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Cortisol and its metabolites in juvenile Siberian sturgeon, *Acipenser baerii* Brandt, 1869 in response to short-term food deprivation

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ABSTRACT

In this study, the effect of short-terms starvation (0, 2, 4 and 8 days) on plasma cortisol, glucose, triglyceride and cholesterol levels and also hepatosomatic index in Siberian sturgeon, *Acipenser baerii*, was investigated. After acclimation to experimental conditions for 10 days using formulated diet, 180 juvenile Siberian sturgeons (mean weight ± S.E.=19.3±0.4, n=15) were randomly distributed among twelve circular, 500l, fiber glass holding tanks with a flow-through system. In this study, control fish (C) were fed with formulated diet to apparent satiation four times daily throughout the experiment. The other three groups were deprived from feed for 2 (T1), 4 (T2) and 8 (T3) days, respectively. Blood samples were taken at the end of the starvation periods for biochemical analyses. Plasma cortisol, triglyceride and cholesterol levels were not significantly different between control and starved fish at the end of the food deprivation periods, but plasma glucose levels were significantly lower in the starved groups, compared to the control fish. HSI index significantly decreased in all starvation groups, except T1, in comparison to the control. The results suggest that energy reserves mobilization during starvation in Siberian sturgeon may be achieved without the involvement of cortisol. Moreover, in this species there are clear indications of metabolic adjustment ability to short periods of food deprivation.

Keywords: Cholesterol, glucose, hepatosomatic index, Siberian sturgeon, starvation, triglyceride

INTRODUCTION

Fishes are exposed to the periods of food deprivation in natural environments, due to several factors (e.g. temperature, spawning migration, reproduction, limited food availability, etc.). In cultural conditions, it can be also imposed by routine aquaculture procedures (Perez-Jimenez et al., 2007; Furne et al., 2008). Both behavioral and physiological strategies are used to meet the energy requirements of the fish during the food deprivation periods and they rely upon their body reserves, primarily on stored fat (Pottinger et al., 2003). The mobilization of stored energy under such conditions is managed by the endocrine system. As verified in other vertebrates, hormonal regulation of metabolism in fishes is a complex process involving several factors, including cortisol, which is characterized in fish as a multi functional hormone involved in metabolic regulation (Wendelaar Bonga, 1997; Mommsen et al., 1999).

In mammals, plasma levels of cortisol are widely reported to be elevated in response to starvation (Ortiz et al., 2001;

Chang et al., 2002). In contrast, some contradictory results have been reported in fish. Pottinger et al. (2003) found cortisol at higher levels in fed fish than starved fish, in Rainbow trout (*Onchorhynchus mykiss*). In literature, data about the effects of food deprivation on plasma cortisol levels in fish are variously reported to be unaffected by starvation (Rainbow trout: Sumpter et al., 1991; Coho salmon, O. kisutch: Vijayan et al., 1993; Holloway et al., 1994; Reddy et al., 1995; Arctic char, *Salvelinus alpinus*: Jorgensen et al., 1999; Chinook salmon, *O. tshawytscha*: Pirhonen et al. 2003), reduced by starvation (Chinook salmon: Barton et al. 1988; Rainbow trout: Farbridge & Leaterland 1992; Pottinger et al., 2003; Channel catfish, *Ictalurus punctatus*: Small, 2005), or increased by starvation (Coho salmon: Varnavsky et al., 1995; Rainbow trout: Blom et al., 2000; Goby, *Gillichthys mirabilis*: Kelley et al., 2001; Arctic charr: Jorgensen et al., 2002; Channel catfish: Peterson & Small, 2004).

Notwithstanding the contradictory results, the precise metabolic role of cortisol in fish under starvation conditions is not comprehensively known (Mommsen et al., 1999). The plasma cortisol probably plays an important role in the mobilization of energy reserves in the food-deprived fish (Barcellos et al., 2010). Blood glucose concentration is maintained at a steady level during periods of food deprivation. This apparent defence of blood glucose against fluctuations or depletion occurs largely at the expense of liver glycogen, at least during the initial stage of starvation (Navarro & Gutierrez, 1995). During fasting the most available fat reserve seems to be triglycerides (Larsson & Lewander, 1973). Starvation can cause a decrease in plasma glucose, triglyceride, and total cholesterol levels during food deprivation periods (Perez-Jimenez et al., 2007). On the contrary, other studies have reported an increase or steady levels of plasma triglyceride at the early stages of starvation (Echevarria et al., 1997; Kirchner et al., 2005).

Obviously, starvation will lead to morphological alterations in fish. Fish liver, one of the organs firstly affected by starvation (Power et al., 2000), has a major role in glucose homeostasis. Therefore, HSI was considered as the tissue supplying energy in short-term starvation. Thus, the fish respond to these situations is an important challenge for aquaculture since it would help to prevent the possible damage for fish health and, subsequently, to optimize the production (Perez-Jimenez et al., 2007). The present study aimed to verify the possible role of cortisol and metabolites of control of intermediary metabolism during feed deprivation in Siberian sturgeon.

MATERIALS AND METHODS

Animals AndExperimental Conditions

The experiment was conducted at the International Sturgeon Research Institute of Rasht (Iran) in October 2009, using a semi-natural condition such as ambient photoperiod and water temperature fluctuations. Prior to the beginning of the experiment, fish were acclimatized to experimental conditions for one week (Bagherzadeh Lakani et al., 2013). During acclimation, fish were fed to apparent satiation four times daily using a feed formulated for sturgeon rearing (containing 45% crude protein, 18% crude fat, 10% ash and 8% moisture). 180 fish averaged 19.3 g in weight were randomly distributed in twelve circular fiber glass tanks with 500l capacity (15 fish per each replication). The tanks water was continuously changed throughout the experiment using Sefid-Rood River after filtration at a flow rate of approximately 5 L min-1. The water in each tank was permanently saturated (up 92%) with oxygen by supplying air continuously through air-stone from an air-blower. Water quality parameters were monitored daily to ensure the fish needs. Water temperature (17.2±1.5^{o}C), pH (7.2±0.2) and ammonia (lower than 0.1 mg l-1) were recorded during the experiment. In this study, the control group (C) was fed on a formulated diet to apparent satiation four times daily throughout the experiment. The other three groups were deprived from the

feed for 2 (T1), 4 (T2) and 8 (T3) days, respectively.

Sample Collection

Three fish per tank (nine fish per treatment) were randomly sampled and sacrificed with a blow to the head, at days 2, 4 and 8 of the experiment. Blood samples were collected from the caudal vein using heparinzed syringes. Plasma was immediately obtained by centrifuging blood samples at 3000 rpm for 10 min (Cataldi*et al*., 1998) and then was stored at -20^{o}C (Bayunova *et al*., 2002) for further measurements of cortisol and its metabolites. Six fish per treatment were then rapidly euthanized, thereafter , whole fish body and liver weights were recorded for calculating hepatosomatic index as according to the following formula: (liver weight (g)/total body weight (g))*100.

Plasma cortisol levels were determined using a previously validated cortisol RIA kit (Pickering *et al*., 1987; Solati & Falahatkar, 2007). Plasma glucose levels were also assayed by a standard enzymatic-colorimetric test, based on the glucose oxidase-peroxidase method using a commercial kit (Greiner-diagnostic, Germany; www.greiner-diagnostic.com). Commercial kits from Pars Azmun (Karaj, Iran; www.parsazmun.com) were used for the determination of triglyceride (kit 1500032) and total cholesterol (kit 1500010).

Statistical Analysis

All data comparing control and starved groups were analyzed by independent t-test while the starved groups' data were analyzed by One-Way ANOVA using a SPSS version 17.0 for Windows software package. Significant differences among means (P<0.05) were determined by the Duncan's multiple range test (Duncan, 1995).

RESULTS

The plasma cortisol levels are represented in Fig. 1. There were no significant differences in plasma cortisol levels among the control and starved groups (P>0.05). The plasma metabolites variation in treatments is depicted in Fig. 2. There were no significant differences between the control and the food starved groups in plasma triglyceride and total cholesterol levels (P>0.05), although plasma glucose levels were significantly (P<0.05) lower in the starved groups than that in the control group. In addition, the levels of plasma total cholesterol were significantly higher in T3 (133.00±16.74) group than in T1 (83.33±4.33) and T2 (83.67±6.23) groups (P<0.05). Starvation induced a significant decrease (P<0.05) in HSI in T2 and T3 groups (Fig. 3).

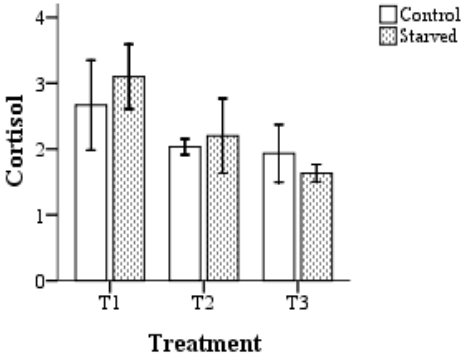

Fig. 1. Plasma cortisol levels of juvenile Siberian sturgeon in control and starved groups. Values are expressed as ng ml^{-1} and they are means±S.E. (n=9). Small letters (columns) indicate statistical differences between the starvation and their control groups, and capital letters (columns) indicate statistical differences between the starvation T_1, T_2 and T_3 groups (P<0.05).

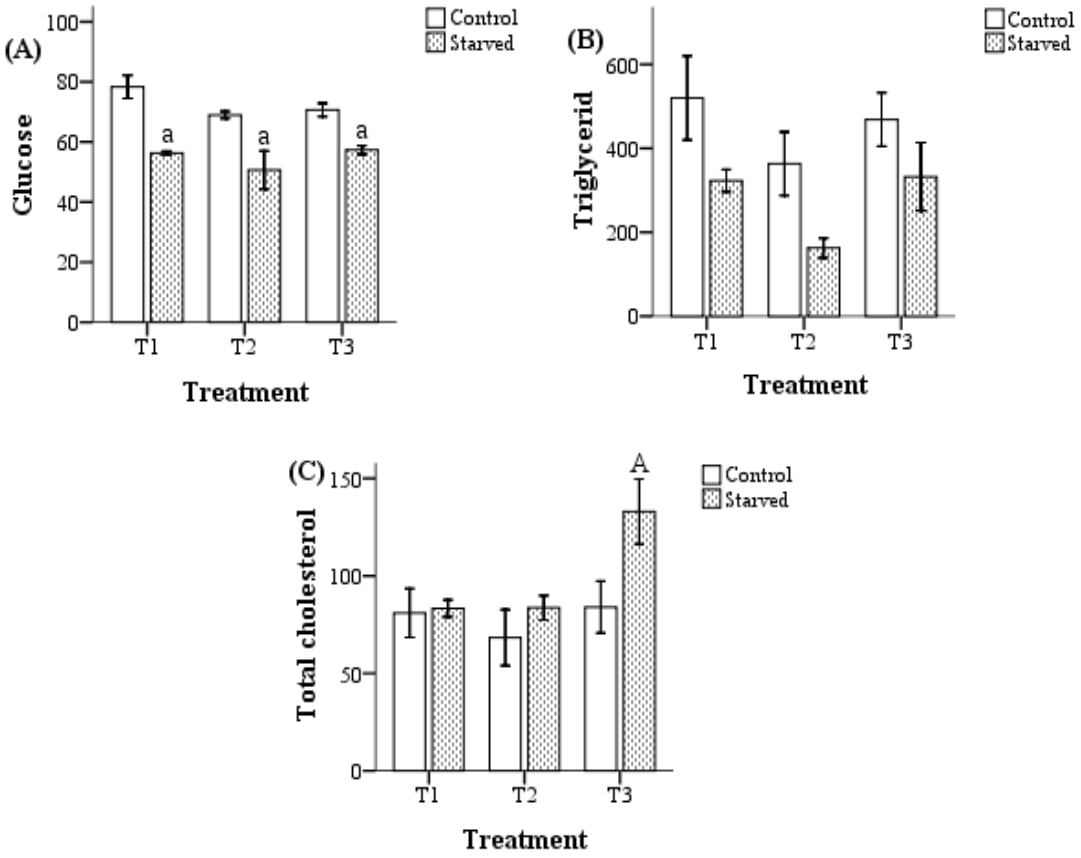

Fig. 2. (A) Plasma glucose, (B) triglyceride and (C) total cholesterol levels of juvenile Siberian sturgeon in control and starved groups. Values are expressed as mg dl^{-1} and they are means±S.E. (n=9). Small letters (columns) indicate statistical differences between the starvation and their control groups, and capital letters (columns) indicate statistical differences between the starvation T_1, T_2 and T_3 groups ($P<0.05$).

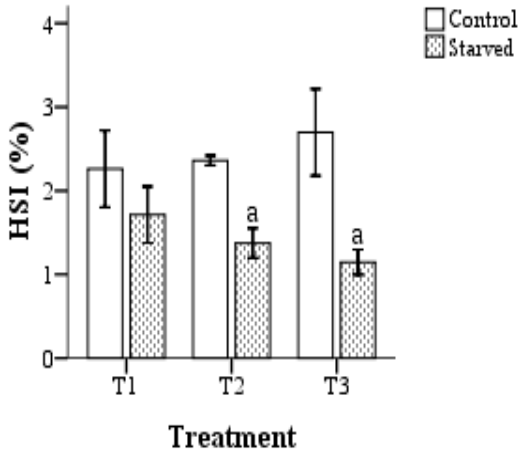

Fig. 3. Hepatosomatic index (HSI = liver weight (g)/body weight (g) × 100) in juvenile Siberian sturgeon. Values are means±S.E. (n=6, number of tanks per group). Small letters (columns) indicate statistical differences between the starvation and their control groups, and capital letters (columns) indicate statistical differences between the starvation T_1, T_2 and T_3 groups ($P<0.05$).

DISCUSSION

Several hormones play a central role in regulating energy reserves utilization during period of starvation in all vertebrates, including fish (De Pedro *et al*., 2003). Metabolic adjustments during food deprivation periods are species-dependent. Intraspecific adjustments to these conditions also depend on different factor such as fish age, reserves availability, past nutritional history, etc. (Navarro & Gutierrez, 1995; Perez-Jimenez *et al*., 2007).

In the present study, plasma cortisol concentrations were almost similar to those previously reported for unstressed Siberian sturgeon (Maxime*et al*., 1995) and in the other sturgeon species (Mommsen *et al*., 1999; Barton, 2002). As shown in Fig. 1, cortisol levels in fish, exposed to starvation for 2 and 4 days,had a non-significant increase in comparison to the control group. By extending the feed restriction period, a slight decrease in cortisol levels was observed. Kelley *et al*. (2001) reported an increase in circulating cortisol levels in Gobies (*G. mirabilis*) starved for 20 days. However, an increase in cortisol levels during starvation has been reported by other studies in fish (Blom*et al*., 2000; Peterson & small, 2004). Peterson and Small (2004) observed that the effect of starvation on plasma cortisol levels in Channel catfish was dependent on the length of the food deprivation.

In the present study, plasma glucose was measured as one of the metabolism indicators. In spite of the fact that plasma glucose levels decline during starvation periods in fish, its level will not be lower than basal metabolism rate (Navarro & Gutierrez, 1995). Kamra (1966) found out that food deprivation brought about a decrease in blood glucose, because of the reliance of some organs on glucose even under the conditions in which metabolism energy is taken more of fat, however, a base blood glucose level is always needed. In this study, reducing plasma glucose level during starvation is in agreement with most previous studies on different species of fish (Sea bream: Power *et al*., 2000; European sea bass, *Dicentrarchus labrax*: Perez-Jimenez *et al*., 2007; Rainbow trout: Ceinos*et al*., 2008). In contrast, Barcellos*et al*. (2010) reported that plasma glucose levels were maintained at a constant level during the different periods of starvation in adult Jundiai *Rhamdia quelen*. It seems that in the majority species of fish maintaining plasma glucose during food deprivation depends on reducing glucose expense rate and then the activation of liver glycogenolysis, particularly in chronic starvation, (Machado *et al*., 1988; Navarro & Gutierrez, 1995).

In this study, despite a decrease in plasma triglyceride and a rise in total cholesterol levels in the starved groups, no significant differences in these variables were observed among these groups and the control group at the end of starvation periods. It is because during the food deprivation period, after the reduction of glycogen reserves, there will also be a decline in plasma glucose level. Consequently, in response to lowered plasma glucose levels, epinephrine and glucagon hormones are released. As a result, gluconeogenesis and lipolysis processes are activated and will bring on the mobilization of the available fat reserves, namely triglycerides (Larsson & lewander, 1973). Hence, fatty acids released due to fat lipolysis will be

carried into the blood. These fatty acids will enter the citric acid cycle and will create energy (Palmegiano *et al*., 1993). This process will also lead to the production of cholesterol (Palmegiano *et al*., 1993). In the agreement with the present results, Ince and Thorpe (1976) studying Pike *Esox Lucius*, Weatherley and Gill (1981) and, Bilinski and Gardner (1968) studying Rainbow trout observed a decrease in fatty acids and an increase in cholesterol level during the starvation period and reported that, this was because of the increased process of gluconeogenesis especially in fat reserves (triglycerides) and the release of cholesterol. Furthermore, Black and Shinner (1986) reported similar results for Rainbow trout in the same process. Larsson and Lewander (1973) observed an increase in fatty acid and a decrease in cholesterol level in European eel (*Anguilla anguilla*). Nonetheless, a considerable decrease in triglyceride, cholesterol and plasma glucose of shrimp *Litopenaeus vannamei* and Common dentex (*Dentex dentex*) was observed in long-term fasting studies (Pascual*et al*., 2006; Perez-Jimenez *et al*., 2012). In contrast, a rise in plasma cholesterol level due to long starvation in some fish has been reported (Simpkins 2002). Such contradictory results are probably caused by differences in fish species and their physiology for satisfying their biological needs during the starvation period (Weatherley & Gill, 1981). In the present study, probably the catabolism of fat reserves and lipoprotein during food deprivation for supplying plasma fatty acid and the production of ketone body as well as the inability to consume cholesterol in the synthesis of bile acids or thyroid hormones (Larsson & Lewander, 1973) are the most important reasons for a dramatic increase in cholesterol levels by the extension the starvation period in T_3 group.

The consumption of hepatic energy reserves as a result of starvation induces decreased liver weight and HSI (Leatherland & Farbridge, 1992; Leiner*et al*., 2000). In this study, the usage of hepatic energy reserves and HSI in T_2 and T_3 groups considerably diminished by lengthening the food deprivation periods. Similar results were reported the depletion of this index (HSI) during starvation periods in fishes. Ali *et al*. (2003) showed a significant reduction in HSI in Common carp (*Cyprinus carpio*) after 8 days of starvation due to consumed liver glycogen. Similarly, Hung *et al*. (1997) stated a significant reduction in HSI in White sturgeon (*Acipenser transmontanus*) after several weeks of starvation. Feed restriction causes noticeable decline in both HSI and liver glycogen in European sea bass (Perez-Jimenez *et al*., 2007). Montserrat *et al*. (2007) observed a drop in HSI in Sea bream after 1, 2 and 3 weeks of starvation. Likewise, HSI in Red porgy (*Pagrus pagrus*) sharply fell during the food deprivation (Caruso *et al*., 2012).

CONCLUSION

In summary, the results of this study revealed that Siberian sturgeon has the capability of metabolic adjustment to short periods of food deprivation without any detrimentally irreversible effects. This species is able to resist to short-term starvation by reducing the rate of metabolism and consuming energy reserves during food deprivation period. In addition, the results of this study are indicative of the relationship between starvation effects and the extension of starvation period. However, the hematological,

biochemical, and immunological factors of this species are suggested to be assessed during food deprivation periods for future studied.

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کورتیزول و متابولیت های بچه تاسماهیان سیبری (*Acipenser baerii*) در پاسخ به گرسنگی کوتاه مدت

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چکیده

ماهیان ممکن است دوره های محرومیت غذایی یا گرسنگی را در طبیعت و آبزی پروری تجربه کنند. این مطالعه با هدف تأثیر دوره های کوتاه مدت گرسنگی بر مقادیر کورتیزول، گلوکز، تری گلیسرید و کلسترول پلاسما و همچنین شاخص کبدی (HSI) در بچه تاسماهیان سیبری *Acipenser baerii* انجام شد. پس از 10 روز سازگاری با شرایط آزمایش تعداد 180 قطعه بچه تاسماهی سیبری *A. baerii* با میانگین وزنی19/32±0/43 گرم به صورت کاملاً تصادفی در 12 مخزن مدور فایبرگلاس 500 لیتری توزیع شدند. در این آزمایش 4 تیمار با سه تکرار در نظر گرفته شد. تیمار شاهد (C) که چهار وعده در روز تا حد سیری ظاهری تغذیه شد. سه تیمار دیگر به ترتیب 2 (T_1)، 4 (T_2)، و 8 (T_3)روز گرسنگی را تجربه کردند. نمونه های خون در پایان دوره های گرسنگی هر یک از تیمارهای آزمایشی گرفته شد. در مقادیر کورتیزول، تری گلیسرید و کلسترول پلاسما بین تیمارهای گرسنگی و شاهد اختلاف معنی داری مشاهده نگردید($P>0/05$)، اما مقادیر گلوکز پلاسما در تیمارهای گرسنگی در مقایسه با تیمار شاهد به طور معنی داری ($P<0/05$) کمتر بود. در مقایسه بین تیمارهای گرسنگی و شاهد، کاهش معنی داری ($P<0/05$) در HSI تحت تأثیر دوره های گرسنگی مشاهده گردید (به استثنای تیمار T_1). نتایج نشان می دهد که مصرف ذخایر انرژی در طی گرسنگی در تاسماهی سیبری *A. baerii* ممکن است بدون دخالت کورتیزول باشد. همچنین تاسماهی سیبری توان سازگاری متابولیکی با گرسنگی کوتاه مدت را دارد.

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