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

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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 (T_3); thyroxin (T_4); 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±23.04Ul^{-1}) and T_4 (6.46±0.88-11.69±1.16 ng ml^{-1}) levels were significantly affected by the starvation period (p<0.05). T_3 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 T_4 levels are responsive to starvation.

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

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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

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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:

G_1: 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.

G_2: fish were fed similar to treatment G_1 for one week and were deprived of feeding during the second week.

G_3: 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 T_3 and T_4 levels were determined by radioimmunoassay technique using T_3 and T_4 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

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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, T_4 level decreased ($p<0.05$) significantly over the deprivation period but T_3 level remained relatively stable during the first week of starvation. After 2 weeks of starvation, the level of T_3 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 ($p<0.05$). G_1: snow trout unfed for 24 h; G_2: snow trout unfed for 1 week; G_3: snow trout unfed for 2 weeks

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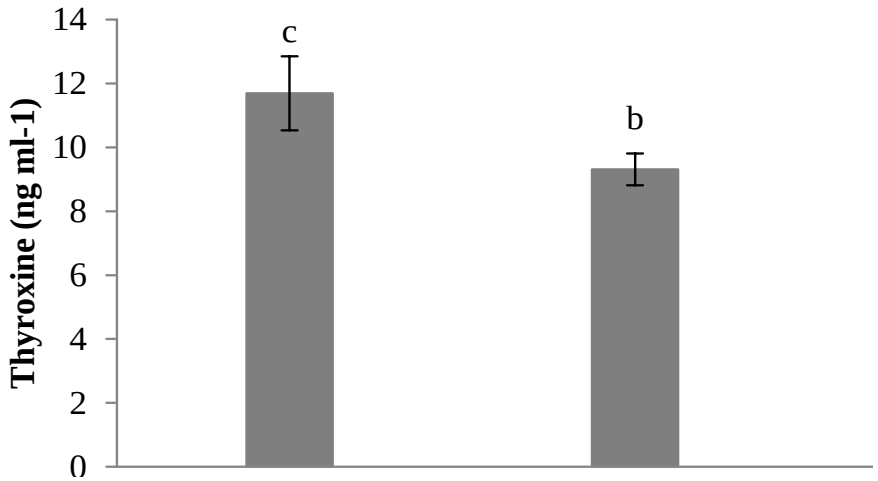

Figure 1: Changes in plasma thyroxin in snow trout (*Schizothorax zarudnyi*) under different feeding protocols (mean±SD). G_1: snow trout unfed for 24 h; G_2: snow trout unfed for 1 week; G_3: snow trout unfed for 2 weeks. Different superscripts on each column indicate significant between treatments (p<0.05).

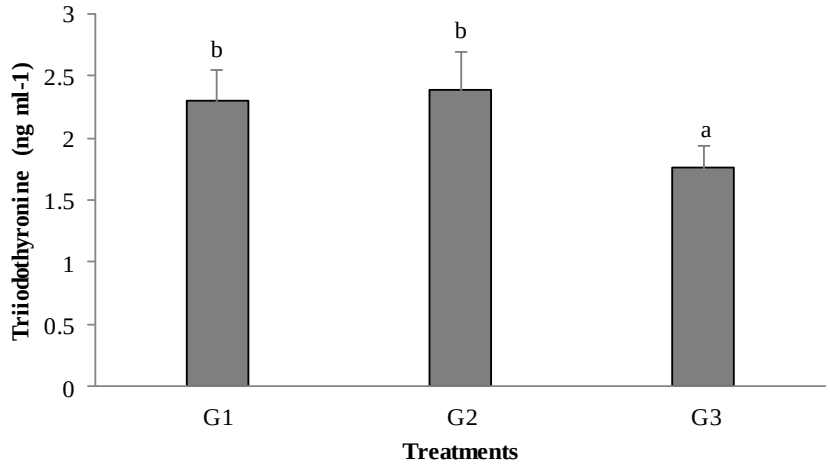

Figure 2: Changes in plasma triiodothyronine in snow trout (*Schizothorax zarudnyi*) under different feeding protocols (mean±SD). G_1: snow trout unfed for 24 h; G_2: snow trout unfed for 1 week; G_3: snow trout unfed for 2 weeks. Different superscripts on each column indicate significant between treatments (p<0.05).

Discussion

In this study, the levels of glucose and triglyceride against AST, T_3 and T_4 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.

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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 G_2 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

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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.

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