

Blood biochemistry fluctuations as influenced by feed provision in juvenile Snow trout (*Schizothorax zarudnyi*)

Khandan Barani H.^{1*}; Gharaei A.¹; Sanchooli N.¹; Miri M.²

Received: March 2017

Accepted: April 2017

Abstract

This study aimed at determining blood biochemical parameters with potential diagnostic value to assess the nutritional status for healthy Snow trout, *Schizothorax zarudnyi*. For this purpose, three treatment groups of Snow trout (average weight, 65.9 ± 5.3 g) were kept unfed for 24 h, 7 and 14 days. During the experiment, a natural photoperiod was adopted, water temperature was maintained at 23.2 ± 1.1 °C, pH at 7.8 ± 0.2 , dissolved oxygen at 7 ± 0.4 mg L⁻¹ and ammonia concentration was kept around zero mg L⁻¹. Fish in all treatment groups were fed twice a day, to apparent satiety. At the end of the trial, the levels of glucose; triglyceride; cholesterol; total protein; inorganic phosphorus; calcium; magnesium; triiodothyronine (T₃); thyroxin (T₄); alkaline phosphatase (ALP); aspartate aminotransferase (AST); Alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were determined using standard clinical methods. The results showed that glucose (71.8 ± 6.64 - 138.6 ± 13.02 mg dl⁻¹), AST (63.53 ± 12.17 - 130.41 ± 23.04 UI⁻¹) and T₄ (6.46 ± 0.88 - 11.69 ± 1.16 ng ml⁻¹) levels were significantly affected by the starvation period ($p < 0.05$). T₃ levels remained relatively stable during the first week of starvation (2.3 ± 0.25 - 2.39 ± 0.31 ng ml⁻¹) and then significantly decreased (1.77 ± 0.16 ng ml⁻¹) in the second week of starvation ($p < 0.05$). Triglycerides levels increased during the first week of starvation (147.40 ± 16.62 to 199.31 ± 31.42 mg dl⁻¹) and decreased significantly ($p < 0.05$) in the second week (163.70 ± 24.63 mg dl⁻¹). These results suggested that in healthy Snow trout juveniles, glucose, AST and T₄ levels are responsive to starvation.

Keywords: Blood biochemistry, Thyroid hormone, Enzymes activity, *Schizothorax zarudnyi*, Starvation

1-Department of Fisheries, Hamoun International Wetland Research Institute, University of Zabol, Zabol, Iran.

2-Department of Natural ecosystems management, Hamoun International Wetland Research Institute, University of Zabol, Zabol, Iran.

*Corresponding author's Email: hashem.barani@uoz.ac.ir

Introduction

Ideal growth performance, competent immunity and general health of fish have been identified to be directly affected by proper feeds and feeding conditions (Pujante *et al.*, 2015; Li *et al.*, 2016). However, under natural environment and farm conditions, fish may experience periods of enforced starvation or restricted food availability in their lives (Navarro and Gutiérrez, 1995; Bayir *et al.*, 2011). Many factors such as environmental evolution, seasonal variations in temperature and reproductive processes can act together or independently to decrease the ability of a fish to capture food (Brown and Murphy, 2004). Moreover, reduction in feeding may occur due to errors in feed estimates or as a part of a feeding program to save food and to improve the management of water quality (Davis and Gaylord, 2011). Under intensive aquaculture, nutritional and health conditions of fish are mainly diagnosed through morphological examination and malnutrition or diseases are discovered by clear visual symptoms or weight losses (Peres *et al.*, 2014).

Besides the above method, the screening of blood chemistry parameters is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in farmed animals because these parameters are specific among different species and sexes (Gharaei *et al.*, 2013). Several studies have shown that blood chemistry analysis can also be used to detect the health of fish and provide an easy way of evaluating early malnutrition, stress,

infectious and non-infectious etiologies, dysfunction and metabolic disturbances and disease that have negative impacts on aquaculture productivity (Peres *et al.*, 2014). This issue is particularly relevant as fish nurtured under intensive conditions are subjected to various stressors that risk their nutrition and immunity status and early diagnosis of such condition would enhance management practices.

Many fish populations worldwide have experienced a drastic reduction, largely due to the effects of the industry and habitat loss. One of the useful ways to replace declining natural stocks is through captive breeding or hatchery programs. Since 1997, the population of snow trout, *Schizothorax zarudnyi*, has been decreasing. The original fish, snow trout *S. zarudnyi* which is an endangered species of endemic fish - of southeast Iran and Afghanistan that belongs to the cyprinid family - shows a lot of promise on the grounds of its wide popularity and hardness in environmental conditions (Rahdari *et al.*, 2014). This species has high market value and is a promising candidate for Iranian aquaculture diversification. However, for the majority of fresh water species, including Snow trout, the data about blood biochemical indicators related to feeding behavior in captivity condition are still missing.

In order to clinically interpret blood data, it is necessary to select the metabolites and/or enzymes that have specificity, sensitivity and predictive or diagnostic value for a specific situation and for a specific species (Kerr, 2008). Hence, the present study is aimed to

determine the most sensitive serum biochemical parameters to starvation, i.e., the serum biochemistry parameters with diagnostic value to assess the nutritional condition of Snow trout.

Materials and methods

In this experiment, healthy Snow trout were obtained from the reproduction center of local commercial fish (Zahak, Iran) and transferred to the live aquatic laboratory in autumn. In order to adapt the fish to experimental conditions, they were stocked in a 2000 l tank for 14 days before the start of the experiment. After the acclimation period, 135 fish with a mean weight of 65.9 ± 5.3 g were randomly distributed into 9 tanks (300-L). This study was performed in three treatment groups and triplicate repeats per group (15 fish per aquarium; 3 aquaria per treatment). Three treatment groups including:

G₁: fish were fed diets up to satiation twice daily at 9:00 and 14:00 h during 14 days, and then were left unfed for 24 h.

G₂: fish were fed similar to treatment G₁ for one week and were deprived of feeding during the second week.

G₃: fish were kept unfed for the entire 2 weeks.

During both acclimatization and experimental periods, fish were fed on a commercial diet (Esfahan Mokammel Company, Isfahan, Iran). The proximate composition of the diet was crude protein 30%, crude fat 9%, moisture 10% and ash 11%. The water quality parameters during the controlled condition included: temperature (23.2 ± 1.1 °C), pH (7.8 ± 0.2), dissolved

oxygen (7 ± 0.4 mg L⁻¹) and ammonia concentration was kept around zero mg L⁻¹ (Palintest 8000). All tanks were maintained under a natural photoperiod (dark–light: 12–12 h, in autumn) condition.

At the end of the trial, three fish were sampled from each tank and anesthetized with MS- 222 (50 mg L⁻¹) following at 24 h starvation. All blood samples (9 fish per each treatment) were drawn gently from the caudal vein by 2.0 ml plastic heparinized and non heparinized syringes attached to 21-gauge needles. Blood sera and plasma were collected by centrifuging blood samples at 5000 rpm for 10 min using a Heraeus Labofuge 400, and then the sera were isolated by micropipette and stored at -20 °C for later analysis (Akrami *et al.*, 2013). The sera samples were analyzed using an auto-analyzer (Selectra-PRO model, Netherlands) and diagnostic kits (Pars Azmoon Co., Tehran, Iran) in order to determine serum metabolites (glucose, cholesterol, triglycerides and total protein), enzyme activities (ALT, ALP, AST and LDH) and electrolytes (Calcium, Magnesium and Inorganic phosphorus) according to the methods described by Gharaei *et al.* (2013). Plasma T₃ and T₄ levels were determined by radioimmunoassay technique using T₃ and T₄ RIA kits (Immunotech, Beckman Culture Company, France). All methods including euthanasia were according to the guidelines provided by International Sturgeon Research Institute (Iran) and European Communities Council directive (86/609/EEC) on the

protection of animals used for scientific purposes.

Statistical analyses were performed using SPSS software, version 16. Normality of biochemical values was assayed by the Kolmogorov–Smirnov test. The homogeneity of variances was tested using Levene’s F test. Differences between various treatments were analyzed using one-way ANOVA. Post hoc comparisons among sample means were tested by Tukey’s test and $p < 0.05$ was taken as the level of significance.

Results

No mortality was observed in fish among all treatment groups and specific signs of disease were not recorded in experimental fish during the acclimatization and experiment periods. Blood biochemistry parameter fluctuations of Snow trout kept unfed for 24h, 7 and 14 days are presented in Table 1. The results of the present study showed that mean glucose levels were affected significantly by the starvation time. The significantly highest and lowest levels of glucose ($p < 0.05$) were observed at 24 h (138.60 ± 13.02 mg dl⁻¹) and 14 day (71.8 ± 6.64 mg dl⁻¹)

starvation, respectively. Mean protein and cholesterol levels were not affected ($p > 0.05$) by starvation period and remained from 3.26 ± 0.37 to 3.88 ± 0.24 and 162.20 ± 17.51 to 174.40 ± 26.36 mg dl⁻¹, respectively. After 7 days of starvation, triglyceride level was significantly ($p < 0.05$) elevated from 147.40 ± 16.62 to 199.31 ± 31.42 mg dl⁻¹. However this starvation made AST levels decrease significantly ($p < 0.05$) from 130.41 ± 23.04 to 63.53 ± 12.17 U l⁻¹. ALP and LDH levels were not significantly affected by starvation, though a trend of reduction in mean values was also observed in these enzymes activity. Also, ALT enzyme activity was not detected for most fish analyzed and were not reported in this manuscript. The study also showed that inorganic phosphorus, calcium and magnesium levels were unaffected ($p > 0.05$) by the starvation period. As shown in figure 1 and 2, T₄ level decreased ($p < 0.05$) significantly over the deprivation period but T₃ level remained relatively stable during the first week of starvation. After 2 weeks of starvation, the level of T₃ decreased ($p < 0.05$) significantly to 1.77 ± 0.16 ng ml⁻¹.

Table 1: biochemical indices of blood in snow trout (*Schizothorax zarudnyi*) maintained under different feeding protocols (mean±SD).

Parameters	Treatment			
	G ₁	G ₂	G ₃	
Metabolites	Glucose (mg dl ⁻¹)	138.60±13.02 ^c	97.2±8.33 ^b	71.8±6.64 ^a
	Total protein (mg dl ⁻¹)	3.88±0.24	3.63±0.40	3.26±0.37
	Triglycerides (mg dl ⁻¹)	147.40±16.62 ^a	199.31±31.42 ^b	163.70±24.63 ^a
	Cholesterol (mg dl ⁻¹)	170.85±21.47	162.20±17.51	174.40±26.36
Enzymes	ALP (U l ⁻¹)	90.79±12.96	83.45±8.23	79.27±14.35 ^a
	AST (U l ⁻¹)	130.41±23.04 ^c	98.87±15.97 ^b	63.53±12.17 ^a
	LDH (U l ⁻¹)	706.61±113.57	697.40±66.31	610.28±78.54
Electrolytes	Inorganic phosphorus (mg dl ⁻¹)	4.99±0.40	5.80±0.22	5.52±0.72
	Calcium (mg dl ⁻¹)	8.59±0.38	8.92±0.43	8.64±0.17
	Magnesium (mg dl ⁻¹)	2.83±0.11	2.86±0.29	2.57±0.35

Different superscripts in the same row indicate significant differences between treatments ($p < 0.05$). G₁: snow trout unfed for 24 h; G₂: snow trout unfed for 1 week; G₃: snow trout unfed for 2 weeks

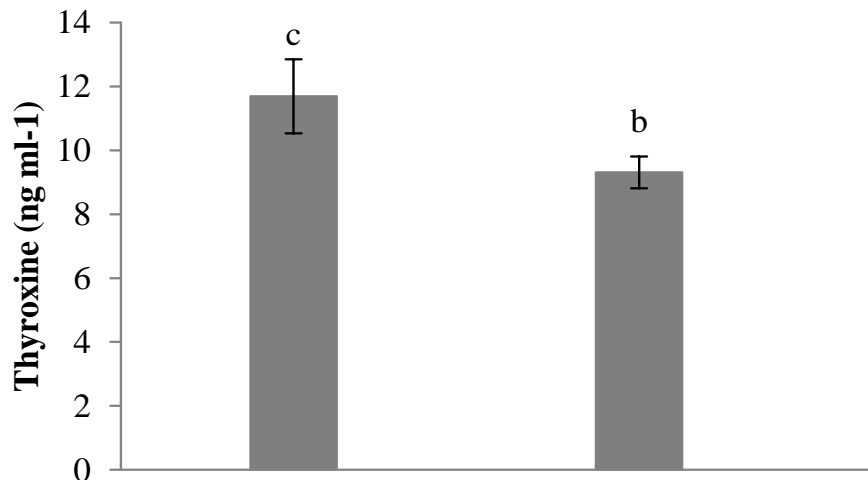


Figure 1: Changes in plasma thyroxine in snow trout (*Schizothorax zarudnyi*) under different feeding protocols (mean±SD). G₁: snow trout unfed for 24 h; G₂: snow trout unfed for 1 week; G₃: snow trout unfed for 2 weeks. Different superscripts on each column indicate significant between treatments ($p<0.05$).

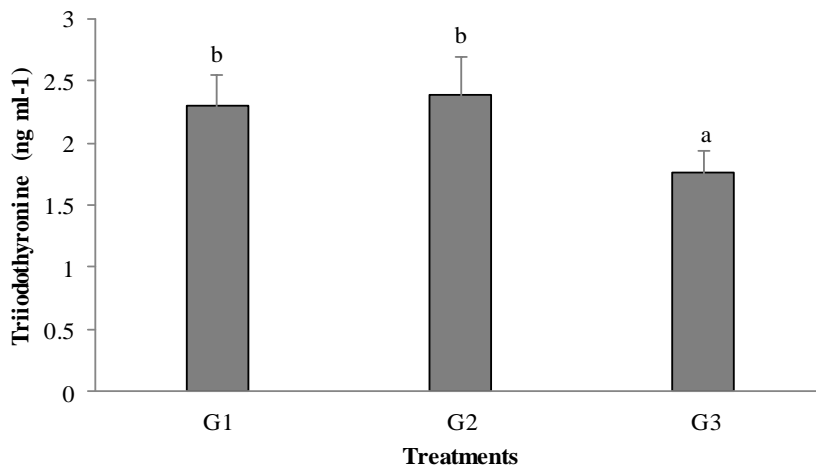


Figure 2: Changes in plasma triiodothyronine in snow trout (*Schizothorax zarudnyi*) under different feeding protocols (mean±SD). G₁: snow trout unfed for 24 h; G₂: snow trout unfed for 1 week; G₃: snow trout unfed for 2 weeks. Different superscripts on each column indicate significant between treatments ($p<0.05$).

Discussion

In this study, the levels of glucose and triglyceride against AST, T₃ and T₄ were increased in fish after starvation. In the aquaculture industry, rapid clinical diagnosis of malnutrition due to exogenous factors, such as management, diseases and stress factors is highly important (Oliva-Teles, 2012; Peres *et al.*, 2014). The analysis of

blood indices is one of the most valuable modern diagnostic tools in terrestrial animals although not yet widely used in aquaculture. Therefore, more studies are required to establish biochemical blood indices and the changes in these parameters for different fish species in various physiological conditions.

The blood glucose level is normally measured to indicate physiological variables in starving animals. The reduction of glucose level induced by starvation in Snow trout that is in agreement with most studies performed in fish (Pérez-Jiménez *et al.*, 2007; Rossi *et al.*, 2015). The lower circulating levels of glucose on starved Snow trout may be due to utilization of glucose, an energetic substrate, to cope with starvation. The extensive mobilization of glucose during starvation gives it some potential as a marker of nutritional condition in Snow trout.

Triglyceride and cholesterol concentration are important to evaluate lipid metabolism. These parameters are used as a major indicator of nutritional status (Pérez-Jimenez *et al.*, 2007; Peres *et al.*, 2014). In this study, there were no significant differences in cholesterol level among all treatment groups. However, triglyceride level was elevated in the deprived fish in G₂ treatment group. It is known that triglycerides are the most available lipid reserve during the early phases of starvation (Navarro and Gutiérrez, 1995). Similarly, Azodi *et al.* (2015) reported that triglyceride level in rainbow trout increased during the starvation period, while cholesterol level remained unchanged. In contrast, many studies reported that the level of triglyceride was decreased in some fish species during starvation (Pérez-Jiménez *et al.*, 2007; Falahatkar, 2012). Therefore, it is suggested that glucose, triglyceride, cholesterol and total protein levels fluctuate with protein

catabolism and glycogenolysis (Vijayan and Moon, 1992). Total triglyceride levels exhibit variable responses; such contradictory results could also result from differences in fish species, past nutritional history, different tissues for lipids storage, and the applied strategies for mobilizing energy reserves as well as duration and severity of starvation (Navarro and Gutiérrez, 1995).

Serum total protein level is known to be an indicator of the nutritional status and provides valuable information about fish metabolism. The findings of the present study showed that feeding strategies had no significant effects on total protein. This is in agreement with results of Azodi *et al.* (2015) and Power *et al.* (2000) who reported that total protein level was not affected by feed deprivation in rainbow trout and seabream. The previous studies proved that duration of deprivation has an important influence on the way in which energy reserves are utilized and metabolic processes are altered (Mehner and Wieser, 1994; Collins and Anderson, 1997).

Enzymes are required for normal cellular metabolism. They are considered as sensitive biochemical indicators and widely used to assess the health of animals (Tahmasebi-Kohyani *et al.*, 2012). The alterations in the enzymatic activities indicate metabolic disorder or organ-specific cell damage in the animal body (Rehulka, 2000; Velmurugan *et al.*, 2008). In the current study, ALP and LDH were not significantly affected by starvation, but a reduction in mean values was observed. However, AST levels were

significantly decreased in unfed animals after 1 and 2 weeks of starvation. This overall reduction of enzyme activity is attributed to a decrease of enzyme synthesis and turnover rates due to lower metabolic demands in unfed fish (Evans and Watterson, 2009). More studies reported that AST activity level decreased during starvation in fish subjected to feed deprivation (Peres *et al.*, 2013; Peres *et al.*, 2014).

Thyroid hormones play an important role in metabolism, growth, development and reproduction of fish (Eales, 2006; Nelson and Habibi, 2008). In our study, starvation induced a significant reduction in thyroid hormones levels (particularly in T_4). Similar results were also recorded in rainbow trout, *Oncorhynchus mykiss* (Raine *et al.*, 2005), channel catfish, *Ictalurus punctatus* (Gaylord *et al.*, 2001) and tench, *Tinca tinca* (De Pedro *et al.*, 2003). The decrease of thyroid activity hormones induced by starvation is an adaptive response to reduce metabolism or growth and preserve nutritional reserves (Leatherland and Farbridge, 1992; Raine *et al.*, 2005). Therefore, thyroid hormone levels seem to have a high potential as predicative diagnostic tools to assess the nutritional status of Snow trout.

It has been demonstrated in some species of fish that blood electrolytes can indicate the nutritional status (Peres *et al.*, 2014). In this study, phosphorus, calcium and magnesium levels were not significantly affected by starvation. Similar results were also reported in common carp (Hoseini and Ghelichpour, 2013). Congleton and

Wagner (2006) indicate that plasma electrolytes decrease only after extended periods of starvation. Also fish can absorb some inorganic nutrients from the aquatic environment. Thus, steady plasma electrolytes levels in Snow trout during starvation probably can be due to short periods of starvation and/or absorbing inorganic nutrients from the water.

The results of the present study showed that starvation (<14 days) significantly influenced some of the measured blood biochemical parameters in Snow trout juveniles. Glucose, AST, T_3 and T_4 levels decreased during starvation and can also be used as a clinical indicator of nutritional status in Snow trout. Moreover, the results of this study would facilitate detection of disease and malnutrition conditions which could affect the production performance of the species in any farming system. However, further studies are required to make sure whether these results can be generalized to include fish with different size/age classes or maturity stages and raised under different culture conditions.

Acknowledgement

Special thanks to Mysam Amiri for helpful contributions. This work was financially supported by Hamoun International Wetland Research Institute, University of Zabol.

References

Akrami, R., Gharaei, A. and Karami, R., 2013. Age and sex specific variation in hematological and serum

- biochemical parameters of beluga (*Huso huso* Linnaeus, 1758). *International Journal of Aquatic Biology*, 3, 132-137.
- Azodi, M., Ebrahimi, E., Motaghi, E. and Morshedi, V., 2015.** Metabolic responses to short starvation and re-feeding in rainbow trout (*Oncorhynchus mykiss*). *Ichthyological Research*, 62, 177-183.
- Bayir, A., Sirkecioglu, A.N., Bayir, M., Halilogly, I., Kocaman, E.M. and Aras, N.M., 2011.** Metabolic responses to prolonged starvation, food restriction, and refeeding in the brown trout (*Salmo trutta*): oxidative stress and antioxidant defenses. *Comparative Biochemistry and Physiology B*, 159, 191-196.
- Brown M.L. and Murphy B.R., 2004.** Seasonal dynamics of direct and indirect condition indices in relation to energy allocation in largemouth bass (*Micropterus salmoides*). *Ecology of Freshwater Fish*, 13, 23-36.
- Collins, A.L. and Anderson, T.A., 1997.** The influence of changes in food availability on the activities of key degradative and metabolic enzymes in the liver and epaxial muscle of the golden perch. *Journal of Fish Biology*, 50, 1158-1165.
- Congleton, J.L. and Wagner, T., 2006.** Blood-chemistry indicators of nutritional status in juvenile salmonids. *Journal of Fish Biology*, 69, 473-490.
- Davis, K.B. and Gaylord, T.G., 2011.** Effect of fasting on body composition and responses to stress in sunshine bass. *Comparative Biochemistry and Physiology A*, 158, 30-36.
- De Pedro, N., Delgado, M.J., Gancedo, B. and Alonso-Bedate, M., 2003.** Changes in glucose, glycogen, thyroid activity and hypothalamic catecholamines in tench by starvation and refeeding. *Journal of Comparative Physiology B*, 173, 475-481.
- Eales, J.G., 2006.** Modes of action and physiological effects of thyroid hormones in fish. In: Reinecke M., Zaccane G., Kapoor B.G. (Eds.), *Fish Endocrinology*. Science Publishers, New York, pp. 767-808.
- Evans, G.O. and Watterson, C.L., 2009.** General enzymology. In: *Animal clinical chemistry, a practical guide for toxicologists and biochemical researchers*, 2nd edn. G. O. Evans (Ed.). CRC Press, New York. pp. 17-36.
- Falahatkar, B., 2012.** The metabolic effects of feeding and fasting in beluga (*Huso huso*). *Marine Environmental Research*, 82, 69-75.
- Gaylord, T.G., MacKenzie, D.S. and Gatlin, D.M., 2001.** Growth performance, body composition and plasma thyroid hormone status of channel catfish (*Ictalurus punctatus*) in response to short-term feed deprivation and refeeding. *Fish Physiology and Biochemistry*, 24, 73-79.
- Gharaei, A., Akrami, R., Ghaffari, M. and Karami, R., 2013.** Determining age- and sex-related changes in serum biochemical and electrolytes profile of beluga (*Huso*

- huso). *Comparative Clinical Pathology*, 22, 923-927.
- Hoseini S.M. and Ghelichpour M., 2013.** Effects of pre-sampling fasting on serum characteristics of common carp (*Cyprinus carpio*). *International Journal of Aquatic Biology*, 1, 6-13.
- Kerr, M.G., 2008.** Veterinary laboratory medicine: Clinical biochemistry and haematology. 2nd Edition. Blackwell Science Ltd, London, 368 P.
- Leatherland J.F. and Farbridge K.J., 1992.** Chronic fasting reduces the response of the thyroid to growth hormone and TSH, and alters the growth hormone-related changes in hepatic 5'-monodeiodinase activity in rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology*, 87, 342-353.
- Li, X.M., Liu, L., Yuan, J.M., Xiao, Y., Fu, S.J. and Zhang, T.G., 2016.** The effect of aerobic exercise and starvation on growth performance and postprandial metabolic response in juvenile southern catfish (*Silurus meridionalis*). *Comparative Biochemistry and Physiology A*, 193, 36-44.
- Mehner, T. and Wieser, W., 1994.** Energetics and metabolic correlates of starvation in juvenile perch (*Perca fluviatilis*). *Journal of Fish Biology*, 45, 325-33.
- Navarro, I. and Gutiérrez, J., 1995.** Fasting and starvation. In: Hochachka, P.W., Mommsen TP (eds) *Biochemistry and molecular biology of fishes*, vol 4. Elsevier, New York, pp. 393-433.
- Nelson, E.R. and Habibi, H.R., 2008.** Functional significance of a truncated thyroid receptor subtype lacking a hormone-binding domain in goldfish. *Endocrinology*, 149, 4702-4709.
- Oliva-Teles, A., 2012.** Nutrition and health of aquaculture fish. *Journal of Fish Diseases*, 35, 83-108.
- Peres, H., Santos, S. and Oliva-Teles, A., 2013.** Selected plasma biochemistry parameters in gilthead seabream (*Sparus aurata*) juveniles. *Journal of Applied Ichthyology*, 29, 630-636.
- Peres, H., Santos, S. and Oliva-Teles, A., 2014.** Blood chemistry profile as indicator of nutritional status in European seabass (*Dicentrarchus labrax*). *Fish Physiology and Biochemistry*, 40, 1339-1347.
- Pérez-Jiménez, A., Guedes, M.J., Morales, A.E. and Oliva-Teles, A., 2007.** Metabolic responses to short starvation and refeeding in (*Dicentrarchus labrax*). Effect of dietary composition. *Aquaculture*, 265, 325-335.
- Power, D.M., Melo, J. and Santos, C.R.A., 2000.** The effect of food deprivation and refeeding on the liver, thyroid hormones and transthyretin in sea bream. *Journal of Fish Biology*, 56, 374-387.
- Pujante, I.M., Martos-Sitcha, J.A., Moyano, F.J., Ruiz-Jarabo, I., Martínez-Rodríguez, G. and Mancera, J.M., 2015.** Starving/re-feeding processes induce metabolic modifications in thick-lipped grey mullet (*Chelon labrosus* Risso,

- 1827). *Comparative Biochemistry and Physiology B*, 180, 57–67.
- Rahdari, A., Gharaei, A. and Ghaffari, M., 2014.** Spawning latency period in hormonal induced reproduction of snow trout (*Schizothorax zarudnyi* Nikolskii, 1897). *Iranian Journal of Biotechnology*, 12, 61-65.
- Raine, J.C., Cameron, C., Vijayan, M.M., MacKenzie, D.S. and Leatherland, J.F., 2005.** Effect of fasting on thyroid hormone levels, and TRa and TRh mRNA accumulation in late-stage embryo and juvenile rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology A*, 140, 452-459.
- Rehulka, J., 2000.** Influence of astaxanthin on growth rate, condition, and some blood indices of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 190, 27–47.
- Rossi, A., Cazenave, J., Bacchetta, C., Campana, M. and Parma, M.J., 2015.** Physiological and metabolic adjustments of (*Hoplosternum littorale*) (Teleostei, Callichthyidae) during starvation. *Ecological indicators*, 56, 161-170.
- Tahmasebi-Kohyani, A., Keyvanshokoo, S., Nematollahi, A., Mahmoudi, N. and Pasha-Zanoosi, H., 2012.** Effects of dietary nucleotides supplementation on rainbow trout (*Oncorhynchus mykiss*) performance and acute stress response. *Fish Physiology and Biochemistry*, 38, 431-440.
- Velmurugan, B., Selvanayagam, M., Cengiz, E.I. and Uysal, E., 2008.** Levels of transaminases, alkaline phosphatase, and protein in tissues of (*Clarias gariepinus*) fingerlings exposed to sublethal concentrations of cadmium chloride. *Environmental Toxicology*, 23, 672–678.
- Vijayan, M.M. and Moon, T.W., 1992.** Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences*, 49, 2260-2266.