

Research Article

The effect of short starvation and re-feeding on growth performance, hematological, and morphological responses in juvenile beluga (*Huso huso*)

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Abstract

A 40-day study was done to evaluate the effect of different feeding strategies on growth performance, hematological, and morphological indices in juvenile beluga (*Huso huso*). For this purpose, one hundred and eighty fish with a mean weight of 34.58 ± 5.32 g were divided into four feeding strategies: F) fed throughout the 40 days, SRF1) 4 intermittent periods of 2-day starvation, 8-day feeding, SRF2) 2 intermittent periods of 4-day starvation, 16-day feeding, SRF3) an 8-day starvation and 32-day feeding. After 40 days, the results showed that some blood indices, including albumin, globulin, cholesterol, and triglyceride levels were not significantly affected by starvation and re-feeding ($p > 0.05$). A significant change was observed in glucose level between treatments and SRF3 had the lowest glucose level. There were significant difference in immunoglobulin levels among treatments ($p < 0.05$). A significant decrease was observed in cortisol levels in starvation and re-feeding treatments compared to the control group ($p < 0.05$). No significant difference was observed in the hepatic and gastrointestinal index ($p > 0.05$). These findings showed that short-term starvations of beluga had no significant negative effect on most biochemical and hematological indices. The results indicated that beluga has the metabolic regulation capability for short periods of starvation.

Keywords: *Huso huso*, Compensatory growth, Starvation, Re-feeding

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Introduction

High meat quality and the remarkable economic value of caviar have led to the expansion of sturgeon culture in the world (Bronzi and Rosenthal, 2014). Beluga or great sturgeon (*Huso huso*) is the most important fish in Caspian Sea. This species is a good candidate for aquaculture due to fast growth, ease of reproduction in captivity, and tolerance of variable rearing conditions (Mohseni *et al.*, 2006). In captive conditions, beluga like other aquaculture species can be subjected to feed deprivation in order to water quality problems, reduce handling stress and mortality due to disease outbreaks, temperature changes, and also save the feed to increase profit of the farm (Caruso *et al.*, 2011; Falahatkar, 2012). The potency of animals to adapt to food shortages and resume growth after a restricted period of fasting has been well determined (McCue, 2010). Studies have shown that numerous species of fish are able to tolerate periods of starvation. However, the response of different organs and tissues of fish during starvation and re-feeding is different among species. Body weight and composition also biochemical and hematological factors, as well as liver enzyme activity in different species of fish in the face of dietary restriction and re-feeding, have been studied (Ali *et al.*, 2003). Some studies have shown that fish increase their chances of surviving in the period of starvation by reducing their energy expenditure from protein circulation, and use their energy reserves to

maintain vital processes (Furne *et al.*, 2008).

Given the relatively long maturation period of sturgeons and its high production cost, better knowledge of sturgeon nutrition and physiology may improve rearing conditions and reduce production cost (Yarmohammadi *et al.*, 2012). A few studies have shown the effects of starvation in members of sturgeon family (Acipenseridae). In Yangtze sturgeon (*Acipenser dabryanus*), the effect of starvation and re-feeding on oxidative stress and antioxidant defenses has been studied (Yang *et al.*, 2019). Moreover, gluconeogenesis during starvation and re-feeding phase has been studied in Siberian sturgeon, *Acipenser baerii* (Liang *et al.*, 2017). Effects of starvation and re-feeding on some hematological and plasma biochemical parameters was studied in Adriatic sturgeon, *Acipenser naccarii* (Furne *et al.*, 2008) and Persian sturgeon, *Acipenser persicus* (Yarmohammadi *et al.*, 2012). Therefore, the present study aimed to address metabolic strategies of beluga in response to a period of fasting and following re-feeding. Consequently, the effect of a starvation and re-feeding cycle was assessed on growth, plasma metabolites (glucose, cholesterol, triglycerides, and total protein), and liver histological changes in Beluga (*Huso huso*). Finally, the effects of multiple fasting or feeding periods on the weight gain/loss of cultured beluga were tested during 40-day starvation and feeding regimens.

Material and methods

Fish culture and experimental condition

This study was conducted at Ghareboron Sturgeon Rearing Complex (Sari, Mazandaran, Iran). Before the experiment, the fish were disinfected with salt (5 present salt bath) to avoid contamination and were acclimated to experimental conditions for 10 days while they fed three times a day (08:00, 13:00, and 18:00) on a commercial food containing 45% crude protein and 15% crude fat. After accumulation, 180 beluga juveniles with a mean initial body weight of 34.58 ± 5.32 g were randomly divided into twelve tanks with 15 fish per tank and this experiment lasted for 40 days (Falahatkar, 2012). Fish were maintained under a stress free condition during the experimental period and each tank was aerated using air stones that were connected to a central air pump (Iran Pash, Urmia, Iran). The tanks were siphoned weekly to exonerate excessive food and feces. In the present study, hole water was applied and water quality factors were monitored periodically, the temperature was $21.3 \pm 1.3^\circ\text{C}$ (mercury thermometer, Zomorodazma Company, Iran), dissolved oxygen was $6\text{--}8$ mg L⁻¹ (Cyberscan Eutech Instruments, DO 110, Singapore), and pH was recorded 7.5 (Hanna Instrument, 8314, USA).

Diet preparation and experimental design

For this study, a basal diet was formulated (Table 1).

Table 1: Formulation of the experimental diets.

Ingredients	%
Fish meal	54
Soybean meal	9
Wheat flour	5
Fish oil	6.5
Soybean oil	6.5
Lecithin	2
Dicalcium phosphate	2
Vitamin premix ¹	3
Mineral premix ²	3
Antioxidant ³	0.02
Antifungus ⁴	0.45
Methionine	1
Lactic acid	2.5
Lysine	1
Filler	4
Total	99.98

¹ Vitamin supplementation 0.5% contained: vitamin A 80,000 IU/kg; vitamin D3 2,000 IU/kg; vitamin k 20 mg/kg; thiamin 60 mg/kg; riboflavin 60 mg/kg; pyridoxine 100 mg/kg; pantothenic acid 150 mg/kg; niacin 300 mg/kg; biotin 2 mg/kg; folic acid 20 mg/kg; vitamin B12 0.1 mg/kg; inositol 300 mg/kg; ascorbic acid 600 mg/kg; choline chloride 3000 mg/kg.

²Mineral Supplementation contained: Co, 100; I, 400; Se, 20; Zn, 10,000; Fe, 6,000; Cu, 600; Mn, 5,000.

³Antioxidant: Butylated hydroxytoluene (BHT).

⁴Anti fungi: Toxiban premix (Component: Aluminosilicate, zeolite, bentonate, propionate ammonium).

The experimental diets were prepared by mixing dry ingredients in a mixer (Pars Electric, Tehran, Iran). After complete mixing, liquid ingredients such as fish oil, soybean oil, and lecithin were weighed and added gradually to the dry mixture to form a soft dough. The mixed dough was extruded through an electric meat grinder and dried at 30°C for 12h in a drying oven and then feeds were prepared based on the fish mouth size. Proximate analysis of each diet,

including moisture, protein, ash, and fat were performed according to AOAC method (Table 2; AOAC, 2007). Diets were packed and stored at -20°C but daily diets were held at 4°C (Mohammadzadeh *et al.*, 2017).

Table 2: Proximate analyses of the experimental diets.

Proximate composition	%
Crude protein	45
Crude lipid	15
Ash	13.5
Moisture	10

Fish in four groups were subjected to the following three periods of

starvation: F) fed throughout the 40 days, SRF1) 4 intermittent periods of 2-day starvation, 8-day feeding, SRF2) 2 intermittent periods of 4-day starvation, 16-day feeding, SRF3) an 8-day starvation and 32-day feeding. Fish from each group were fed by hand until apparent satiety throughout the experiment.

Growth performance

After the end of the feeding trial, growth performance was determined and feed utilization was calculated as follows:

Weight gain (g) = final weight (g) - initial weight (g)

Feed conversion ratio (FCR) = dry weight of feed given (g)/WG (g)

Survival rate(%)= 100×(number of fish stocked - umber of fish died)/number of fish stocked

Sample collection and biochemical analysis

At the end of the experiment, a total of 3 fish were randomly sampled from each tank (9 fish every treatment). First, fish were anesthetized in diluted clove oil (100 mg L⁻¹). Blood samples were collected from behind of the anal fin and transformed into non-heparinized tubes. To obtain the serum, non-heparinized blood was centrifuged (1600×g for 10 min) to separate supernatant and stored at -20°C for later analysis (Yarmohammadi *et al.*, 2012). Concentrations of cholesterol, triglyceride, total protein, and glucose were measured using standard kits from Pars Azmoon (Karaj, Iran). Albumin, Globulin, and Immunoglobulin assay

was measured using standard kits (Biosystem Company, Spain). Plasma cortisol was determined by using competitive enzyme immunoassay kits (ELISA Micro wells, Diaplus, USA).

Histomorphometric tests

First, Three fish from every treatment (one fish from each tank) were anesthetized in diluted clove oil (100 mg L⁻¹), and liver and gill were removed, then after washing with physiological serum they were fixed in 70% alcohol for 24 hours. After that, the samples were transferred to 10% buffered formalin solution. Histomorphometric indices were studied using the hematoxylin-eosin staining method. Hepatosomatic index

and gastrointestinal index were measured using the following formulas (Mohanta *et al.*, 2008):

Hepatosomatic Index (HSI) = $100 \times (\text{liver weight} / \text{total body weight})$

Gastrointestinal index (GSI) = $100 \times (\text{gastrointestinal weight} / \text{total body weight})$

Statistical analysis

Before analysis, homogeneity of variances and normality of the data was checked using the Levene and Kolmogorov-Smirnov tests, respectively. Then, data were analyzed by one-way analysis of variance (ANOVA). Duncan test was applied to compare the significant differences among the treatments ($p < 0.05$) with SPSS version 18 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at $p < 0.05$ for all analyses. Data were expressed as mean \pm Standard Deviation.

Results

Growth performance in beluga juveniles during the starvation and re-feeding cycle was presented in Table 3. The survival rate was generally high (80-100%) over 40 days trial and it was affected the starvation and re-feeding period and the lowest content was obtained in SRF3 ($p < 0.05$). There was significant difference in weight gain among treatments and the highest weight gain was observed in the control group ($p < 0.05$). FCR was affected by starvation and re-feeding cycle and lowest FCR was observed in SRF1 and SRF2 treatments ($p < 0.05$).

Table 3: Mean \pm SD values of growth performance in beluga (*Huso huso*) juveniles during the starvation and re-feeding cycle.

Treatments	F	SRF1	SRF2	SRF3
Weight gain (g)	114.58 \pm 16.73 ^a	104.33 \pm 5.25 ^a	101.75 \pm 9.36 ^a	79.41 \pm 8.64 ^b
FCR	0.71 \pm 0.10 ^{ab}	0.65 \pm 0.07 ^b	0.65 \pm 0.08 ^b	0.85 \pm 0.07 ^a
Survival rate (%)	95.55 \pm 3.85 ^{ab}	95.55 \pm 7.69 ^{ab}	100.00 \pm 5.00 ^a	86.66 \pm 6.66 ^b

F) fed throughout the 40 days, SRF1) 4 intermittent periods of 2-day starvation, 8-day feeding, SRF2) 2 intermittent periods of 4-day starvation, 16-day feeding, SRF3) an 8-day starvation, and 32-day feeding. Different letters in the same row indicate significant differences among treatments.

The results of hepatosomatic index and gastrointestinal index are presented in Table 4 and there were no significant difference among experimental treatments ($p > 0.05$).

The biochemical and hematological indices of beluga during the starvation and re-feeding cycle are shown in Tables 5 and 6. At the end of the trial,

plasma glucose was affected by starvation and re-feeding period, and the lowest content was observed in SRF3 treatment ($p < 0.05$). There was significant difference among treatments in total immunoglobulin and the highest rate was related to the SRF2 treatment and the lowest rate was recorded in the control group (F). Starvation and re-

feeding treatments were not albumin, globulin, cholesterol, and significantly different in total protein, triglycerides ($p>0.05$).

Table 4: Mean±SD values of the Hepatosomatic Index (HSI) and Gastrointestinal Index (GSI) of beluga (*Huso huso*) after a short term starvation and re-feeding (n=3 from each tank).

Treatments	F	SRF1	SRF2	SRF3
HIS	4.07 ± 0.38	3.82 ± 0.41	3.53 ± 0.69	3.32 ± 0.09
GSI	14.83 ± 1.18	12.68 ± 2.88	11.43 ± 3.05	11.52 ± 2.14

F) fed throughout the 40 days, SRF1) 4 intermittent periods of 2-day starvation, 8-day feeding, SRF2) 2 intermittent periods of 4-day starvation, 16-day feeding, SRF3) an 8-day starvation, and 32-day feeding. Different letters in the same row indicate significant differences among treatments.

Table 5: Mean±SD values of hematological indices of beluga (*Huso huso*) after a short term starvation and re-feeding (n=3 from each tank).

Indices	F	SRF1	SRF2	SRF3
Albumin (g/dl)	0.53 ± 0.06	0.52 ± 0.04	0.5 ± 0.04	0.6 ± 0.03
Globulin (g/dl)	2.17 ± 0.34	2.08 ± 0.14	2.09 ± 0.19	1.86 ± 0.20
Immunoglobulin (mg/ml)	15.66 ± 1.3 ^c	16.96 ± 0.87 ^{ab}	17.6 ± 1.02 ^a	16.73 ± 1.12 ^b

F) fed throughout the 40 days, SRF1) 4 intermittent periods of 2-day starvation, 8-day feeding, SRF2) 2 intermittent periods of 4-day starvation, 16-day feeding, SRF3) an 8-day starvation, and 32-day feeding. Different letters in the same row indicate significant differences among treatments.

Table 6: Mean±SD values of biochemical indices of beluga (*Huso huso*) after a short term starvation and re-feeding (n=3 from each tank).

Indices	F	SRF1	SRF2	SRF3
Total protein (g/dl)	2.72 ± 0.30	2.61 ± 0.14	2.60 ± 0.18	2.47 ± 0.22
Glucose (mg/dl)	44.21 ± 7.80 ^a	43.69 ± 6.45 ^a	39.11 ± 5.13 ^{ab}	33.44 ± 4.05 ^b
Cholesterol (mg/dl)	39.86 ± 6.86	38.95 ± 8.88	34.36 ± 10.01	32.51 ± 6.21
Triglyceride (mg/dl)	206.79 ± 23.58	195.37 ± 38.19	191.83 ± 11.22	193.27 ± 16.01

F) fed throughout the 40 days, SRF1) 4 intermittent periods of 2-day starvation, 8-day feeding, SRF2) 2 intermittent periods of 4-day starvation, 16-day feeding, SRF3) an 8-day starvation, and 32-day feeding. Different letters in the same row indicate significant differences among treatments.

Cortisol levels in beluga during the starvation and re-feeding cycle are shown in Figure 1 and Table 7. Significant decrease was observed in cortisol levels in the starvation-treated treatments (SRF1, SRF2, and SRF3) compared to those in the control group ($p<0.05$). There was no significant difference between the SRF1 and SRF2 treatments ($p>0.05$).

The Histomorphometric results of the liver (Fig. 2) and gills (Fig. 3) in fish with short terms of starvation and re-feeding are shown in Table 6. The structure of the liver and gill did not show any anomalies and different feeding periods did not cause a significant change in liver and gill ($p>0.05$).

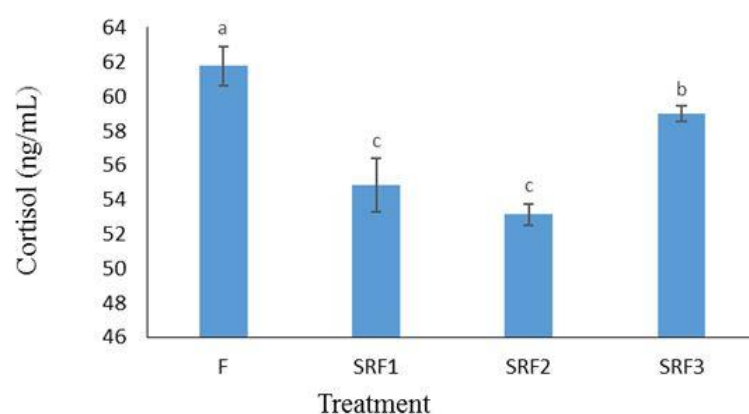


Figure 1: Cortisol levels of beluga (*Huso huso*) after a short term starvation and re-feeding (n=3 from each tank). F) fed throughout the 40 days, SRF1) 4 intermittent periods of 2-day starvation, 8-day feeding, SRF2) 2 intermittent periods of 4-day starvation, 16-day feeding, SRF3) an 8-day starvation, and 32-day feeding. Different letters indicate significant differences among treatments.

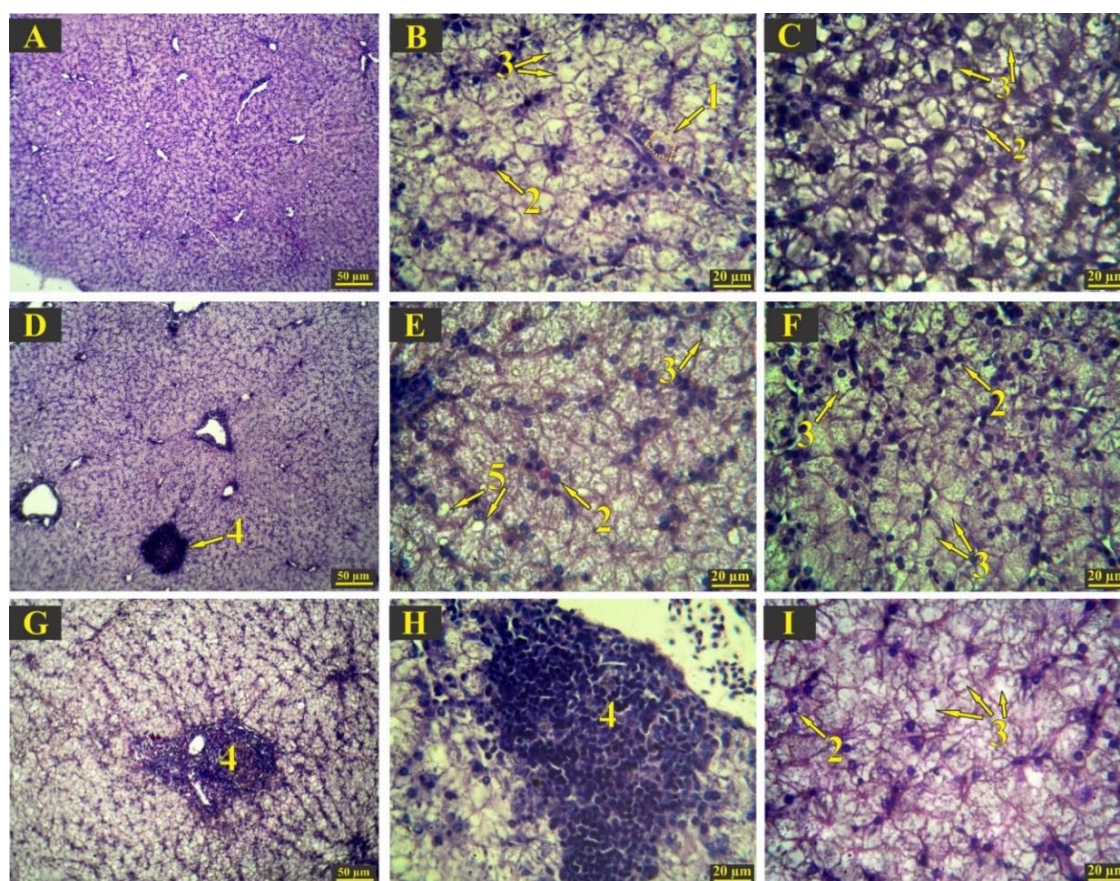


Figure 2: Liver section of beluga (*Huso huso*) after a short term starvation and re-feeding (H & E, $\times 100$ and $\times 400$); A: healthy liver; B, C, F, I: Hepatocellular ballooning; D, G, H: Lobular inflammation; E: Moderate hepatic steatosis; 1. Hepatocyte; 2. Hepatocyte nucleus; 3. Clinical hepatocyte; 4. Lobular inflammation; 5. Hepatic steatosis.

Table 7: Liver and gill histology of beluga (*Huso huso*) after a short term starvation and re-feeding (n=3 from each treatment).

Liver	Indices			
	F	SRF1	SRF2	SRF3
Hepatocyte nucleus diameter (mm)	7.04 ± 1.02	6.80 ± 1.24	6.26 ± 1.15	6.61 ± 0.50
Hepatocyte cell diameter (mm)	16.67 ± 3.25	25.28 ± 2.25	14.33 ± 2.86	15.69 ± 2.12
Nucleus capacity (%)	27.81 ± 6.62	22.11 ± 13.38	23.13 ± 3.82	32.32 ± 2.52
Cytoplasm capacity (%)	72.18 ± 6.62	88.87 ± 13.38	76.85 ± 3.82	67.67 ± 2.50
Gill				
Primary lamellae length (mm)	2317.84 ± 461.94	2511.02 ± 62.26	2421.13 ± 352.87	2866.37 ± 106.68
Primary lamellae density (mm)	238.31 ± 28.17	276.02 ± 19.83	214.44 ± 19.90	246.83 ± 57.05
Secondary lamellae length (mm)	132.88 ± 10.56	165.93 ± 33.79	140.13 ± 16.81	144.27 ± 23.25
Secondary lamellae density (mm)	21.69 ± 6.83	15.47 ± 4.30	20.55 ± 7.71	23.63 ± 12.54

F) fed throughout the 40 days, SRF1) 4 intermittent periods of 2-day starvation, 8-day feeding, SRF2) 2 intermittent periods of 4-day starvation, 16-day feeding, SRF3) an 8-day starvation, and 32-day feeding.

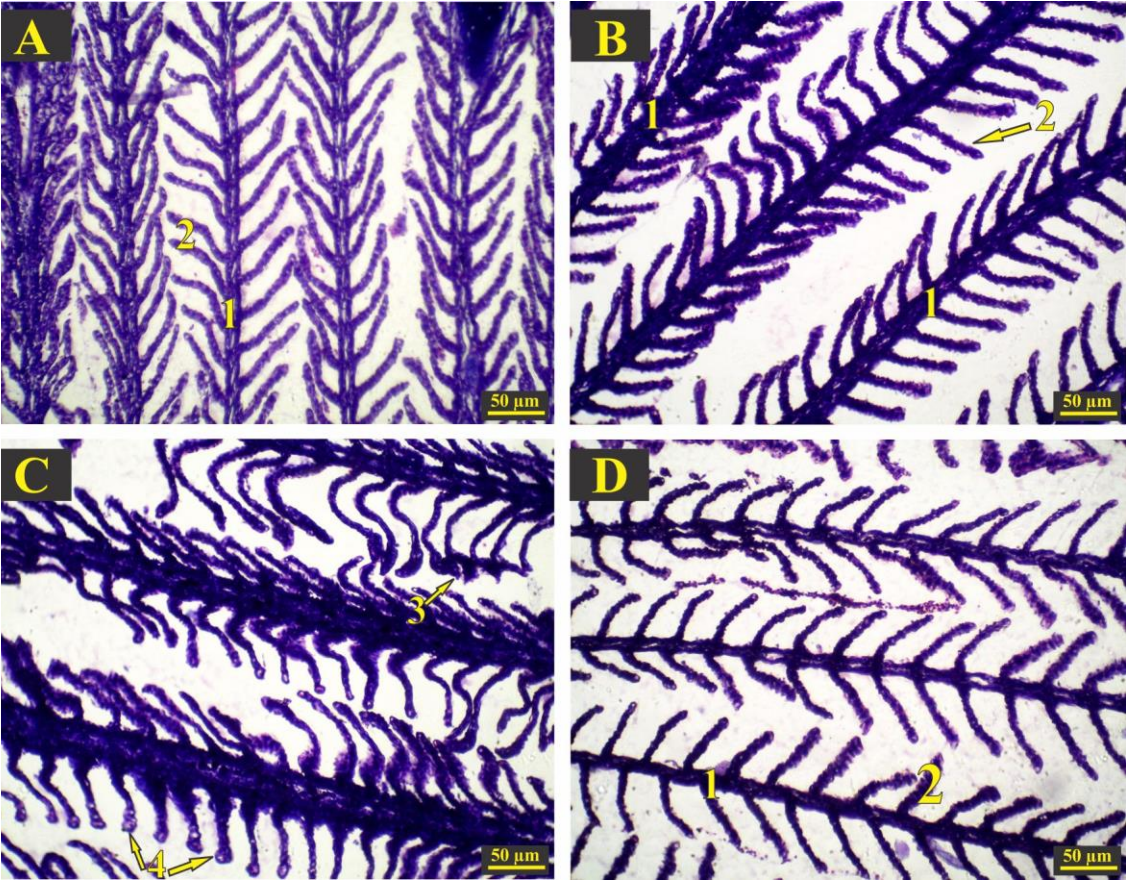


Figure 3: Gill section of beluga (*Huso huso*) after a short term starvation and re-feeding. (H & E, × 100); A: 1. Primary lamellae 2. Secondary lamellae B: 1. Primary lamellae 2. Secondary lamellae C: 3. Moderate fusion 4. Moderate lamellar hyperplasia D: 1. Primary lamellae 2. Secondary lamellae

Discussion

In this study, there was no significant difference in weight gain between SRF1, SRF2, and the control group. These results showed that fish can adapt to periods of starvation so that treatments 2 and 4 days of starvation had full compensatory growth. But the 8-day starvation treatment (SRF3) had less weight gain than the control treatment, which showed a relative compensatory growth. There is much evidence that factors such as severity and duration of food deprivation, dietary composition, age, species, as well as sex, and sexual maturity affect compensatory growth. Despite the differences in the experimental species, the results of this study were consistent with the results of Abdel-Tawwab *et al.* (2006) and Mohanta *et al.* (2017).

In this study, plasma glucose level was affected by starvation and re-feeding period and 8 days of starvation decreased glucose plasma levels in beluga. The lowest content obtained in SRF3, indicating that beluga is unable to maintain plasma glucose levels for 8 days starvation. In agreement with this study, Power *et al.* (2000) in sea bream (*Sparus aurata*), Abdel-Tawwab *et al.* (2006) in Nile tilapia (*Oreochromis niloticus*), Pérez-Jiménez *et al.* (2007, 2012) in European sea bass (*Dicentrarchus labrax*) and (*Dentex dentex*) have reported a significant reduction in plasma glucose levels after starvation. Although glycogenolysis occurs in sturgeon (Babaei *et al.*, 2020), glucose demands and a minor content

of hepatic glycogen would explain the notable decrease of glycemia in starved Beluga.

In the present study, feeding strategies did not significantly affect plasma cholesterol and triglyceride levels, but the total plasma cholesterol and triglyceride declined during food deprivation. Food deprivation can decrease cholesterol and triglyceride levels (Pérez-Jiménez *et al.*, 2007). Similarly, a reduction in plasma cholesterol and triglyceride levels was observed in different species after starvation (Pérez-Jiménez *et al.*, 2007, 2012; Caruso *et al.*, 2011; Eslamloo *et al.*, 2017). The liver lipid content was used in response to food deprivation in beluga and it seems triglycerides are the most available lipid during starvation.

In the present research, starvation and re-feeding had no significant effect on total protein. Furen *et al.* (2012) reported, no conclusive change was found after starvation and re-feeding in plasma protein levels in trout (*Oncorhynchus mykiss*) and *A. naccarii*. Hajimoradi *et al.* (2007) reported no significant effect in protein levels after starvation and re-feeding. The increase and/or steadiness in plasma protein levels during starvation indicate that fish do not use this substrate as an energy source. Our results are consistent with previous findings, which postulate the use of the energy reserves in the muscle of fish subjected to starvation by utilizing glycogen, lipid reserves, and structural protein as energy source (Furen *et al.*, 2012).

The elevated plasma cortisol levels probably play a role in the mobilization of energy substrates in food-deprived fish (Barcellos *et al.*, 2010). In this study, the results showed that food deprivation decreased cortisol levels. Other studies showed decreased cortisol levels in fasted fish (Small and Peterson, 2005). In the current literature, data about the effects of fasting on cortisol levels were inconsistent. Some research reported no effect of starvation on cortisol levels (Yarmohammadi *et al.*, 2015). Others report decreased cortisol levels in fasted fish (Small and Peterson, 2005; Furen *et al.*, 2012) and some others reported increased cortisol levels (Kelley *et al.*, 2001; Peterson and Small, 2004). Peterson and Small (2004), observed that the effect of fasting on plasma cortisol levels in channel catfish (*Ictalurus punctatus*) was dependent on fast length. Due to these highly variable results, it is not safe to speculate about the effects of fasting on cortisol levels. In current work, beluga showed a decreasing trend in HIS and GIS during starvation and re-feeding, although no statistical difference was detected among the data. A reduction in HSI and GSI values was expected in fasted fish (Barcellos *et al.*, 2010). Similarly, the HIS was significantly lower in fasted fish than that in fish fed continuously to apparent satiation in *Rhamdia quelen* (Barcellos *et al.*, 2010). At the end of the experiment, the authors found lower values of HSI and GSI in fasted fish than those in the continuously fed group

(control group), indicating the importance of liver and visceral reserves as a source of energy during starvation in beluga. Following our findings, Nogueira *et al.* (2010) reported a decreasing trend in HSI in red porgy (*Pagrus pagrus*) after food deprivation. Commonly, fish undergoing a period of starvation satisfy the energy requirements by utilizing lipid stores (Weatherley and Gill, 1987). Because the liver is the first storage site where lipid and glycogen are depleted in fish, we expected to observe a negative relationship between fasting and HSI.

In the present study, 2 to 4 days of starvation reduced the size of liver cells, but this trend was not significant compared to the control treatment. However, the findings of the current study were different from the findings of some other studies. A research reported that starvation causes atrophy of liver hepatocytes and degeneration of liver tissue in fish. The hepatocyte atrophy occurred after 30 days of fasting in the neotropical traira (*Hoplias malabaricus*) and it may reflect mobilization of glycogen reserves (Rios *et al.*, 2005). Differences in results may be related to shorter starvation period in the present study.

In conclusion, the results of this study demonstrated that metabolic responses in beluga that were subjected to different treatments (partial or complete period of starvation and feeding) were significantly influenced. Physiological indices were also affected

by feeding strategies because of the effect of fasting/re-feeding on energy mobilization and changes in hematological and biochemical indices.

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