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Original Article

Relationship between cortisol and glucose as physiological stress indicators during growth season in juvenile Siberian sturgeon *Acipenser baerii*

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Abstract
The relationship between cortisol and glucose was studied in two-year old farmed Siberian sturgeon *Acipenser baerii* with a body weight of 340±30 g (mean ± SD) and total length of 45±1 cm from August to November 2017. Thirty-six individuals were randomly selected and stocked in some 3×300 L fiberglass tanks. Blood samples were collected monthly from the tagged fish. The mean cortisol in August, September, October and November were 4.7±0.9, 23.5±3.0, 6.3±0.9 and 7.4±0.9 ng mL^{-1} respectively whereas glucose concentrations were 44.6±0.4, 27.3±0.6, 49.2±0.7 and 48.5±0.7 mg dL^{-1} respectively indicating a significant increase in cortisol and decrease in glucose in September, may be due to the exposure to prolonged high temperature (26–28.5°C). Although it was expected to happen naturally due to hyperglycemia of cortisol but no such phenomena was detected. Our results suggest that the consumption of glucose for maintenance of homeostasis and physiological status is a mechanism against the non-optimal thermal regime. This mechanism consumes glucose at a rate higher than that produced by cortisol, causing significant decrease of plasma glucose. In general, there was a reverse relationship between cortisol and glucose concentration during the experiment in Siberian sturgeons.

Keywords: Cortisol; glucose; physiology; rearing; temperature.

1 | INTRODUCTION

Sturgeons of the family Acipenseridae are at stake due to over-exploitation for caviar and habitat destruction (Gisbert *et al.* 2014; Abdali and Eagderi 2015; Eagderi *et al.* 2017). Hence, culture of sturgeons has been encouraged, which may decrease the fishing pressure and subsequently assist to restore the wild stocks (UNEP and WCMC 2010). Siberian sturgeon, *Acipenser baerii* is a non-anadromous migratory species, living in deep parts of large rivers, spawning over stone-gravel or gravel-sand substrates with moderate to swift current (Kottelat and Freyhof 2007). It can tolerate low temperatures, lives in freshwater and consumes a wide range of food items (Holcik 1989; Koksal *et al.* 2000), which make it a suitable species for aquaculture (Hasanpour *et al.* 2015).

Cortisol and glucose are two stress indicators being released into the blood stream as primary and secondary responses of fishes at the time of stress (Martinez-Alvarez *et al.* 2002). Cortisol is hyperglycemic causing increased blood glucose through gluconeogenesis (Mommsen *et al.* 1999). In sturgeons, cortisol and glucose, as stress indicators, have been studied in Adriatic sturgeon *Acipenser naccarii* (Martinez-Alvarez *et al.* 2002; Cataldi *et al.* 1998), green sturgeon *A. medirostris* (Lankford *et al.* 2003) and Siberian sturgeon *A. baerii* (Maxime *et al.* 1995; Hamlin *et al.* 2007; Williot *et al.* 2011; Hasanalipour *et al.* 2013). In the present study, the interest to study the relationship between these two stress indicators has arisen from firstly, glucose response induced by cortisol is different among fish species, *i.e.* the hyperglycemia is expected after an increase of cortisol but Mammsen *et al.* (1999) pointed out that plasma glycemia depends on the fish species and may increase, decrease or remain unaltered. Secondly, measurement of the cortisol and glucose in the stress studies of Siberian sturgeon have mainly focused on acute stress, for example, nitrite inducing effects (Hamlin 2006), hypoxia (Maxime *et al.* 1995), capture and confinement (Hamline *et al.* 2007), and reproductive procedures (Williot *et al.* 2011), however, there is no report on cortisol-glucose changes as physiological status indicators during growth period, hence this study aimed to assess changes of these two indicators in Siberian sturgeon during August to November as part of its growing season.

2 | METHODOLOGY

This study was performed at the International Sturgeon Research Institute of the Caspian Sea (Rasht, Iran) from August to November 2017. Thirty-six two year old farmed Siberian sturgeon of both sexes with a similar size (mean ± SD; body weight 340 ± 30 g, total length 45 ± 1 cm) were selected for the experiment. They were randomly stocked into three 300 L round fiberglass tanks (diameter ≈ 0.8 m, height ≈ 0.6 m) containing 250 L aerated water as three replicates (Each tank contains 12 fish). Then they were tagged using FDX PIT Microchip 2×12 mm tags under dorsal-fin base using injector syringe. Water flow rate into the tanks was 1 L s^{-1} being supplied from the Sefid River. Water was constantly drained using a drainage valve installed on the bottom of the tanks. Tanks were kept under a 14:10 (light : dark) photoperiod throughout the experiment. Dissolved oxygen and temperature were measured daily using a portable DO meter (Hanna). Fish were fed a manual diet (36% protein, 14% fat, 10% ash, 4% fiber, 11% moisture) at rate of 1.5–2% of live weight four times a day (at 0700, 1200, 1700 and 2200). Specimens were acclimated to the experimental condition for two weeks. Blood was sampled monthly (on 15 August, 15 September, 15 October, and 15 November). Feeding was stopped 24 hours before sampling. The animals were not anesthetized before blood sampling as anesthesia may create stressful conditions and considered inappropriate for cortisol measurement (Barton *et al.* 1998; Marino *et al.* 2001; Papoutsoglou *et al.* 2007).

At each sampling time, three tagged fish were selected from each tank (*i.e.* each specimen was sampled for taking blood once) and two mL blood was taken from the caudal vein of each specimens using a heparinized 2-mL syringe (Trenzado *et al.* 2003). Blood samples were stored in ice and transferred to the laboratory where plasma was separated using centrifugation at 7000 rpm during seven minutes based on Rotllant *et al.* (2001). A 100-µL blood sample from each specimen was transferred to an eppendorf tube and stored at –20°C for later analyses. Cortisol was measured using radioimmunoassay (Rotllant *et al.* 2001) and glucose by enzymatic method (Weil *et al.* 2001). Statistical analyses were performed using SPSS 11 with an α significance level of 0.05. The analysis of repeated measures ANOVA was employed to examine significance differences between means of glucose and cortisol concentrations measured over time.

3 | RESULTS AND DISCUSSION

The results of the cortisol and glucose concentrations of the blood in the farmed Siberian sturgeon during growing season indicate a significant increase in the plasma cortisol level and significant decrease in glucose level in September (both $P < 0.05$; Figure 1). No significant difference was detected among sampling times in cortisol concentration ($P > 0.05$). Mean temperature and oxygen are shown in Table 1.

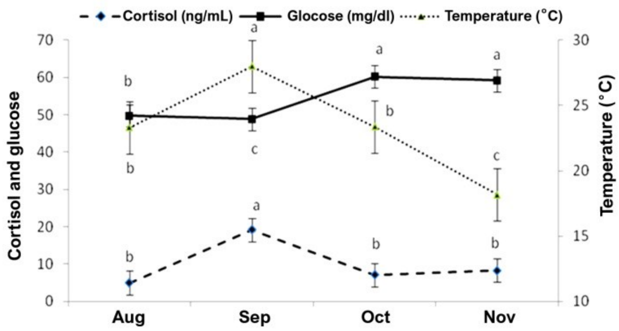

FIGURE 1 Changes in cortisol and glucose concentrations of blood (mean ± standard error) in the farmed Siberian sturgeon during growing season with different temperatures. Significant differences between months were shown with different letters ($P < 0.05$).

TABLE 1 Changes of temperature and oxygen during cultured period (mean ± standard error).

Parameter	Aug	Sep	Oct	Nov
Oxygen (mgL^{-1})	6.3±0.13^{b}	5.8±0.11^{c}	6.8±0.12^{a}	6.7±0.11^{a}
Temperature (°C)	23.3±0.21^{b}	28.0±0.25^{a}	23.4±0.36^{c}	18.6±0.32^{d}

The mean of cortisol concentration in August was comparable with those reported as resting cortisol level by Maxime *et al.* (1995) for the same species indicating that Siberian sturgeon in our study was under normal cultural conditions at the beginning of the experiment. Glucose concentration in August was also similar to resting glucose level in cultured female Siberian sturgeon (Hamlin *et al.* 2007). In September, Siberian sturgeon exposed to higher mean temperature and mean cortisol has increased significantly from 5.01 to 19.18 ng mL^{-1} as a result of thermal stress. The cortisol-caused hyperglycemia was expected in September but this phenomenon was not recorded in this study. The Siberian sturgeon may maintain homeostasis to overcome unfavorable environmental conditions (cumulative effects of high temperature and decreased oxygen) and such response required a high amount of energy which may result in use of excessive glucose, exceeding the supply capability of cortisol. Therefore, glucose concentration decreased significantly in individuals. In October and November, water temperature was comparatively lower and no thermal stress was expected resulting in a significant decrease in cortisol level. Glucose concentration was influenced by decreased cortisol concentration. Inducing effects of temperature increase on cortisol concentration had been found in *A. naccarri* (Cataldi *et al.* 1998) and *A. medirostris* (Lankford *et al.* 2003). We did not monitor water chemistry of the tanks but our flow-through rate was almost twice of those of other studies on sturgeons (e.g. Jodun *et al.* 2002; Ghomi *et al.* 2011) suggesting good water quality. The concentration of cortisol in August–September (19.8 vs. 5.01 ng mL^{-1}) in the present study may be due to thermal effect (Figure 1) and this was found similar to the thermal stress response of juvenile green sturgeon (19.1 vs. 4.4 ng mL^{-1}) reported by Lankford *et al.* (2003) suggesting probability of similar stress response of these two sturgeon species against thermal stress.

There is no unanimous finding on growth temperature of Siberian sturgeon. While Holcik (1989) believes that Siberian sturgeon is compatible to low temperatures. Ruban (2005) found that potential growth of Siberian sturgeon in warm waters is high. According to FAO (2019), temperature range tolerated by the Siberian sturgeon is 25–26°C, hence, the temperature measured in September (26–28.5°C with mean of 27.99°C) may be stressful for this species and in response to the stressor cortisol increased significantly.

Plasma glucose concentration in Adriatic sturgeon exposed to increasing salinity, despite an increase in plasma cortisol concentration, was unaltered. This is in agreement with Martínez-Álvarez *et al.* (2002) on the use of glucose for osmoregulatory mechanisms showing glucose consumption exceeded its production by cortisol. In a review by Martinez-Porchase *et al.* (2009), it was argued that no significant change or even decrease in plasma glucose during chronic stress can be due to consumption of glucose to maintain homeostasis. Type of glucose response to cortisol is affected by factors such as fish species, developmental stage, diet and metabolic state of the animal (Mommsen *et al.* 1999; Barton *et al.* 1988) and time of exposure to stress (short or long time). Siberian sturgeons exposed to hypoxia stress (Maxime *et al.* 1995) and handling stress (Hamlin *et al.* 2007), may result in significant increase in cortisol and glucose concentrations. In rainbow trout, plasma glucose concentration also increased when exposed to long term chronic stress (Barton *et al.* 1987) while in response to handling stress it remained unaltered (Vijayan 1994). In present study, it was found that there is an inverse relationship between cortisol and glucose concentration in Siberian sturgeon during growth season. In other words, significant increase of cortisol concentration was associated with significant decrease of glucose. Finally, we suggest that for general assessment of physiological status of cultured fish, measurement of cortisol and glucose simultaneously might be an appropriate strategy that can be used to design management protocols for aquaculture to minimize stressful impacts of operational procedures.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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SE data interpretation, manuscript preparation**;**
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EÇ critical review of the manuscript.

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