Bulgarian Chemical Communications, Special Edition H, (pp. 119 – 123) 2017

Changes in blood biochemical parameters and enzyme activity of juvenile Chinese sturgeon *Acipenser sinensis* during starvation

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Received June 23 2017; Revised July 20, 2017

In recent years, the number of Chinese sturgeon (*Acipenser sinensis*) has decreased, due to food shortage and starvation as a result of human activity. In this study, the effects of starvation on the metabolism of juvenile sturgeon were examined by measuring their blood parameters over time to assess the utilization of energy resource and identify biomarkers of stressed sturgeon populations. Plasma was sampled every six days during 49 days of starvation. The results showed that the total protein concentration of sturgeon increased slightly during the initial period of the experiment, and then declined significantly after 25 days. It has become apparent that the concentration of albumin (ALB) declined significantly after 31 days of starvation. Glucose (GLU) concentration was decreased significantly after only 7 days of starvation, and dripped significantly again after 31 days. Triglyceride (TGL) and cholesterol (CHOL) levels were steady, until as late as 25 days after starvation. Other parameters including urea, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase also reduced significantly. In contrast, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and alkaline phosphatase were less affected. The activity of alanine aminotransferase (ALT) decreased significantly after 13 and 37 days of starvation. The activity of lysozyme (LSZ) increased generally and there were significant difference between 43-day starvation group and 4 starvation groups of 1-19 days. Therefore, these results indicate that carbohydrates were metabolized preferentially in the blood of juvenile Chinese sturgeon, then proteins, and finally lipids. Furthermore, GLU, ALB, CHOL, and TGL can be used as indicators to nutritional condition. Obvious effects occurred in the blood of juvenile Chinese sturgeon after 13 days of starvation.

Key words: Chinese sturgeon, Blood, Physiology, Nutrient utilization, Starvation.

INTRODUCTION

Chinese sturgeon (*Acipenser sinensis* Gray 1835) are an anadromous species that live only in the Changjiang (Yangtze) River in China. This species is on the Red List of Threatened Species, International Union for Conservation of Nature (IUCN), and has been given Category I Protection in China [1, 2]. Since the sturgeon lifecycle includes a transition from birth in fresh water to salt water for maturity, juveniles concentrate at the river estuary from May through September annually to develop their capacity for osmoregulation by salinity challenge. At this site, the juvenile sturgeon mainly feed on demersal fish, polychaeta, amphipoda, and some small-sized benthic invertebrates including shrimp, crabs, and bivalves [3]. However, because of overfishing, water pollution, beach reclamation, and other human activity, food biomass of Chinese sturgeon in the Changjiang River estuary has declined significantly in recent years, indicating that juvenile Chinese sturgeon face scarce and starvation in the Changjiang River. Therefore, we require a non-lethal method to monitor the nutritional status of this species for the conservation purpose.

Some types of biochemical parameters in blood have been used as the indicators of nutritional status in fishes, the relative predominance of three body constituents (carbohydrates, lipids and protein) depends on the duration of starvation, and if the temporal changes in blood biochemical parameters reflect the change in the relative predominance, the degree of nutritional stress in juvenile Chinese sturgeon may estimate more precisely. However, the nature of metabolic changes experienced during starvation largely depends on the species and duration of the fasting period. Certain fish, including goldfish, carp, rainbow trout [4], and porgy [5], preserve glycogen while metabolizing lipids and/or proteins. Other species, such as cod [6], tilapia [7], and coho salmon [8], conserve proteins and lipids while partially depleting glycogen stores. Evaluating the blood chemical parameters of fish could provide essential information on their metabolism during conditions of starvation.

Previous studies on starvation have mainly focused on teleost, which undergo natural starvation during winter months; whereas little literature is available on the effects of starvation on non-teleost species. Therefore, Chinese sturgeon

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were used in the present study to gain a better understanding of the strategy that non-teleost fish species use to cope with starvation. The main objectives of this study were to: (1) investigate blood changes in juvenile sturgeon in response to starvation stress; (2) assess which energy resource juvenile sturgeon used preferentially during different starvation periods; (3) identify blood biochemical parameters that can potentially be used as indices of substrate utilization and nutritional state; (4) evaluate the maximum time juvenile Chinese sturgeon can suffer. These information would provide a better understanding of the metabolism of Chinese sturgeon, and could be used to further develop conservation strategies.

EXPERIMENTAL

Materials

One hundred juvenile Chinese sturgeons (7 months old) were obtained from the Chinese Sturgeon Research Institute in Yichang, China. The sturgeons were transferred to the Aquaculture Laboratory (East China Sea Fisheries Research Institute, Shanghai, China) and reared indoors in 2-m-diameter circular fiberglass tanks. Fish were stocked for 15 days under ambient light and temperature prior to experimentation in order to acclimate them to laboratory conditions. They were also fed a commercial diet (Shandong Shengsuo Feed Company, Yantai City, China) to apparent satiety 3 times per day (08:00, 14:00, and 20:00 h).

After 15 days of acclimation, 162 active juveniles (mean body length = 16.0±0.8 cm, mean body weight = 21.00±4.01 g) were chosen to be cultured in 3 separate tanks for the starvation experiment (54 fish per tank). The mean lengths and weights of fish did not differ among 3 groups. Water quality was measured daily during the experiment. Water temperature, pH, and dissolved oxygen levels were determined to be 19.2±1.4 °C, 7.4±0.3, and 6.1±0.5 mg/L, respectively. Fish were exposed to a 12 h light-dark photoperiod using overhead fluorescent lights.

Experimental treatments and sampling procedures

The study was carried out from April to May 2008. No sturgeon mortalities occurred during the experiment. Fish were fasted for 24 h prior to the first blood sampling (day 1). Thereafter, samples were taken every 6 days during total 48 days of starvation. Upon each sampling occasion, six fish were randomly selected from each tank, always at the same time of day. Juvenile sturgeon were immediately anaesthetized by immersion in tricaine methanesulphonate at 100 mg/L. Special care was taken to maintain the fish as unstressed as possible. Fish were immersed in the anaesthetic solution until they reached the stage of complete immobility. Thereafter, blood samples of each fish were taken within 2 minutes by puncturing the caudal vein with a disposable sterilized syringe. Blood was immediately transferred into 1.5 mL Eppendorf tubes.

Analysis of samples

Blood was allowed to stand for 120 minutes at 4 °C to achieve complete clotting, then centrifuged at 10 000 × *g* for 10 min at 4 °C. Serum samples were then analyzed, together with the standard material, using a BS-200 Auto Chemistry Analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen City, China). Serum glucose (GLU), total protein (TP), cholesterol (CHOL), triglyceride (TGL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), albumin (ALB), high-density lipoprotein cholesterol (HDLC), low density lipoprotein cholesterol (LDLC), and urea (UREA) were determined using a commercially available kit (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen City, China) according to the manufacturer's instructions. For analytical quality control of the chemical methods used, the certified reference materials was used to ensure accuracy of the coefficient of variation for each determination method was <5%.

The levels of ALT and LSZ were determined with respective kits bought from Nanjing Jiancheng Bioengineering Institute (Jiangshu province, China), according to the manufacturer's instructions. For measurement of LSZ, serum, kit reagents, and distilled water were mixed uniformly and placed into a 37 °C water bath for 15 minutes. Subsequently, the mixtures were placed into a 0 °C ice-water bath for 3 minutes, and were further transferred into 1-cm cuvettes for analysis.

Statistical analysis

All statistical analyses were performed using one-way analysis of variance (ANOVA). Significant differences among means were determined by Duncan's multiple range test. Difference were accepted as significant when $P<0.05$. All data were analysed with Statistics software version 6.0 (StatSoft Inc., Tulsa, OK, USA) and described as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

As shown in Table 1, chemical analysis determined that TP levels increased slightly during the initial period of starvation, and then continually declined to significantly reduced levels by day 25 ($P<0.05$). After 25 days, TP levels remained steady, until it reached its lowest level of 5.1 g/L at the end of experiment (49 days). Similarly, ALB levels remained relatively constant with a slight decrease during the first 25 days of starvation, then decreased significantly by 31 days ($P<0.05$).

In the experiment, GLU levels of sturgeon decreased significantly over time during starvation (Table 1). GLU concentration was significantly decreased ($P<0.05$) after only 7 days of starvation,

and continued to decrease until it was again significantly reduced (P<0.05) after 31 days.

In the experiment, the CHOL levels of sturgeon increased slightly during the first 19 days starvation, then decreased significantly by 25 days (P<0.05) (Table 2). TGL levels also decreased over time during the 49 days of starvation. Its concentration was significantly reduced (P<0.05) after 25 days of starvation, and again after 43 days (P<0.05), and finally reached its lowest level of 5.23 mmol/L on day 49 (Table 2). Of note, the concentrations of CHOL, TGL, and UREA were all significantly decreased by day 49, compared to corresponding starting levels of each (Table 2). In addition, HDLC, LDLC and ALP levels also did not experience significant changes during 49 days of starvation (Table 3). The range of ALP, LDLC, HDLC and LDH were 93.4±4.7–102.9 ± 19.5, 0.20 ± 0.05–0.27 ± 0.10, 0.30 ± 0.08–0.32 ± 0.14, 922.0 ±54.9–992.5±57.8 respectively. The results of statistical tests were P > 0.05 for ALP, LDLC, HDLC and LDH (Table 4). The activity of alanine aminotransferase decreased significantly after 13 and 37 days of starvation. The levels ranged from 42.1 to 134.6 U/L (Figure 1). The activity of lysozyme increased generally and there were significant difference between 43-day starvation group and 4 starvation groups of 1-19 days (Figure 2). Obvious effects occurred in the blood of juvenile Chinese sturgeon after 13 days of starvation.

Evaluations of nutritional health of animals are based mainly on measurements of available energy because the quantity of energy reserves correlates with survival or the adaptive aspects of life-history variation [9, 10]. However, it has been difficult to estimate the nutritional condition of animals living in their natural conditions. Blood biochemical parameters have been used as semi-quantitative indicators of nutritional condition for some fishes [11, 12]. The lethal method is not appropriate for the conservation of Chinese sturgeon, because the fish is faced with the risk of extinction. Non-lethal blood sampling may prove particularly useful for evaluating the nutritional condition of sturgeon.

For instance, blood TP concentration provides information about fish metabolism, as it may be used as a back-up energy source during severe stress [13]. Previous studies showed that TP concentration decreased significantly after a 7-day fasting period in gilthead sea bream [14]. Friedrich et al. (2001) also found that the crude protein level of carp (*Cyprinus carpio*) significantly decreased after 12 weeks without food [15]. ALB is a major component of plasma proteins, and is a biomarker of many diseases and other stress situations [16]. In the current study, TP concentration was determined to be increased slightly during the initial period of starvation, and then declined significantly after 25 days. And ALB concentration declined significantly after 31 days of starvation. These results suggested that during prolonged periods of starvation, sturgeon used protein as one of their energy sources.

Blood carbohydrates play an important role in the regulation of blood GLU levels. GLU is of the utmost of importance in metabolism and can be used as an indicator of stress [17]. Various patterns of change in plasma GLU levels have been described in fasting fish [14, 18]. In the current study, GLU levels of juvenile Chinese sturgeon decreased during starvation, with significant drops detected after 7 days of starvation, and again after 31 days. Similarly, plasma GLU concentrations were decreased in fasting brown trout *Salmo trutta* [19]. Generally, fish use liver glycogen for maintaining metabolic functions, thus supplying energy during short starvation [11, 20]. However, active glycogenolysis produced to counteract fasting is not enough to maintain GLU, thus the concentration of GLU is decreased again [21].

In addition, Macfarlane et al. (1990) previously found that HDLC increased significantly in striped bass (*Morone saxatilis*) during starvation [22]. However, the levels of HDLC and LDLC were fairly unaffected, and exhibited no particular pattern, during starvation of sturgeon. The discrepancy between the present study and the striped bass study may be due to species difference or the physiological statuses of fish, but this issue requires further research for clarification.

During starvation, fish rely on a few main energy resources, firstly carbohydrates, secondly lipids and finally proteins. Physiologists typically describe three phases of fasting for vertebrate animals, mainly according to their measureable changes of glucose, lipids, and proteins [23]. The specific energy source used is highly dependent on the species and duration of the fasting period used. Some fish preserve glycogen, while metabolizing lipids and/or proteins [5]. Other species conserve protein and lipids, while partially depleting glycogen [8]. In the present study, GLU concentration of juvenile Chinese sturgeon decreased significantly after 7 days of starvation, while TP, ALB, CHOL, and TGL levels decreased significantly after 25 days starvation. These data indicated that during starvation, carbohydrates were metabolized preferentially in juvenile Chinese sturgeon, followed by proteins and lipids.

Many fish species can tolerate long periods of starvation in nature. However, the starvation time different fish can sustain vary widely across species, and the overall effects of long-term starvation on different organs and tissues are also different. The neotropical fish *Hoplias malabaricus* can survive food deprivation for periods of up to 180 days, without experiencing obvious reductions in metabolism; these studies also showed perivisceral fat bodies were finally exhausted after 240 days of starvation [24]. In the present study, the concentrations of TP, ALB, GLU, CHOL, and TGL were significantly changed by ~25 or ~31 days of starvation. Therefore, Chinese sturgeon would survive at least during 49 days of starvation at 19˚C.

Table 1. TP, ALB and GLU expression in serum of juvenile Chinese sturgeon during starvation.

Starvation (days)	TP (g/L)	ALB (g/L)	GLU (mmol/L)
1	7.6 ± 0.8 a	3.6 ± 0.5 a	5.86 ± 0.69 a
7	9.4 ± 1.2 b	3.5 ± 0.4 a	4.47 ± 0.87 b
13	8.8 ± 1.1 ab	3.2 ± 0.1 a	4.38 ± 0.60 b
19	8.9 ± 0.5 b	3.4 ± 0.2 a	4.14 ± 0.22 b
25	6.0 ± 1.1 c	3.1 ± 0.7 a	3.89 ± 0.39 b
31	5.5 ± 0.7 c	2.2 ± 0.3 b	2.34 ± 0.17 c
37	5.6 ± 0.5 c	2.0 ± 0.4 b	2.73 ± 0.22 c
43	5.4 ± 0.9 c	2.4 ± 0.3 b	2.38 ± 0.50 c
49	5. 1 ± 0.4 c	2.2 ± 0.3 b	2.11 ± 0.57 c

Mean ± standard deviation. Values sharing same letters differ non-significangtly ($P > 0.05$). The same below.

Table 2. CHOL, TGL and UREA expression in serum of juvenile Chinese sturgeon during starvation.

Starvation (days)	CHOL (mmol/L)	TGL (mmol/L)	UREA (mmol/L)
1	2.00 ± 0.35 a	10.42 ± 0.94	1.36 ± 0.21 a
7	2.24 ± 0.45 a	10.20 ± 0.77	1.47 ± 0.16 a
13	2.12 ± 0.28 a	11.46 ± 0.22	1.18 ± 0.14
19	2.34 ± 0.63 a	10.40 ± 2.08	1.00 ± 0.30
25	1.17 ± 0.45 b	7.82 ± 0.72 b	0.96 ± 0.18
31	1.25 ± 0.65 b	7.66 ± 0.59 b	0.84 ± 0.21 c
37	1.15 ± 0.52 b	6.67 ± 0.49	0.86 ± 0.16 c
43	1.13 ± 0.43 b	5.92 ± 0.88 c	0.85 ± 0.17 c
49	1.06 ± 0.35 b	5.23 ± 1.08 c	0.80 ± 0.15 c

LSZs are enzymes that damage bacterial cell walls by catalyzing the hydrolysis of 1, 4-beta-linkages. LSZ activity has previously been detected in serum, organs, and eggs of fish species [25]. Two LSZs have previously been purified and LSZ cDNA has been cloned from the head kidney of rainbow trout (*Oncorhynchus mykiss*) [26]. Furthermore, Fevolden *et al*. suggested that increases in LSZ activity could be interpreted as a response signal to stress in fish, and that the activity change was dependent on the type and intensity of the stress [27].

Table 3. HDLC, LDLC and ALP expression in serum of juvenile Chinese sturgeon during starvation.

Starvation (days)	HDLC (U/L)	LDLC (U/L)	ALP (U/L)
1	0.30 ± 0.08 a	0.20 ± 0.05 a	93.4 ± 4.7 a
7	0.36 ± 0.05 a	0.30 ± 0.12 a	95.1 ± 3.0 a
13	0.32 ± 0.07 a	0.20 ± 0.06 a	98.0 ± 7.4 a
19	0.44 ± 0.06 a	0.44 ± 0.15 a	116.0 ± 5.6 a
25	0.39 ± 0.11 a	0.28 ± 0.18 a	110.6 ± 23.7 a
31	0.41 ± 0.06 a	0.46 ± 0.30 a	116.2 ± 6.2 a
37	0.42 ± 0.06 a	0.24 ± 0.09 a	114.5 ± 6.8 a
43	0.34 ± 0.15 a	0.40 ± 0.07 a	115.3 ± 24.7 a
49	0.32 ± 0.14 a	0.27 ± 0.10 a	102.9 ± 19.5 a

Table 4. ALT, AST and LDH expression in serum of juvenile Chinese sturgeon during starvation.

Starvation (days)	ALT (U/L)	AST (U/L)	LDH (U/L)
1	134.6 ± 36.6	387.2 ± 128.1	922.0 ±54.9 ab
7	114.0 ± 14.6	420.8 ± 83.4 a	1098.2 ±124.8
13	73.4 ± 10.9 b	356.8 ± 43.1	1113.2 ±113.0
19	76.6 ± 16.1 b	416.8 ± 36.7 a	1276.6±40.6
25	54.3 ± 11.6	339.8 ± 20.0	1203.9±113.6
31	53.2 ± 12.5	310.1 ± 52.4 b	994.5±74.6 c
37	45.6 ± 6.4 c	330.1 ± 54.8	1039.1±135.0
43	45.4 ± 10.3 c	356.8 ± 40.6	1021.4±76.2
49	42.1 ± 4.1 c	302.0 ± 10.2 b	992.5±57.8 ac

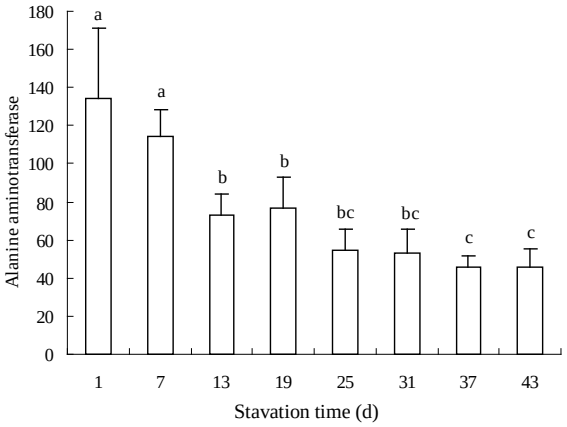

Fig. 1. Effect of starvation on activity of alanine aminotransferase in juvenile Chinese sturgeon.

In contrast, Mock *et al*. reported that chronic stress resulted in a reduced LSZ activity [28]. Therefore, it seems that the trends of LSZ activity changes may vary for different fish species, and upon different stressors. In the present study, as the starvation time increased, the LSZ activity of juvenile Chinese sturgeon generally increased. This finding suggests that the non-specific immunity of Chinese sturgeon was fairly maintained during the

starvation period. The activity of lysozyme (LSZ) increased significantly until 43-day starvation.

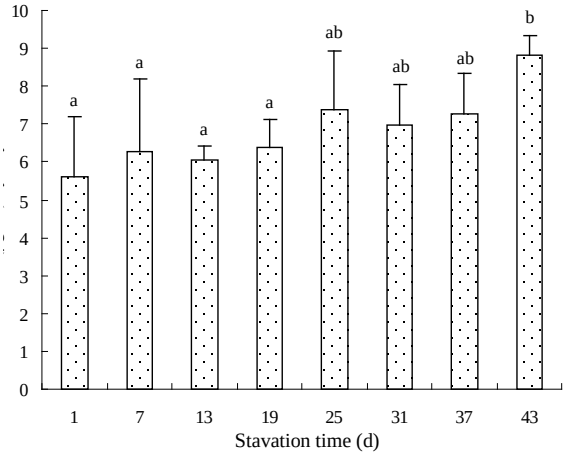

Fig. 2. Effect of starvation on activity of lysozyme in juvenile Chinese sturgeon.

CONCLUSION

Chinese sturgeon in starvation showed significant differences in many blood-chemistry levels, including: TP, ALB, GLU, CHOL, TGL, UREA, ALT, AST, and LDH. Based on our data, carbohydrates are likely metabolized preferentially in the blood of juvenile Chinese sturgeon, followed by proteins and lipids. Of note, GLU, ALB, CHOL, and TGL levels could potentially be used as indices of nutritional condition in multivariate blood-chemistry data sets. Obvious effects on activities of ALT and LSZ in the blood of juvenile Chinese sturgeon after 13 days of starvation. In addition, starvation can be suffered by juvenile Chinese sturgeon for up to 13-31 days. Taken together, the findings may reflect the evolutionary position occupied by sturgeon, as the utilization of stored metabolites during starvation appears to be intermediate to that of elasmobranches and teleost. This information would provide a better understanding of Chinese sturgeon, and could be used to further develop conservation strategies.

Acknowledgements: *The project was financially supported by the Special Research Fund for the National Non-profit Institutes of China (East China Sea Fisheries Research Institute 2011M08).*

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