Some biochemical responses of *Salmo trutta caspius* in response to transport stress

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Received: June 2020 **Accepted:** August 2020

Abstract

One of the most important and influential stress causing problem and secondary diseases in fish is transport stress. The aim of this study was to evaluate the physiological effects of acute stress of fish transportation on some biochemicals in *Salmo trutta caspius*. A total of 100 fish were transported in plastic bags for 6 h and then released in 300-l tanks. Blood samples were taken after 6, 12, 24 and 48 h after a 6-h transportation (n=15). Based on the results, blood glucose increased compared to the basal value ($p<0.05$) after 6 h but the value was decreased at 12 and 24 h compared to that of 6 h. Cortisol value was increased significantly ($p<0.05$) in all sampling times. Unexpectedly, protein content was significantly increased ($p<0.05$) at 24h. On the other hands, other parameters uch as Na^+, Cl^-, K^+, did not show a significant variation after transportation ($p>0.05$).

Keywords: *Salmo trutta caspius*, stress, transport, blood, biochemical

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Introduction

The physiology of fish can be affected by water parameters changes. Water parameters can unpleasantly alter during fish transport. Handling and transportation are the crucial predisposing factors provoke more acute or chronic stress in fish (Meinelt *et al.,* 2008). Stress is well-known to be a non-specific response of animal to any environmental stimulation out of normal level (Wu *et al*., 2015). Handling of live fish, particularly within hatchery phase, is a routine technique in aquaculture management.different types of acute and chronic stress, such as fluctuation in water temperature, dissolved oxygen depletion, increase of ammonia, alteration in pH and occasionally salinity were performed by handling, packing, shipping, stocking in pond, are expected and result in unpredictable mortality and loss (Pan *et al.,* 2010).

Fish movement is a world essential practive, concerning several aims: (i) human feeding (Marçalo *et al.,* 2008; Reglero *et al.,* 2013); (ii) handling the fry from hatchery centers to growout ponds (de Abreu *et al.,* 2008) and fishing in lake (angling); (iii) handling of wild

fish for rearing practices (Marçalo *et al.,* 2008; Oyoo-Okoth *et al.*, 2011), stocking of fish for rehabilitation programmes (Gomes *et al.,* 2003), for unrestricted aquaria of city (Correia *et al*., 2008; Harmon, 2009), ornamental activities (Brinn *et al.*, 2012; Gomes *et al.*, 2009; Wright *et al.,* 2007), or even for study (Corrêa *et al.*, 2014).

Aquaculture management often described handling for different aims (Stieglitz *et al.,* 2012). Suitable handling with determined regulations and less stressful procedures are very crutial for fish aquaculturists because they can promote the benefit of their practices by retarding stress results in fish mortality (Gomes *et al.*, 2003). Research on farmed animals that characterize responses to stress make aquaculture to be an economic activity with higher profitability (Sampaio and Freire, 2016). Also Stress performs by handling and movement usually result in loss of fish quality and marketing particularly due to losing freshness, lack of appropriate muscle texture, loss of filet weight, rigor mortis, muscle pH alteration and water holding capacity (Jittinandana *et al.,* 2005). Although, Caspian brown trout (*Salmo trutta caspius*) is known as endangered species, naturally its habitat located in southern basin of Caspian Sea. Nowadays, it has been ehabilitated, their cultured stocks gradually increased and maintained for enhancement, protection of wild populations and aquaculture objectives (Sarvi *et al.,* 2006).

The aim of this study was to determine stress responses including some biochemical parameters of *Salmo trutta caspius* to handling and transport.

Material and methods

Fish and rearing condition

The experiment was carried out using 100 *Salmo trutta caspius* transported from Cold Water Fishes Research Center, Tonekabon. The initial Fish weight and total length were 16.99±2.35cm and 54.18±16.26 g, respectively. They were then transported in plastic bags at a density of 1 kg m^{-3}, reached the research farm in Inland Water Aquaculture Research Center, Bandar-e Anzali, after 6h and were promptly stocked in 300-L tanks. Water quality parameters were monitored and recorded at the beginning and the end of the study (Table 1).

Table 1. Water quality parameters during the period of study

Temperature (°C)	19
Dissolved oxygen(mg L^{-1})	6.9±0.82
Ammonium (mg L^{-1})	0.91±0.21
pH	7.64±0.04

Oxygen content and temperature were measured *in situ* (WTW pH 235, Germany), and pH and NH^{4+} were measured in a laboratory using commercial test kits from Aquamerck (Merck, Germany).

Sampling design

The study was based on a blood sample that was prepared randomly at sampling times. The fish were anesthetized and blood samples were taken by heparinized syringes from caudal vein

of 15 fish, at 0, 6, 12, 24 and 48 h after transportation. The fish were stunned and discarded after sampling. The total length and body weight were also measured.

Biochemical assay

For biochemical assay, blood samples were immediately transferred to sterile tubes and the serum was separated by centrifugationat at 5000g for 5 min (Hettich D7200, Germany). Thus, plasma separated and stored at -20 ºC until use. Serum total protein was measured by Biuret method using biochemistry kit (ZiestChemie, Iran) using a spectrophotometer (Model M70; Bausch & Lomb Pharma NV, Brussel, Belgium) at 540 nm. Also glucose and cortisol were measured in plasma, applying diagnostic colorimetric kits (ZiestChemieIran) using a spectrophotometer**.** Serum value of Na^+, K^+ and Cl^- were determined by flame photometric and calorimetrically method, separately and cortisol was assayed by RIA and Immunotech kits (IM 1841, Prague, Czech Republic).

Statistical analyses

data are given as mean±SD. The One-Way ANOVA test was used to determine the differences among groups followed by Duncan's multiple comparisons test while a significant difference was observed ($p < 0.05$) in the ANOVA analysis using SPSS 16.0 software (Chicago, USA).

Results

Table 1 shows the normal values of water quality parameters during the period of the study. No mortality was recorded among the fish during the experimental period. The results (mean±SD) of analysis of the biochemical parameters in *Salmo trutta caspius* are presented in Figure 1-3 and Tables 2.

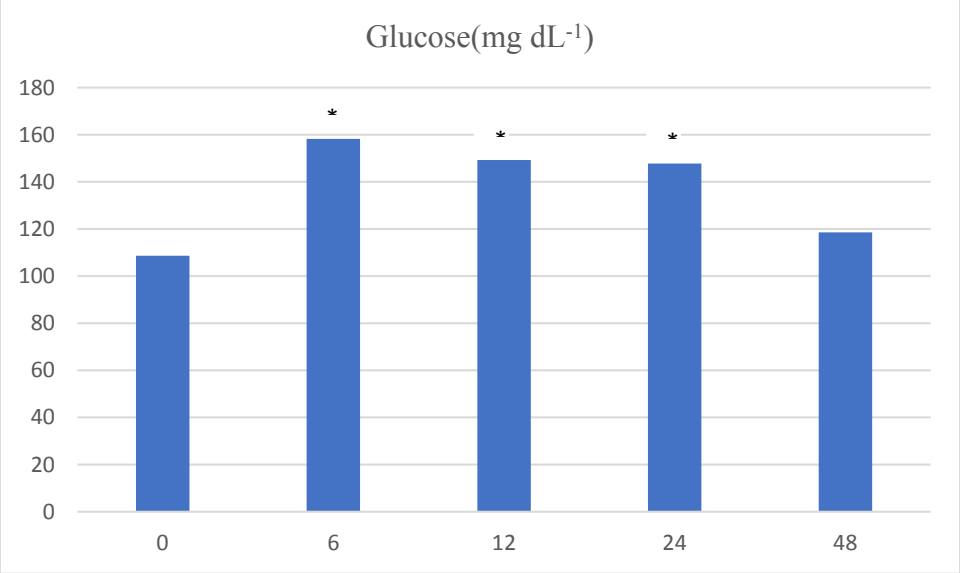

Figure 1. Effect of transportation on blood glucose value at different times. * shows p<0.05.

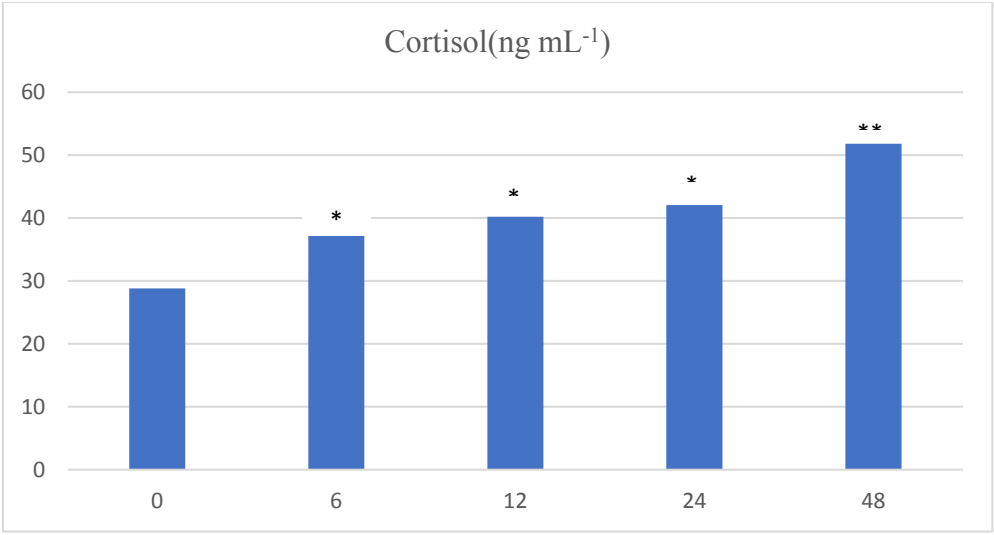

Figure 2. Effect of transportation on blood cortisol value at different times. *, ** shows p<0.05.

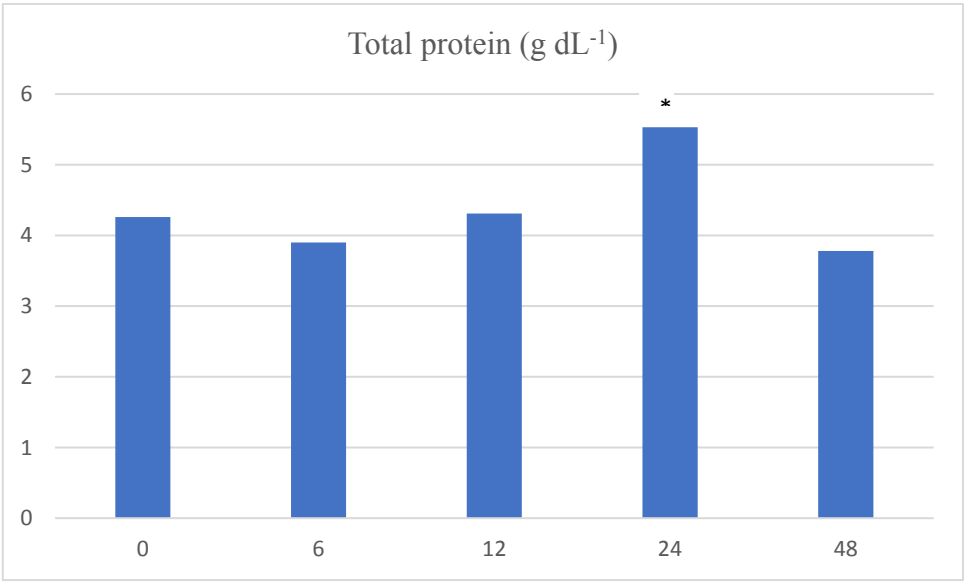

Figure 3. Effect of transportation on total protein value at different times**.** * shows p<0.05.

Table 2. Effect of transportation on blood biochemical parameters

	0	**6**	**12**	**24**	**48**
Na^+(mEq L^{-1})	166.8±13.07[a]	166.4±13.24 [a]	168.50±14.05 [a]	167.25±7.13 [a]	166.4±9.23 [a]
Cl^-(mEq L^{-1})	112.5±5.19 [a]	109.4±3.64 [a]	107.25±2.50 [a]	111±3.60 [a]	114.20±4.08 [a]
K^+(mEq L^{-1})	2.16±0.69 [a]	2.26±.047 [a]	2.90±0.79 [a]	2.59±0.28 [a]	2.57±0.35 [a]

Values are given as mean ± SD. Different letters denote significant differences (p < 0.05) between values of each treatment.

Blood glucose was increased (p<0.05) after 6 h (158.21±30.05 mg dL^{-1}) compared to the basal values (108.61±27.75 mg dL^{-1}. At 12 (149.0±0.25 mg dL^{-1}) and 24 h (147.0±0.75 mg dL^{-1}) of the experiment, the values were decreased compared to samples were analazed

after 6 h (108.6 mg dL^{-1}). Unexpectedly, the value of the samples were remarkably at 48 hour (118.52±9.03), it was insignificantly ($p>0.05$) higher than the basal values (149.25±9.94 mg dL^{-1}). Cortisol significantly increased ($p<0.05$) in all sampling times (6, 12, 24 and 48 h) compared to control values. In the 48-hour sampling, this rate (51.80±11.57 ng mL^{-1}) was significantly ($p<0.05$) higher than all sampling times carrying out at 0, 6, 12 and 24 h (28.8±7.92, 37.38±2.78, 40.21±4.62, 42.05±5.73, respectively). Protein content showed no significant difference ($p>0.05$) among the values of sampling times 0, 6, 12 (4.26±0.68, 3.9±0.47, 4.31±0.72 g dL^{-1}, respectively) and significantly increased (5.53±0.54 g dL^{-1}, $p<0.05$). Its value significantly ($p<0.05$) decreased at 48 h 3.78±0.54 g dL^{-1}). and did not show a significant difference ($p>0.05$) in comparison to the value of the samples were analyzed at 0, 6, 12 and 24 h. On the other hand, blood Na^+, Cl^- and K^+ content did not show a significant difference ($p>0.05$) at different times (Tables 2).

Discussion

As fish are vulnerable to sudden changes in environmental parameters, necessary care should be considered. During the period of study, water quality parameters were checked and it should be expressed that the transport media had no adverse effect on fish condition.

After exposure to stress, activation of hypothalamo-pituitary-interrenal response eventually result in an increase in the plasma cortisol level (Herman *et al.,* 2011). This increase is also observed in the present study. Fish, like other vertebrates cortisol, plays a crucial role in the restoration of homeostasis during stress or afterwards (Goos and Consten, 2002). An upsurge in the levels of plasma cortisol is a primary response to stress (Kalamarz-Kubiak, 2018). In this study, the plasma cortisol level in fish slightly increased after 6 h of transport. This initial increase may be caused by netting within pre-transport handling that is similar with other findings (Dobšíková *et al.,* 2009; Ruane *et al.,* 2002). Plasma cortisol also grew up lightly during transport, which could be continued with the extension of transport time reported while they reared in a red drum (Tacchi *et al.,* 2015).

In this study, glucose was increased significantly at the time of capture and decreased after 12 h, but did not return to basal values. As stated, the response of fish to a stress is described with a series of biochemical and physiological alterations, which in turns results in discharging stress hormones including cortisol into the blood (Stankevičiūtė *et al.,* 2018). These alterations promote the tolerance of a fish to manage stress in order to keep its normal condition or homeostatic state (Barton, 2002). As glucose mobilization is controlled primarily by catecholamines, an increase of plasma catecholamine level almost is occurred after wild capture (Pankhurst, 2011). it can be concluded that the increase of plasma glucose levels recorded from fish samples at the beginning of this study caused by stress-induced changes.

Also It had been showed that blood osmolality and electrolyte concentrations are

affected by fish handling and transportation (Urbinati *et al.*, 2004). This Osmotic fluctuation is caused directly by changes in branchial permeability to water and electrolytes (Henry *et al.*, 2012). In this study, plasma Na^+, K^+ and Cl^- didn't change during the experiment. Similar to vertebrate brain, fish brain also coordinates the spatial and temporal physiological activities through the activation/inactivation of their specific neuronal groups. The membrane-bound of Na+/K+-ATPase (NKA) that maintains the asymmetric distribution of Na^+ and K^+ in neuronal cells shows synaptic and neuronal activity and is connected to neuronal functions (Foo *et al.*, 2012) and the increased NKA activity after hypoxia stress observed in another study (Peter and Simi, 2017). On the contrary, induction of hypoxia stress in immune-challenged fish brain shows a shift in the pattern of NKA activity, and this further points that hypoxia stress has a direct control on brain Na^+/K^+-pump function (Peter and Simi, 2017). Unlike the present study, an increase of ion content in blood had been described in other species (Barcelos *et al.*, 2020; Narra *et al.*, 2017).

Although the protein and glucose content of *Salmo truuta caspius* tended to be decreased after 24 h post stress but cortisol did not show any decrease up to 48 h post stress. Thus, it is concluded that the transport stress in this species could be prolonged up to 48 h after transport stress.

Conflicts of interest

None of the authors has any conflicts of interest to declare.

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