content, and resistance of channel catfish *Ictalurus punctatus* challenged with *Edwardsiella ictaluri*', *Journal of the World Aquaculture Society*, 34(1): 18-28.
- Liu, W. Wei, Q. Wen, H. Jiang, M. Wu, F. & Shi, Y. (2011) 'Compensatory growth in juvenile Chinese sturgeon (*Acipenser sinensis*): effects of starvation and subsequent feeding on growth and body composition', *Journal of Applied Ichthyology*, 27(2): 749-754.
- Mahajan, C. & Dheer, T. (1983) 'Haematological and haematopoietic responses to starvation in an air-breathing fish *Channapunctatus Bloch*', *Journal of fish biology*, 22(1): 111-123.
- Mohseni, M. Sajjadi, M. & Pourkazemi, M. (2007) 'Growth performance and body composition of sub-yearling Persian sturgeon, (*Acipenser persicus*, Borodin, 1897), fed different dietary protein and lipid levels', *Journal of Applied Ichthyology*, 23(3): 204-208.
- Mommsen, T. P., Vijayan, M. M. & Moon, T. W. (1999) 'Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation', *Reviews in Fish Biology and Fisheries*, 9(3): 211-268.
- Montserrat, N., Gabillard, J., Capilla, E., Navarro, M. & Gutiérrez, J. (2007) 'Role of insulin, insulin-like growth factors, and muscle regulatory factors in the compensatory growth of the trout (*Oncorhynchus mykiss*)', *General and comparative endocrinology*, 150(3): 462-472.
- Montserrat, N., Gómez-Requeni, P., Bellini, G., Capilla, E., Pérez-Sánchez, J., Navarro, I. & Gutiérrez, J. (2007) 'Distinct role of insulin and IGF-I and its receptors in white

- skeletal muscle during the compensatory growth of gilthead sea bream (*Sparus aurata*), *Aquaculture*, 267(1): 188-198.
- Moon, T. & Johnston, I. (1981) 'Amino acid transport and interconversions in tissues of freshly caught and food-deprived plaice, *Pleuronectes platessa* L', *Journal of fish biology*, 19(6): 653-663.
- Morshedi, V. Ashouri, G. Kochanian, P. Yavari, V. Bahmani, M. Pourdehghani, M. Yazdani, M. Fashtami, H. & Azodi, M. (2011) 'Effects of short-term starvation on hematological parameters in cultured juvenile Beluga', *Journal of Veterinary Research*, 66(4): 363-368.
- Murachi, S. (1959) 'Haemoglobin content, erythrocyte sedimentation rate and haematocrit of the blood in the young of the carp (*Cyprinus carpio*)', *J. Fac. Fish Anim. Husb. Hiroshima Univ*, 2:241-247.
- Navarro, I. & Gutiérrez, J. (1995) 'Fasting and starvation', *Biochemistry and molecular biology of fishes*, 4: 393-434.
- Nicieza, A. G. & Metcalfe, N. B. (1997) 'Growth compensation in juvenile Atlantic salmon: responses to depressed temperature and food availability', *Ecology*, 78(8):2385-2400.
- Polakof, S. Ceinos, R. M. Fernández-Durán, B. Míguez, J. M. & Soengas, J. L. (2007) 'Daily changes in parameters of energy metabolism in brain of rainbow trout: dependence on feeding', *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 146(2): 265-273.
- Pottinger, T. G. Rand-Weaver, M. & Sumpter, J. P. (2003) 'Overwinter fasting and re-feeding in rainbow trout: plasma growth hormone and cortisol levels in relation to energy mobilisation', *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 136(3): 403-417.
- Pourkazemi, M. Skibinski, D. & A Beardmore, J. (1999) 'Application of mtDNA d-loop region for the study of Russian sturgeon population structure from Iranian coastline of the Caspian Sea', *Journal of Applied Ichthyology*, 15(4-5):23-28.
- Rios, F. S. Oba, E. T. Fernandes, M. N. Kalinin, A. L. & Rantin, F. T. (2005) 'Erythrocyte senescence and haematological changes induced by starvation in the neotropical fish traíra, *Hoplias malabaricus* (Characiformes, Erythrinidae)', *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 140(3):281-287.
- Rowley, A. F. (1990) 'Collection, separation and identification of fish leucocytes', *Techniques in fish immunology*, 1, 113-136.
- Sano, T. (1962) 'Haematological studies of the culture of fishes in Japan. 5. Application of a protein refractometer on fish serum', *Journal of the Tokyo University of Fisheries*, 48: 99-104.
- Shahsavani, D. Mohri, M. & Kanani, H. G. (2010) 'Determination of normal values of some blood serum enzymes in *Acipenser stellatus* Pallas', *Fish Physiology and Biochemistry*, 36(1): 39-43.
- Sumpter, J. Le Bail, P. Pickering, A. Pottinger, T. & Carragher, J. (1991) 'The effect of starvation on growth and plasma growth hormone concentrations of rainbow trout, *Oncorhynchus mykiss*', *General and comparative endocrinology*, 83(1): 94-102.
- Thrall, M. A. Weiser, G. Allison, R. & Campbell, T. W. (2012) *Veterinary hematology and clinical chemistry*, Wiley-Blackwell.
- Tian, X. & Qin, J. G. (2003) 'A single phase of food deprivation provoked compensatory growth in barramundi *Lates calcarifer*', *Aquaculture*, 224(1): 169-179.
- Vijayan, M. & Moon, T. (1994) 'The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven', *Canadian Journal of Zoology*, 72(3): 379-386.
- Wang, Y. Cui, Y. Yang, Y. & Cai, F. (2000) 'Compensatory growth in hybrid tilapia, *Oreochromis mossambicus* × *O. niloticus*, reared in seawater', *Aquaculture*, 189(1):101-108.
- Weber, T. E. & Bosworth, B. G. (2005) 'Effects of 28 day exposure to cold temperature or feed restriction on growth, body composition, and expression of genes

- related to muscle growth and metabolism in channel catfish', *Aquaculture*, 246(1):483-492.
- Yarmohammadi, M. Shabani, A. Pourkazemi, M. Soltanloo, H. & Imanpour, M. (2012) 'Effect of starvation and re-feeding on growth performance and content of plasma lipids, glucose and insulin in cultured juvenile Persian sturgeon (*Acipenser persicus* Borodin, 1897)', *Journal of Applied Ichthyology*, 28(5): 692-696.
- Zammit, V. A. & Newsholme, E. A. (1979) 'Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobranch fish', *Biochemical Journal*, 184(2): 313.
- Zhu, X. Xie, S. Lei, W. Cui, Y. Yang, Y. & Wootton, R. J. (2005) 'Compensatory growth in the Chinese longsnout catfish, *Leiocassis longirostris* following feed deprivation: Temporal patterns in growth, nutrient deposition, feed intake and body composition', *Aquaculture*, 248(1): 307-314.

Archive of SID

## تأثیر محرومیت غذایی و تغذیه مجدد بر روی برخی از شاخص‌های هماتولوژیک و بیوشیمیایی پلاسما در تاسماهی ایرانی جوان (*Acipenser persicus* Borodin 1897)

م. یارمحمدی<sup>۱\*</sup>، م. پورکاظمی<sup>۲</sup>، ر. کاظمی<sup>۱</sup>، م. پوردهقانی<sup>۱</sup>، م. حسن زاده صابر<sup>۱</sup>، ل. عزیززاده<sup>۱</sup>

۱- موسسه تحقیقات بین المللی تاسماهیان دریای خزر، سازمان تحقیقات، آموزش و ترویج کشاورزی، رشت - ایران

۲- موسسه تحقیقات علوم شیلاتی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران - ایران

(تاریخ دریافت: ۹۳/۷/۱۴ - تاریخ پذیرش: ۹۴/۱/۱۷)

### چکیده

در این پژوهش، تأثیر محرومیت غذایی و تغذیه مجدد بر روی پارامترهای رشد، هماتولوژیک و بیوشیمیایی خون تاسماهی ایرانی جوان بررسی گردید. سیصد و هفتاد و پنج قطعه بچه ماهی ( $10.8 \pm 0.63$ ) در بین پنج تیمار آزمایشی توزیع گردید. بطوریکه تیمار کنترل سه مرتبه در روز در حد سیری و چهار تیمار دیگر به ترتیب یک، دو، سه و چهار هفته دارای محرومیت غذایی بودند و سپس به مدت چهار هفته در طول دوره غذا دهی مجدد، سه با در روز در حد سیری تغذیه شدند. نتایج نشان داد که برخی از پارامترهای مورد مطالعه از جمله وزن نهایی، رشد ویژه، افزایش وزن بدن، آنزیم‌های پلاسما (ALT، AST و ALP) و شاخص‌های هماتولوژیک (MCH، MCHC، MCV) بطور معنی داری تحت تأثیر رژیم غذایی قرار گرفتند. در حالیکه برخی از شاخص‌های پلاسما از جمله کورتیزول، هماتوکریت، لنفوسیت‌ها، نوتروفیل‌ها، ائوزونوفیل‌ها و مونوسیت‌ها تحت تأثیر تیمارهای غذایی و تغذیه مجدد قرار نگرفتند. بر اساس نتایج این پژوهش، دوره‌های کوتاه گرسنگی بر روی عملکرد رشد دارای تأثیر منفی نمی‌باشد و بیشتر شاخص‌های بیوشیمیایی و هماتولوژیکی در تاسماهی ایرانی بعد از برقراری تغذیه مجدد جبران می‌گردد.

\* مولف مسئول