Iranian Journal of Fisheries Sciences
10.22092/ijfs.2019.118343

Effects of starvation and refeeding regimes on haematological and biochemical parameters, body composition and growth performance of Caspian brown trout parr (*Salmo trutta caspius* Kessler 1877)

Rahmati F.[1]; Falahatkar B.[2]; Khara H.[1*]

Received: August 2015 Accepted: February 2016

Abstract

This study was carried out to investigate the effects of starvation and feeding regimes on growth performance, haematological 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 growth rate (GR), special growth rate (SGR), condition factor (K) and hepatosomatic index (HSI) decreased as the length of starvation periods increased (p<0.05). The haemoglobin content and haematocrit did not seem to be affected by starvation (p>0.05) while the highest values of red blood cells (RBCs) and white blood cells (WBCs) were observed in the SSS group (p<0.05). Also, the lower values of MCH (haemoglobin concentration in one RBC) and MCV (mean volume of one RBC) were observed in the SSS group (p<0.05). The lipid content of body tissue decreased with increased length of the starvation period (p<0.05), wherase the total protein, ash and moisture 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, Haematological, *Salmo trutta caspius*

1-Department of Fisheries, Lahijan Branch, Islamic Azad University, Lahijan, Iran, P.O. Box: 1616

2- Department of Fisheries, Faculty of Natural Resource, University of Guilan, Sowmeh Sara, Iran,
P.O. Box: 1144

*Corresponding author's Email: h.khara1974@yahoo.com

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 Kazlauskiene, 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 Kazlauskiene, 1999) and haematological parameters (Mahajan and Dheer, 1983; Heming and Paleczny, 1987; Stepanowska *et al*., 2006). As well as this, 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 trutta 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 fry 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 availibility and financial aspects. The aim of the present study is to describe changes induced by starvation on body composition, growth and haematological 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). During the experiment, the water temperature was 11±0.1 ^{0}C, dissolved oxygen was 8±0.5 mgL 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, haematological parameters and body composition were analysed.

Table 1: The starvation and feeding regimes used in the present study.

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 weeksfeeding, three weeks fstarvation
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:

Growth rate (g)=W_2-W_1, where

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

W_2: the total weight after the experiment period;

Specific Growth Rate (SGR %) = (Ln w_2- Ln W_1) / the total number of experiment days × 100,

where W_1: total weight of fish in beginning of the experiment,

W_2: the total weight after the experiment period;

Feed Conversion Ratio (FCR) = amount of feed used (g) / total fish weight gain (g) × 100;

Hepatosomatic index (HSI %) = total weight of liver / total body weight × 100.

Measurment of haematological parameters

The haematological parameters included the number of red and white blood cells (RBC and WBC), haematocrit (%), haemoglobin concentration, mean of haemoglobin percent (MCHC), mean volume of one RBC (MCV) and also the haemoglobin concentration in one RBC (MCH). The blood samples were taken from the caudal vein of fish using heparinized syringe. The microhaematocrit capillary tubes were used for the measurment of haematocrit values according to Rehulka *et al*. (2005). the haemoglobin values were determined by Cyanmethemoglobin 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 haemoglobin concentration was measured by spectrophotometry at the wave-length of 540 nm. Red blood cell count (RBC) and white blood cells were determined using the chamber method using Neubauers haemocytometer (Drabkin 1945).

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

MCV (fl) = (haematocrit value) / total number of RBCs (million. mm^{-3}) × 10

MCH (pg)=(haemoglobin concentration) / total number of RBCs

(million. mm^{-3}) × 10

MCHC (g dL^{-1})=(haemoglobin concentration) / (haematocrit value)× 100

Measurment of biochemical parameters

After blood sampling, 2 mL blood from each fish was allocated for analysis of glucose, triglyceride and cholesterol. To this end, at first, the blood samples were centrifuged (350 g for 10 min) and then the separated serum samples stored at -20 °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, Iran) using an Auto-analyser (Photic 100 Lab system).

Analysis of body composition

12 fish were considered for the analysis of body composition in terms of total protein, total lipid, ash and moisture. For this purpose, at first, the pure meat was prepared after discharge of viscera and blood and also cutting of head, skin and fins. Afterward, the pure meat of each fish was squeezed and hemogenized in a grinder and mixer respectively. By weighing the meat samples before and after incubation at 105 °C in an oven (HERAEUS INSTRUMENTS, D-63450 Hanau, Germany) for a period of 24 h, the body moisture was measured as follows:

Moisture (%) = (initial weight before incubation – final weight after incubation) × 100

The total 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 ^{o}C for 5 h inside a furnance to burn. Afterward, the burned samples were cooled in a desiccator for 30 min. at the end, the total ash was measured as follows:

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

where W_2 refers to the weight of the ashed 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 calculated by ANOVA, the Tukey test was applied to identify which means were different.

Results

The lowest values of growth indices were observed in the SSS group (Table 2, $p<0.05$). The GR, SGR, K and HSI decreased as the length of starvation periods increased (Table 2, $p<0.05$). The haemoglobin content and haematocrit did not seem to be affected by starvation (Table 3, $p>0.05$) while the highest values of red blood cells (RBCs) and white blood cells (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 not significant differences between experimental groups in terms of MCHC values (Table 3, $p>0.05$). The lipid percent of body tissue decreased with increasing lengths of starvation periods (Table 4, $p<0.05$) wherase the total protein, ash and moisture 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, $p<0.05$).

Discussion

Our results showed that starvation has significant effects on growth, plasma biochemical parameters and body composition of the Caspian brown trout. Therefore, we will discuss these effects in clasified sections as follows:

Growth parameters

In the present study, starvation had adverse impacts on growth indices. The growth rate, special growth rate, condition factor and hepatosomatic index 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 peresent study can be the response to starvation and lack of food intake. Some studies demonstrated that the hepatosomatic index decreased after starvation due to the decrease in lipid and glycogen stores of the liver (Blasco *et al*., 1992; Wang *et al*., 2005). Generally, condition factors are 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 food conversion ratio decreased. However such decreases were not significant for groups with one feeding period at least.

Haematological parameters

In this study, the haemoglobin content and haematocrit did not seem to be affected by starvation. Conflicting results exist in scientific literature concerning the effects of starvation on blood haemoglobin content and haematocrit value. For example, Sano (1962), Smirnova (1965) and Johansson-Sjobeck *et al*. (1975) reported an increase in the haematocrit value in response to starvation periods in the Japanese eel, *Anguilla japonica*, the burbot, *Lota lota* and the 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 haematocrit and haemoglobin 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 (Nikinmaa

and Salama, 1998; Holland and Forster, 1966). Therefore, changes in RBC number could be associated with changes in metabolic levels. Also, the RBC count is 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 plasma 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 (i.e. SSS group), althought its value was not occasionally significant compared to some other experimental groups. One assumption could be dehydration of RBC due to starvation as reported proviously by Rios et al. (2005). In such situations, the volume of each RBC decreases and its haemoglobin content is concentrated.

Biochemical parameters of body tissue and blood

In the present study, the lipid percent of tissue decreased with increasing periods of starvation whereas the total 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 (Vinagre *et al*., 2007; Clifford and Brich, 1983; Love, 1980, 1988). 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, tiglyceride 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 immune system. Thus, organized and regular feeding is necessary for good and healthy rearing of this species.

Acknowledgments

The authors express their sincere appreciation to the people who gave their time, advice and support to this study, including: the manager (Mr.

Rezvani) and staff of the Kalardasht Salmonids Reproduction centre (KSRC), for providing fish and technical assistance, Mr. Maziar Akbarabadi, Kaviani and Khodadadi.

References

Black, D. and Love, R.M., 1986. The sequential mobilisation and restoration of energy reserves in tissues of Atlantic Cod during starvation and refeeding. *J. Comp. Physiol. Biochem*, 156(**B**), 469-479.

Blaxhall, P.C. and Daisley, K.W., 1973. Routine hematological methods for use with fish blood. *J. Fish. Biol*, 5, 771-781.

Blasco, J., Fernandez, J. and Gutierrez, J., 1992. Fasting and refeeding in carp (*Cyprinus carpio* L.). The mobilization of reserves and plasma metabolites and hormune variations. *J Comp. Physiol. Biochem*, 162, 539-456.

Blazer, V.S., 1992. Nutrition and disease resistance in fish. *Annu. Rev. Fish. Dis*, 1992, 309–323.

Clifford, H.C. and Brick, R.W., 1983. Nutritional physiology of the freshwater shrimp *Macrobrachium rosenbergii* (DeMan). I. Substrate metabolism in fasting juvenile shrimp. *Comp. Biochem. Physiol*., 74 B, 561–568.

Dave, G., Johansson-Sjobeck, M.L., Larsson, A., Lewander, K. and Lidman, U., 1975. Metabolic and haematological effects of starvation in the European eel, *Anguilla anguilla* L. I. carbohydrate, lipid, protein and inorganic ion metabolism. *Comp Biochem Physiol*, A 52, 423–430.

Drabkin, D.R., 1945. Crystallographic and optical properties of human hemoglobin. A proposal for standartization of hemoglobin. *American Journal of Medical Sciences*, 209, 268270.

Heming, T.A. and Paleczny, E., 1987. Compositional changes in skin muscle and blood serum during starvation of trout. *Aquaculture*, 66, 265.

Holland, R.A.B. and Forster, R.E., 1966. The effects of size of red cells on the kinetics of their oxygen uptake. *Journal of General Physiology*, 49, 727– 742.

Hung, S.S.O., Liu, W., Li, H., Storebakken, T. and Cui, Y., 1997. Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. *Aquaculture*, 151, 357–363.

Inui, Y. and Ohshima, Y., 1966. Effect of starvation on metabolism and chemical composition of eels. *Bulletin of the Japanese Society of Scientific Fisheries*, 32, 492–501

Jobling, M., 1980. Effects of starvation on proximate chemical composition and energy utilization in plaice, *Pleuronectes platessa* L. *Journal of Fish Biology*, 17, 325–334.

Johansson-Sjobeck, M.L., Dave, G., Larsson, A., Lewander, K. and Lidman, U., 1975. Metabolic and haematological effects of starvation in the European eel *Anguilla anguilla* L. II. Hematology. *Comparative Biochemistry and Physiology A*, 52, 431–434.

Kawatsa, H., 1966. Studies on the anemia of fish. I Anaemia of rainbow trout caused by starvation. *Bulletin of Freshwater Fisheries Research Laboratory*, 15, 167–173

Kiabi, B.H., Abdoli, A. and Naderi, M., 1999. Status of fish fauna in the south Caspian Basin of Iran. *Zoology in the Middle East*, 18, 57-65.

Kiron, V., Watanabe, T., Fukuda, H., Okamoto, N. and Takeuchi, T., 1995. Protein nutrition and defence mechanisms in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology A*, 111, 351–359.

Krogdhal, A. and Bakke-McKellep A.M., 2005. Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.). *Comparative Biochemistry and Physiology A*, 141, 450–460.

Larsson, A. and Lewander, K., 1973. Metabolic effects of starvation in the eel, *Anguilla anguilla* L. *Comparative Biochemistry and Physiology A,* 44, 367–374.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* 193(**1**), 265–75.

Love, R.M., 1980. The chemical biology of fishes. Vol 2. Academic Press, London New York.

Love, R.M., 1988. The food fishes. Farrand Press, London. pp. 286-354

Machado, C.R., Garofalo, M.A.R., Roselino, J.E.S., Kettelhut, I.C. and Migliorini, R.H., 1988. Effects of starvation, refeeding, and insulin on energy-linked metabolic processes in catfish (*Rhamdia hilarii*) adapted to a carbohydrate-rich diet. *Gen Comp Endocrnol*, 71, 429-437.

Mahajan, C.L. and Dheer, T.R., 1983. Haematological and haematopoietic responses to starvation in anair-breathing fish Channa punctatus Bloch. *J Fish Biol*, 22, 111–123.

Murachi, S., 1959. Haemoglobin content, erythrocytese dimentation 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. and Gutierrez, J., 1995. Fasting and starvation. In: Hochachka PW, Mommsen TP (eds) Biochemistry and molecular biology of fishes, vol4. Elsevier, Amsterdam. pp. 393–434.

Niksirat, H. and Abdoli, A., 2009. On the status of the critically endangered Caspian Brown trout, *Salmo trutta caspius*, during recent decades in the southern Caspian Sea Basin. *Zoology in the Middle East*, 46, 55-60.

Nikinmaa, M. and Salama, A., 1998. Oxygen transport in fish. In: Perry, S.F., Tufts, B. (Eds.), Fish Physiology, vol. 17. Academic Press, New York, pp. 141– 183.

Olivereau, M. and Olivereau, J.M., 1997. Long-term starvation in the European eel: general effects and responses of pituitary growth hormone-(GH) and somatostatin-

(SL) secreting cells. *Fish Physiol Biochem*, 17, 261–269.

Pascual, P., Pedrajas, J.R., Toribio, F., Lopez-Barea, J. and Peinado, J., 2003. Effect of food deprivation on oxidative stress biomarkers in fish (*Sparus aurata*). *Chem Biol Interact*, 145, 191–199.

Rehulka, J., Minark, B., Adamec, V. and Rehulka, E., 2005. Investigation of physiological and pathological levels of total plasma protein in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research*, 36, 22-32.

Rios, F.S., Oba, E.T., Fernandes, M.N., Kalinin, A.L. and Rantin, F.T., 2005. Erythrocyte senescence and haematological changes induced by starvation in the neotropical fish traira (*Hoplias malabaricus*). *Comparative Biochemistry and Physiology, Part A*, 140, 281– 287.

Sakai, D.K., 1983. Lytic and bactericidal properties of salmonid sera. *J Fish Biol*, 23, 457–466.

Sano, T., 1962. Haematological studies of the culture fishes in Japan.6. Variation in blood constituents of Japanese eel, *Anguilla japonica*, during starvation. *J Tokyo Univ Fish*, 48, 105.

Sargent, J., Henderson, R.J. and Tocher, D.R., 1989. The lipids, p. 153-218. In Halver J.E (ed.) fish nutrition. 2nd ed. Academic Press, San Diego, CA.

Smirnova, L.J., 1965. Blood indices of the burbot during prolonged total fasting and subsequent feeding. *Dokl Akad Sci USSR Biol Sci Sect,* 160, 107–109

Stepanowska, K., Nedzarek, A. and Rakusa-Suszczewki, S., 2006. Effects of starvation on the biochemical composition of blood and body tissue in the Antarctic fish Notothenia coriiceps (Richardson,1844) and excreted metabolic products. *Polar Bio sci*, 20, 46–54

Sullivan, K.M. and Somero, G.N., 1983. Size-and diet-related variations in enzymic activity and tissue composition in the Sable fish, *Anoploma fimbria*. *Biol Bull*, 164, 315–326.

Sumpter, J.P., LeBail, P.Y., Pickering, A.D., Pottinger, T.G. and Carragher, J.F., 1991, The effect of starvation on growth and plasma hormone concentrations of rainbow trout (*Oncorhynchus mykiss*). *Gen Comp Endocrinol*, 83, 94–102.

Tesch, F.W., 1968. Age and growth. In: Methods for assessment of fish production in freshwaters. W.E. Ricker (Ed.). Blackwell Scientific Publications, Oxford, pp. 93–123.

Vinagre, A.S., Nunes do Amaral, A.P., Ribarcki, F.P., Fraga da Silveira, E. and Périco, E., 2007. Seasonal variation of energy metabolism in ghost crab *Ocypode quadrata* at Siriu Beach (Brazil). *Comparative Biochemistry and Physiology, A*, 146, 514-519.

Vosyliene, M.Z. and Kazlauskiene, N., 1999. Alterations in fish health state parameters after exposure to different stressors. *Acta Zool Lituanica Hydrobiol*, 9, 83–94

Wang, Y., Cui, Y., Yang, Y. and Cai, F., 2005. Partial compensatory growth in hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*) following food deprivation. *Journal of Applied Ichthyology*, 21, 389-393.