

Effects of various feeding and starvation strategies on growth, hematological and biochemical parameters, and body composition of Caspian brown trout (*Salmo caspius* Kessler 1877) parr

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Abstract

This study was carried out to investigate the effects of starvation and feeding regimes on growth performance, hematological and biochemical parameters of blood and body composition of Caspian brown trout parr. For this purpose, 900 fish (average weight: 12.5 ± 1 g) were stocked in 300-l tanks (18 tanks at a stocking rate of 50 fish in each tank) using an open system. Six experimental groups composed of feeding and starvation regimes were considered for the experiment as follows: FFF (six weeks feeding), SSS (six weeks starvation), SFS (two weeks starvation + two weeks feeding + two weeks starvation), FSF (two weeks feeding + two weeks starvation + two weeks feeding), FS (three weeks feeding + three weeks starvation), and SF (three weeks starvation + three weeks feeding). According to results obtained, the weight gain, special growth rate, condition factor and hepatosomatic index decreased as the length of starvation periods increased ($p < 0.05$). The hemoglobin content and hematocrit did not seem to be affected by starvation ($p > 0.05$), while the highest values of red blood cells and white blood cells were observed in the SSS group ($p < 0.05$). Moreover, the lower values of mean corpuscular hemoglobin and mean corpuscular volumewere observed in the SSS group ($p < 0.05$). The lipid content of body decreased with increased length of the starvation period ($p < 0.05$), wherase the crude protein, ash and moisture contents showed no differences between the experimental groups ($p > 0.05$). In conclusion, our results showed that starvation has significant physiological and morphological effects on Caspian brown trout parr.

Keywords: Starvation, Body composition, Growth performance, Hematology, *Salmo caspius*

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Introduction

Starvation is a common situation that fish species may experience in the wild, as a part of their life cycle, both as a consequence of seasonal changes in water temperature or migration that may cause a lack of food or, to a greater extent, food depletion. In aquaculture conditions, starvation is not frequent, but farmers may adopt similar conditions for the cultured fish to avoid risks of overproduction (Krogdhal and Bakke-McKellep, 2005). Several studies demonstrated that starvation has numerous effects on physiological and morphological properties of fish including: growth, development (Sumpter *et al.*, 1991; Navarro and Gutierrez, 1995; Olivereau and Olivereau, 1997), cardio-respiratory system (Vosyliene and Kazlauskienė, 1999), body composition and energy consumption (Inui and Ohshima, 1966; Dave *et al.*, 1975; Jobling, 1980), immune system (Sakai, 1983; Sullivan and Somero, 1983), morphological, biochemical (Hung *et al.*, 1997; Vosyliene and Kazlauskienė, 1999) and hematological parameters (Mahajan and Dheer, 1983; Heming and Paleczny, 1987; Stepanowska *et al.*, 2006). In addition, starvation mobilizes the nutrient and energy reserves stored in the liver and skeletal muscles (Dave *et al.*, 1975) and also increases the hepatic anti-oxidant enzymes (Pascual *et al.*, 2003).

The Caspian brown trout, *Salmo caspius*, is a critically endangered anadromous species that has been considered for a biological conservation program in the southern part of the

Caspian Sea (Kiabi *et al.*, 1999; Niksirat and Abdoli, 2009). Overfishing, water pollution, construction of dams and poaching of adults and immature fish are the main factors that threaten the existence of Caspian brown trout (Kiabi *et al.*, 1999). Similar to other anadromous fish, the Caspian brown trout does not feed for a long period when it migrates towards spawning rivers. Moreover in the hatchery, captured fish from the wild do not feed for a long period until they are adapted to hatchery conditions. Various feeding regimes might be used for juveniles depending on food availability and financial aspects. The aim of the present study is to describe changes induced by starvation on body composition, growth and hematological and plasma biochemical parameters of the endangered Caspian brown trout.

Materials and methods

The experiment was carried out through six weeks at the Kalardasht Salmonids Reproduction Centre (KSRC), Iran. A total number of 900 Caspian brown trout parr (total weight 12.5 ± 1 g and total length 11.2 ± 1 cm) were distributed in 300-l tanks (18 tanks at a stocking rate of 50 fish in each tank). Altogether, the six experimental treatments including feeding and starvation regimes were considered for the experiment (Table 1). Six experimental groups composed of feeding and starvation regimes were considered for the experiment as follows: FFF (six weeks feeding), SSS (six weeks starvation), SFS (two weeks starvation + two weeks feeding + two weeks

starvation), FSF (two weeks feeding + two weeks starvation + two weeks feeding), FS (three weeks feeding + three weeks starvation), and SF (three weeks starvation + three weeks feeding). During the experiment, the water temperature was 11 ± 0.1 °C, dissolved oxygen was 8 ± 0.5 mg/L and pH was 8.0 ± 0.2 . During feeding

periods, the parrs were fed daily with commercial feeds (produced by Behparvar Company; total protein: 50.8%, lipid: 17.1%, ash: 10.1% and carbohydrate: 9.4%) three times including: 9:00, 13:00 and 16:00 hours. After the course of the experiment, the growth, hematological parameters and body composition were analysed.

Table 1: The starvation and feeding regimes used in the present study (Falahatkar, 2012).

Treatments	Feeding and starvation periods
T ₁ (FFF)	Six weeks feeding
T ₂ (FSF)	Two weeks feeding, two weeks starvation, two weeks feeding
T ₃ (SFS)	Two weeks starvation, two weeks feeding, two weeks starvation
T ₄ (FS)	Three weeks feeding, three weeks starvation
T ₅ (SF)	Three weeks starvation, three weeks feeding
T ₆ (SSS)	Six weeks starvation

Measurment of growth parameters

The growth parameters were measured according to the following formulae:

$$\text{Weight gain (WG; g)} = W_2 - W_1$$

Where:

W₁: total weight of fish in the beginning of the experiment

W₂: total weight at the end of the experiment period

Specific growth rate (SGR; %/day) = $100 \times (\ln W_2 - \ln W_1) / \text{total number of experiment days}$

where:

W₁: weight of fish in beginning of the experiment

W₂: weight of fish at the end of the experiment period

Condition factor (K) = $100 \times (\text{fish weight} / \text{total length}^3)$

Feed conversion ratio (FCR) = $\text{weight gain (g)} / \text{feed intake total fish (g)}$;

Hepatosomatic index (his; %) = $100 \times (\text{total weight of liver} / \text{total body weight})$.

Measurment of hematological parameters

The hematological parameters including the number of red and white blood cells (RBC and WBC), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were measured. The blood samples were taken from the caudal vein of fish using heparinized syringe. The microhematocrit capillary tubes were used for the measurment of hematocrit values according to Řehulka (2005). The hemoglobin values were determined by Cyanmethemoglobin method according to Blaxhall and Daisley (1973). In this regard, 20 µl uncoagulated blood was mixed with 50 µl Drabkin's solution and then placed in a dark environment for 5-10 min. Then, the hemoglobin concentration was measured by spectrophotometry at the wave-length of 540 nm. the number of RBCs and WBCs were determined

using the chamber method using Neubauer's hemocytometer (Drabkin 1945).

The MCV, MCH and MCHC values were calculated as follows:

MCV (fl) = (hematocrit value) / total number of RBCs (million mm^{-3}) $\times 10$

MCH (pg/cell) = (hemoglobin concentration) / total number of RBCs (million mm^{-3}) $\times 10$

MCHC (g dL^{-1}) = (hemoglobin concentration) / (hematocrit value) $\times 100$

Measurement of biochemical parameters

After blood sampling, 2 mL of blood from each fish was allocated for analysis of glucose, triglyceride and cholesterol. To this, the blood samples were centrifuged (1500 g for 10 min) and then the separated plasma samples stored at $-20\text{ }^{\circ}\text{C}$ until biochemical analysis. The biochemical parameters (i.e. glucose, triglyceride and cholesterol) were measured by a colorimetric method (standard analysis kits from Pars Azmoon Company, Karaj, Iran) using an Auto-analyser (Photic 100 Lab system).

Analysis of body composition

Twelve fish were considered for the analysis of body composition in terms of crude protein, crude lipid, ash and moisture contents. For this purpose, at first, the pure meat was prepared after excluding the viscera and also cutting of head, skin and fins. Afterward, the pure meat of each fish was squeezed and homogenized in a grinder and mixer respectively. By weighing the meat samples before and after

incubation at $105\text{ }^{\circ}\text{C}$ in an oven (Heraeus Instrument, D-63450 Hanau, Germany) for a period of almost 24 h, the body moisture was measured as follows:

Moisture (%) = (initial weight before incubation – final weight after incubation) $\times 100$

The crude protein was assayed according to Lowry's *et al.* (1951) by Kjeltec Analyzer Unit 2300. Also, the total lipid content was measured by FOSS set (Soxtec 2050).

To measure the ash content, the tissues samples (each sample with 0.5 g weight) were placed in porcelain crucibles and then kept at $550\text{ }^{\circ}\text{C}$ for 5 h inside a furnace to burn. Afterward, the burned samples were cooled in a desiccator for 30 min. At the end, the ash content was measured as follows:

Ash (%) = $(W_2 / W_1) \times 100$

where W_2 refers to the weight of the ash sample, and W_1 refer to the original weight of the samples.

Statistical analysis

The SPSS software was used for data analysis. The percentage data were converted by angular transformation ($\arcsin \sqrt{p}$) since these data did not have a normal distribution. One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were distinguished by ANOVA, the Tukey test was applied to identify which means were different at the level of $p < 0.05$.

Results

The lowest values of growth indices were observed in the SSS group (Table 2, $p < 0.05$). The WG, SGR, K and HSI decreased as the length of starvation periods increased (Table 2, $p < 0.05$).

The hemoglobin and hematocrit values did not seem to be affected by starvation (Table 3, $p > 0.05$), while the highest values of RBCs and WBCs were observed in SSS group (Table 3, $p < 0.05$). Also, the lower values of MCH and MCV were observed in SSS group (Table 3, $p < 0.05$). There was no

significant differences between experimental groups in terms of MCHC values (Table 3, $p > 0.05$).

The lipid percent of body tissue decreased with increasing length of starvation periods (Table 4, $p < 0.05$), whereas the crude protein, ash and moisture contents showed no differences between experimental groups (Table 4, $p > 0.05$). The lowest values of glucose, triglyceride and cholesterol were observed in SSS group (Table 5, $p < 0.05$).

Table 2: The growth parameters of Caspian brown trout parr in the experimental groups. Means with same superscripts are not significantly different ($p > 0.05$).

Treatments	FFF	FS	SF	FSF	SFS	SSS
Final weight (g)	^a 0.5 ± 14.98	^a 0.3 ± 13.61	^{ab} 50. ± 13.11	^a 0.4 ± 15.27	^b 0.09 ± 12.13	0.3 ^b ± 12.3
Weight gain (%)	^a 0.2 ± 25.52	^{ab} 0.5 ± 16.58	^{ab} 0.3 ± 12.37	^a 0.2 ± 28.21	^{bc} 0.5 ± 1.27	^c 0.2 ± 6.17
Condition factor	^a 0.008 ± 0.92	^{abc} 0.01 ± 0.85	^{abc} 0.03 ± 0.86	^{ab} 0.01 ± 0.91	^{bc} 0.03 ± 0.81	^c 0.00 ± 0.81
SGR (%/day)	^a 0.4 ± 0.54	^{ab} 0.9 ± 0.36	^{ab} 0.5 ± 0.28	^a 0.6 ± 0.59	^{bc} 0.5 ± 0.03	^c 0.5 ± 0.15
FCR	^{ab} 0.22 ± 2.63	^a 0.92 ± 3.29	^a 1.05 ± 2.99	0.15 ^{ab} ± 2.58	-	-
HSI (%)	0.2 ± 1.58	0.1 ± 1.19	0.2 ± 1.96	1.1 ± 1.59	0.07 ± 1	0.4 ± 0.93

Table 3: The hematological parameters of Caspian brown trout parr in the experimental groups. Means with same superscripts are not significantly different ($p > 0.05$).

Parameters	FFF	FS	SF	FSF	SFS	SSS
WBC ($\times 10^3 l^{-1}$)	11 ± 0.2 ^{ab}	7 ± 1b ^c	5.5 ± 0.5 ^c	10 ± 0.7 ^{abc}	8 ± 1 ^{abc}	12 ± 1.3 ^a
RBC ($\times 10^4 l^{-1}$)	53.7 ± 5.7 ^{abc}	33.7 ± 5.8 ^c	40 ± 8b ^c	56.2 ± 7.9 ^{abc}	67.5 ± 76.5 ^{ab}	72.5 ± 4.6 ^a
Hemoglobin (g dl ⁻¹)	7.4 ± 1 ^a	7.7 ± 0.5 ^a	7.7 ± 0.4 ^a	9.1 ± 0.4 ^a	9.3 ± 0.6 ^a	8.6 ± 0.7 ^a
Hematocrit (%)	28 ± 3 ^a	24 ± 2 ^a	26 ± 3 ^a	26 ± 2 ^a	35 ± 2 ^a	27 ± 4 ^a
MCV (fl)	520 ± 5 ^{ab}	730 ± 95 ^a	670 ± 78 ^a	470 ± 27 ^{ab}	520 ± 26 ^{ab}	370 ± 40 ^b
MCH (pg/cell)	140 ± 8 ^a	240 ± 32 ^a	200 ± 47 ^a	170 ± 14 ^a	140 ± 3 ^a	120 ± 10 ^a
MCHC (g dl ⁻¹)	2.64 ± 0.15 ^a	3.24 ± 10.15 ^a	3.04 ± 0.35 ^a	3.52 ± 0.19 ^a	2.65 ± 0.03 ^a	3.24 ± 0.19 ^a

RBC: Red blood cells, WBC: White blood cells, MCV: mean corpuscular volume one, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular haemoglobin concentration.

Table 4: The body composition of Caspian brown trout parr in the experimental groups. Means with same superscripts are not significantly different ($p > 0.05$).

Parameters	FFF	FS	SF	FSF	SFS	SSS
Protein (%)	16.2 ± 0.5 ^a	15.7 ± 0.9 ^a	17 ± 0.7 ^a	17.1 ± 0.3 ^a	17.7 ± 0.2 ^a	16.8 ± 0.1 ^a
Lipid (%)	7 ± 0.5 ^a	3.8 ± 0.3 ^b	4.8 ± 0.2 ^{ab}	4.1 ± 0.2 ^b	3.3 ± 0.6 ^b	3.1 ± 0.5 ^b
Ash (%)	2 ± 0.5 ^a	4.5 ± 0.9 ^a	2.6 ± 0.5 ^a	4.6 ± 0.4 ^a	3.1 ± 0.7 ^a	2.5 ± 0.3 ^a
Moisture (%)	74.8 ± 0.7 ^a	76 ± 0.2 ^a	75.6 ± 1.4 ^a	74.2 ± 0.6 ^a	75.9 ± 0.8 ^a	77.6 ± 0.1 ^a

Table 5: The blood biochemical parameters of Caspian brown trout parr in the experimental groups. Means with same superscripts are not significantly different ($p > 0.05$).

Parameters	FFF	FS	SF	FSF	SFS	SSS
Glucose (mg dl ⁻¹)	160 ± 3 ^a	99 ± 2 ^c	113 ± 3 ^b	105 ± 2 ^{bc}	93 ± 3 ^c	97 ± 2 ^d
Triglyceride (mg dl ⁻¹)	402 ± 4 ^a	321 ± 2 ^b	266 ± 4 ^c	331 ± 3 ^b	229 ± 4 ^d	97 ± 3 ^e
Cholesterol (mg dl ⁻¹)	275 ± 3 ^a	253 ± 4 ^b	190 ± 3 ^c	219 ± 3 ^c	168 ± 3 ^e	126 ± 4 ^f

Discussion

Our results showed that starvation has significant effects on growth, plasma biochemical parameters and body composition of the Caspian brown trout.

Growth parameters

In the present study, starvation had adverse impacts on growth indices. The WG, SGR, K and HSI decreased as the starvation periods increased. It is obvious that nutrition is very important in fish growth. Proteins are necessary for tissue production and also lipids and carbohydrates are required for energy demands. Thus, the decrease of growth indices in the present study can be the response to starvation and lack of food intake. Some studies demonstrated that the HSI decreased after starvation due to the decrease in lipid and glycogen stores of the liver (Blasco *et al.*, 1992; Wang *et al.*, 2005). Generally, K is used to compare the condition, fatness, or well-being (Tesch, 1968) of fish, based on the assumption that heavier fish of a given length are in better condition. In the present study, by increasing the length of feeding period, the FCR decreased. However such decreases were not significant for groups with one feeding period at least.

Hematological parameters

In this study, the hemoglobin content and hematocrit did not seem to be affected by starvation. Conflicting results exist in scientific literature concerning the effects of starvation on blood hemoglobin content and hematocrit value. For example, Sano

(1962), Smirnova (1965) and Johansson-Sjoberg *et al.* (1975) reported an increase in the hematocrit value in response to starvation periods in Japanese eel, *Anguilla japonica*, burbot, *Lota lota* and European eel, *Anguilla anguilla*, respectively, while Murachi (1959) and Kawatsa (1966) reported a decrease in these parameters in starved carp, *Cyprinus carpio* and rainbow trout, *Oncorhynchus mykiss*, respectively. Also, Larsson and Lewander (1973) showed that starvation did not affect the hematocrit and hemoglobin values of starved European eel.

In Caspian brown trout, the highest values of RBCs and WBCs were observed in fish that were subject to 6 weeks of starvation (i.e. SSS group). The number of RBCs is an indicator of oxygen transfer efficiency from respiratory organs to tissues (Holland and Forster, 1966; Nikinmaa and Salama, 1998). Therefore, changes in the number RBC could be associated with changes in metabolic levels. Also, the RBC count show the status of the fish immune system. Some studies demonstrated that the fish immune system could be affected by its nutritional situation (Blazer, 1989; Kiron *et al.*, 1995). Generally, the fish under starvation has a weaker immune system than fish with appropriate feeding. Thus, the starved fish is prone to pathogen attacks and usually its WBC level is higher than fish with adequate feeding.

According to our results, the lower values of MCH and MCV were observed in fish starved for 6 weeks

(SSS group), although its value was not occasionally significant compared to some other experimental groups. One assumption could be dehydration of the blood due to starvation as reported previously by Rios *et al.* (2005). In such situations, the volume of each RBC decreases and its hemoglobin content is concentrated.

Body and plasma biochemical parameters

In the present study, the lipid percent of tissue decreased with increasing periods of starvation whereas the crude protein, ash and moisture exhibited no differences between experimental groups. Many studies have reported decreasing energy stores in tissue in response to starvation. In fish, usually the liver glycogen and lipids are the first energy resources that are used for providing of energy during starvation periods (Black and Love, 1984). Of course, the nature of the energy resource (i.e. protein, lipid or carbohydrate) is different depending on species, duration of starvation, environmental and nutritional conditions, reproductive stage and fish age (Love, 1980, 1988; Clifford and Brich, 1983; Vinagre *et al.*, 2007). In our study, the moisture content of tissue was statistically equal among the experimental groups. The moisture content of tissue is also used as an indicator of the nutritional condition of fish (Sargeut *et al.*, 1989). In this respect, as the lipid content of body tissues is used to provide energy to starved fish, the moisture content of tissue increases due to the oxydation of lipids and thus production of water and

carbon dioxide (Sargeut *et al.*, 1989). In the present study, the lowest values of glucose, triglyceride and cholesterol were observed in the SSS group. This is likely the response to more consumption of energetic compounds of blood in response to acute starvation.

In conclusion, our results showed that starvation has significant physiological and morphological effects on Caspian brown trout parr. The main effects were decrease in growth and probable weakening of the blood parameters. Thus, it is necessary to optimize the feeding strategy during unfavorable rearing condition.

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