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The Influence of Photoperiod in Farming Beluga Sturgeon (*Huso huso*): Evaluation by Growth and Health Parameters in Serum

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Abstract: Data on the concentrations of some blood constituents of reared Beluga sturgeon, *Huso huso*, including Serum cortisol, glucose, triglyceride, cholesterol, osmolality, Na⁺, K⁺, Ca²⁺, ALP that reared under different light regimes were measured. The light regimes were consist of natural photoperiod (NP), continuous dark (0L:24D), continuous light (24L:0D) and long day regime (16L:8D) and tested on 4 group of 30 one year old reared Beluga for 6 month which sampled four times during the experiment. No significant difference in serum cortisol and ALP levels was found between treatments ($p>0.05$). Elevations of serum cortisol, glucose, cholesterol and triglyceride concentrations were reported in continues dark regime. Increase of serum osmolality, Na⁺, K⁺ and decrease of Ca²⁺ were also associated with increase of cortisol levels. Results are compared with the few data available in chondrosteans fish and with those on teleosts.

Key words: Beluga, photoperiod, growth indices, physiological parameters

INTRODUCTION

Chondrosteans fishes, comprised of the sturgeons and paddlefishes, are anadromous and potamodromous species of the Northern Hemisphere. The evolutionary history of which goes back to 100 million years, apparently originated as a group in fresh water in the early Triassic Period and includes 28 species, of which 6 species inhabit the Caspian basin (Bahmani *et al.*, 2001; Barton *et al.*, 2000).

Hematological studies are valuable tools in determining the physiological conditions in fish. Therefore blood parameters are increasingly used as indicators of the physiological condition or sublethal stress response in fish to endogenous or exogenous changes (Cataldi *et al.*, 1998; Belanger *et al.*, 2001). The possibility of evaluation depends on availability of reference values as close as possible to normal values of various blood components considered as reliable descriptors of healthy fish under natural conditions (Cataldi *et al.*, 1998). It is clear that the environment in which fish live and the condition governing them influence the metabolic content in blood (Bullis, 1993).

Taking into account the long evolutionary history of bony fishes and the many adaptations to different environments, no species can be used as a representative model for all fish. This explains the rapidly expanding literature on the chemical properties of fish blood. However, at present, few reports deal with the blood of primitive fish such as the chondrosteans, as is evident from the extensive review study (McDonald and Milligan, 1992; Cataldi *et al.*, 1998; Bahmani *et al.*, 2001). The hematological study of sturgeons is in its preliminary stages and a few studies conducted in this regard can be cited (Bahmani *et al.*, 2001).

Liver is one of the main digestive glands in fishes and can produce different enzymes such as alkaline phosphatase (ALP) in hepatocyte. The levels of this enzyme is an appreciate indicator for diagnosing hepatic and osteal problems (Sknobrg *et al.*, 1997).

The Beluga sturgeon, *Huso huso*, is an anadromous species endemic to the waters of Caspian Sea and rivers following into it. Different aspects about ecology and biology of this primitive bony fish are well studied. The stocks of this valuable species have been greatly reduced by the impact of over fishing and habitat deterioration (Bahmani *et al.*, 2001). Interest in this species was recently aroused by successful attempts at artificial reproduction, specially because of its high growth rate (Bahmani *et al.*, 2001), which suggest that *Huso huso* may be suitable for fish farming and restocking. Therefore it is important to provide reference values for an assessment on status of this species in both natural and different rearing conditions for best management.

For a long time, the influence of environmental factors on fish has been studied in respect to their effects on growth and reproduction. Photoperiod has been considered as one of the most important growth promoting factor in several fish species and not only can affect feeding behavior but also their physiological condition (Boeuf and Le Bail, 1999; Jonassen *et al.*, 2000; Simensen *et al.*, 2000; Kissil *et al.*, 2001). Different light regimes also can be considered as physical stressor in aquaculture (Boeuf and Le Bail, 1999). Commercial sturgeon aquaculture is a recent development that utilizes wild fish and domestication of sturgeon is slow due to long generation intervals. Sturgeon culture systems are intensive and involve various management stressors such as handling, crowding, transportation, biopsy, hormonally induced spawning and rearing condition such as photoperiod, oxygen levels and finally levels of different physical and chemical factors. Knowledge of the stress response in these fish may help to improve management, production and animal welfare.

In this initial study, growth indices and concentrations of some components of the serum were measured in one year old beluga which reared under artificial conditions (at the International Sturgeon research institute) and different light regimes including Natural Photoperiod (NP), continuous dark (0L: 24D), continuous light (24L: 0D) and long day regime (16L: 8D) for 6 month for determining the best condition for its culture and getting the maximum economic profit during minimum time.

MATERIALS AND METHODS

Origin and Maintenance of Fish

Fifty six reared beluga sturgeon (*Huso huso*), derived from artificial reproduction and were reared at the International Sturgeon Research Institute, Rasht, Gilan Province, Iran. This study was carried out in February up to September of 2006. Fish were maintained in Square fiberglass tanks, with volume of 1,000 L, supplied with fresh water at 15°C, 7.3 pH, 7 ppm dissolved oxygen and 0.1 L sec⁻¹ discharge. Sturgeons were fed on commercial dry pellets (45% protein, 14% fat, 10% carbohydrate, Chine Co, Iran). Fishes were acclimated for 15 days before starting experiment.

Photoperiod Regimes Design

Four groups (with two repetitions) of 30 (both male and female) one year old reared Beluga (n = 56), which had Mean±SEM weight and total length of 475±2.91 g and 49.13±0.22 cm, respectively, were subjected to four different light regimes including natural photoperiod (NP), continuous dark (0L:24D), continuous light (24L:0D) and long day regime (16L:8D) from April to October. The fluctuations of temperature and dissolved oxygen were constant during the experiment. Growth was checked 12 times (weight (wt.) and Total Length (TL)) and condition factor (K-factor), Growth Rate (GR), Specific Growth Rate (SGR) and Food Consumption Ratio (FCR) were calculated according to following formula (Kissil *et al.*, 2001):

$$K = (\text{Weight (g)}/\text{Length}^3 \text{ (cm)}) \times 100$$

$$\text{SGR} = (\ln W_2 - W_1) \text{ (g)}/t_2 - t_1 \text{ (day)}$$

$$\text{GR} = W_2 - W_1 \text{ (g)}/t_2 - t_1 \text{ (day)}$$

Sampling Condition

Sturgeons were sampled after anesthesia in 140 mg L⁻¹ buffered solution of tricaine methane sulphonate (MS₂₂₂), which induced the cessation body and opercular movements within 4-8 min after exposure. Fishes were fasted 24 h before sampling. Two milliliters of blood specimens were collected from the caudal vein. Blood sampling was conducted 4 times during experiment from all sturgeons.

Sampling from liver of 20 sturgeons (5 samples from each treatment) was conducted at the end of experiment.

Laboratory Tests and Analytical Methods

Clotted blood was centrifuged at 3000 x g for 10 min and the analysis were performed immediately. Osmolality was determined by a cryoscopic method (Osmometer, Rebling, Germany). Na⁺ and K⁺ concentrations were measured using Flame photometer (Corning 480, Jenway, England) and Ca²⁺, glucose, ALP, triglyceride and cholesterol concentrations were determined using Spectrophotometer by enzymatic method (RA-1000, Technicon, USA). Cortisol concentration was measured using Coat-A-Co Cortisol (Kontron Analytical MDA 312) and radioimmunoassay software. The kits were supplied by Diagnostic Product Corporation.

Histological investigation was conducted by using the hematoxinilin-Eosin (H and E) staining method (Hung *et al.*, 1990).

Differential White Blood Cell Counts

Blood drawn from the caudal aorta with unheparinized syringe and for differential white blood cells, blood smears were prepared by staining with Giemsa (Merck, Germany) (Stoskopfe, 1993). The stained blood films were examined by a light microscope (Nikon E600, Japan) under immersion oil (100x). Three hundred cells were counted from each slide and in a parallel row.

Data Analysis

Range values, means and standard error of Mean±SEM were determined for all the parameters examined. Differences between groups were determined using t-test and Kruskal Wallis. Significance was accepted at p<0.05.

RESULTS

The Effect of Photoperiod on Growth Indices

The effect of different light regimes on mean values of growth indices were presented in this research on one year old reared Beluga Sturgeon.

The results reveal that extended of day length has positive effects on growth rate of beluga and their growth was progressively faster under continues light. The maximum total length, weight, condition factor, growth rate and specific rate (SGR) and minimum FCR were found in continues light regime (Table 1). A significant difference in either growth indices of beluga was detected between continues light and long day regimes with control and continues dark treatments.

Table 1: The Mean±SEM of growth indices in one year old reared beluga sturgeon (n = 30 in each treatment)

Treatments	TL (cm)	Wt. (g)	Cf (%)	FCR	GR (g)	SGR (%)
Control	54.51±16.53	719.02±23.91	0.41±0.02	2.5±0.10	4.71±1.72	0.62±0.11
0L:24D	54.43±19.48	707.39±20.82	0.42±0.07	2.2±0.10	4.12±1.63	0.59±0.10
24L:0D	59.13±21.62	916.61±33.34	0.46±0.07	1.5±0.10	6.18±1.77	0.87±0.14
16L:8D	58.12±22.56	885.47±27.02	0.45±0.08	1.9±0.10	5.63±1.93	0.73±0.13

Table 2: The Mean±SEM of serum stress and biochemical levels in one year old reared beluga sturgeon (n = 30 in each treatment)

Treatments	Cortisol (nmol dL ⁻¹)	Glucose (mg dL ⁻¹)	Triglyceride (mg dL ⁻¹)	Cholesterol (mg dL ⁻¹)
Control	16.26±2.79	41.17±2.75	347.68±24.79	67.21±3.80
0L:24D	23.84±3.06	54.39±2.89	397.60±20.11	91.41±6.51
24L:0D	18.00±1.81	50.18±2.12	366.30±19.43	71.11±4.95
16L:8D	18.72±2.21	53.01±2.62	387.60±23.18	84.55±5.71

Table 3: The Mean±SEM of serum Osmotic constituents in one year old reared beluga sturgeon (n = 30 in each treatment)

Treatments	Osmolality (mOsm kg ⁻¹)	Na ⁺ (mEq L ⁻¹)	K ⁺ (mEq L ⁻¹)	Ca ⁺² (mg dL ⁻¹)
Control	260.27±0.90	144.1±1.89	2.96±0.07	8.73±0.14
0L:24D	276.94±1.18	133.7±1.75	4.10±0.06	7.25±0.12
24L:0D	270.07±1.03	139.1±1.12	3.50±0.09	7.32±0.14
16L:8D	272.90±1.27	136.2±1.62	3.90±0.08	7.37±0.14

Table 4: The Mean±SEM of ALP levels in one year old reared beluga sturgeon (n = 30 in each treatment)

Treatments	ALP (IU L ⁻¹)
Control	328.83±13.68
0L:24D	281.76±11.83
24L:0D	279.78±13.40
16L:8D	311.77±24.10

The Effect of Photoperiod on Stress and Biochemical Indices

Significant elevation ($p < 0.05$) of serum glucose, triglyceride and cholesterol concentrations were observed in continues dark, long day regime and continues light treatments, respectively (Table 2). No significant difference in serum cortisol level was found between treatments ($p > 0.05$). The level of serum cortisol, glucose, cholesterol and triglyceride in control was lower than other treatments.

The Effect of Photoperiod on Osmolality and Ion Concentration

Table 3, the trend of osmolality and K⁺ concentrations were towards higher levels in continues dark, long day regime and continues light treatments, respectively. Significant difference in serum osmolality and ion concentration was detected between continues dark, long day and continues light regimes with control ($p < 0.05$).

The lowest and highest levels of Ca⁺² and Na⁺ were also observed in continues dark and control, respectively.

The Effect of Photoperiod on Hepatic Indices

The mean level of ALP was lower in continues dark, long day regime and continues light treatments in comparison to control (Table 4). No significant difference was detected between all treatments.

Pearson coefficient showed no correlation between Cortisol and ALP levels ($r = 0.001$, $p > 0.05$) ($r = 0.001$, $p > 0.05$). According to Fig. 1, no abnormality was found in liver.

The Effect of Photoperiod on Cellular Immunity System

The maximum percent of neutrophil and eosinophil and minimum percent of Lymphocyte was detected in continues dark treatment. Lymphosytosis was accompanied by neutropenia and eosiopenia and also basopenia was accompanied by monocytosis in all treatment (Table 5).

Table 5: The Mean $\pm$ SEM leukocyte fluctuation in one year old reared beluga sturgeon (n = 30 in each treatment)

Treatments	Lymphocyte	Neutrophil	Eosinophil (%)	Monocyte	Basophil
Control	57.89 $\pm$ 1.10	32.18 $\pm$ 1.31	9.92 $\pm$ 1.83	1.57 $\pm$ 0.10	0.00
0L:24D	56.42 $\pm$ 1.06	32.96 $\pm$ 1.29	9.95 $\pm$ 0.81	0.32 $\pm$ 0.05	0.18 $\pm$ 0.05
24L:0D	59.31 $\pm$ 1.10	29.75 $\pm$ 1.24	9.68 $\pm$ 1.05	0.35 $\pm$ 0.06	0.17 $\pm$ 0.06
16L:8D	59.00 $\pm$ 1.16	30.16 $\pm$ 1.41	9.86 $\pm$ 1.06	0.38 $\pm$ 0.07	0.14 $\pm$ 0.04

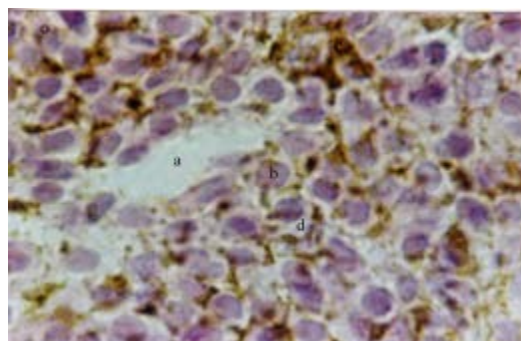


Fig. 1: Hepatic cells in one year old reared beluga sturgeon, Hematoxylin-Eosin(100X) (a) Gall tract, (b) Hepatocyte nucleus, (c) Lipid and (d) Hepatocyte

Study on lymphocyte, neutrophil and eosinophil showed that no significant difference was found between all groups but statistical analysis on monocyte and basophil fluctuations detected significant difference between control and other groups. Basophil was reported for the first in beluga and also sturgeons.

DISCUSSION

The effect of photoperiod on somatic growth of beluga is in its preliminary stage and a few studies conducted in this regard can be cited (Trenkler and Semenikova, 1995). The results of this study revealed that long day length has positive effect on somatic growth of beluga through better food conversion. This result demonstrated that food absorption in beluga directly correlated to the amount of light to which the fish were exposed. Fish growth and food conversion efficiency were closely correlated and were generally highest in the increasing photoperiod (Boef and Le Bail, 1999).

Positive effects of photoperiod on growth through better food conversion have been recorded in other species like Atlantic salmon (Berg *et al.*, 1992), Rainbow trout (Mortensen and Damsgard, 1993), halibut (Hallaraker and Stefansson, 1995), gilthead seabream (Silva-Garcia, 1996) and turbot (Imsland *et al.*, 1997).

Study on growth indices showed that no significant difference was detected between continues dark and control treatment. No same result was report on beluga in juvenile stage and this will prove that food intake in beluga does not correlated to their vision ability.

In sturgeon (Cataldi *et al.*, 1998; Barton *et al.*, 2000; Belanger *et al.*, 2001) as well as in teleost, the increase of serum cortisol level was considered as a primary indicator of stress response. The other parameters were also liable to change in response to stress or environmental variations (Cataldi *et al.*, 1998; Weerd and Komen, 1998; Barton *et al.*, 2000).

Beluga reacts to different day length and light regimes and increase of cortisol was observed in continues dark, long day regime and continues light treatments, respectively in comparison to natural photoperiod but no significant difference was found between all treatments. Therefore, different light

regimes can be conducted as one of the stressor in beluga and can active HPI axis in this species during their culture but physiological acclimatization has occurred in this species during 6 months of experiment.

Cortisol resting levels (unstressed fish) have been reported between 1 ng mL⁻¹ (3.70 nmol dL⁻¹) and 10 ng mL⁻¹ (37.02 nmol dL⁻¹) in salmonids (Pottinger and Moran, 1993), 9.4 ng mL⁻¹ (34.82 nmol dL⁻¹) in cannulated Adriatic sturgeon (Di Marco *et al.*, 1999) and also 83.6 nmol L⁻¹ (3.32 ng mL⁻¹) by cardiac puncture (Cataldi *et al.*, 1998), 8.60 ng mL⁻¹ in cannulated white sturgeon (Belanger *et al.*, 2001), 16.26 nmol dL⁻¹ (4.40 ng mL⁻¹) in beluga by bleeding from caudal vein in control treatment. The resting cortisol levels in beluga was found significantly lower than cannulated Adriatic and white sturgeon and higher than Adriatic sturgeon which bleeding by cardiac puncture. Plasma cortisol level in sturgeon reflects different sampling techniques, fish age, size, season and rearing temperature (Cataldi *et al.*, 1998; Di Marco *et al.*, 1999). Sex and reproductive stage may affect resting plasma cortisol levels in sturgeon (Belanger *et al.*, 2001).

Significant elevation of serum glucose, cholesterol and triglyceride levels were accompanied by increasing secretion of cortisol in continues dark, long day regime and continues light treatments. Increases in plasma glucose, triglyceride and cholesterol following stress are considered to occur in response to the stress-induced release of catecholamines, chiefly epinephrine and nor epinephrine, into circulation (Mazeaud *et al.*, 1977; Wedemeyer *et al.*, 1990; Gamperl *et al.*, 1994). Presumably catecholamines, among other functions, serve to mobilize energy reserves through glycolysis as an adaptive response to meet increased metabolic demands necessary for enabling the fish to cope with the stress (Gamperl *et al.*, 1994; Reid *et al.*, 1998).

In this study, the effect of photoperiod on salt and water balance was also demonstrated in beluga. The levels of osmolality and potassium increased in different light regimes in comparison to control treatments. The highest level of cortisol was also observed in these treatments in beluga. Increase of osmolality due to elevation of cortisol has been reported in teleosts (Shrimpton, 1996; Shrimpton and McCormic, 1998; Nolan *et al.*, 1999; Deane *et al.*, 2000) but the effect of cortisol on potassium levels was different in different species (Eddy, 1981; Shrimpton, 1996; Cataldi *et al.*, 1998).

The trend of Ca⁺² and Na⁺ concentrations were towards lower levels in continues dark, long day regime and continues light treatments, respectively in comparison to control. Decrease of Ca⁺² and Na⁺ due to increase of cortisol were demonstrated in teleost and sturgeon (Eddy, 1981; Wendelaar Bonga, 1993; Hazon and Balment, 1998; Nolan *et al.*, 1998; Cataldi *et al.*, 1998). The results reveal that cortisol has hypocalcemic effects in fishes (Cataldi *et al.*, 1998; Wendelaar Bonga, 1993; Hazon and Balment, 1998). Furthermore in sturgeons Na⁺ contribute to over 75% of total osmolality as in teleosts (Cataldi *et al.*, 1998). Moreover in sturgeon as in euryhaline anadromous teleost species osmolality and ion concentrations can be linked to physiological condition, age, fish size, species, sex and sexual maturation (Cataldi *et al.*, 1998).

The maximum percent of neutrophil and eosinophil and minimum percent of lymphocyte was detected in continues dark treatment. There is an interaction between HPI axis and cellular immunity system.

Stress is well known for its immune-suppressive actions both in mammals in lower vertebrates. Corticosteroids produced by the activated hypothalamus-pituitary-renal axis are considered to be important mediators of these effects on immunity (Hazon and Balment, 1998; Weyts *et al.*, 1998; Wendelaar Bonga, 1993). Cortisol has negative effect on fluctuation of leukocytes in fishes (Ellis, 1981; Houston, 1990; Barton and Zitzow, 1995). Lymphocytopenia and neutrophilia usually occur in fishes in response to different stressors because cortisol decrease apoptosis in neutrophil (Weyts *et al.*, 1998) and increase cytolytic response in lymphocyte (Pickering, 1984; Benfey and Biron, 2000). The response of eosinophil, monocyte and basophil are not same to different stressor. Statistical analysis on lymphocyte, neutrophil and eosinophil showed no significant difference between all treatments and physiological acclimation was proved in beluga in this research.

Study on ALP levels showed no significant difference between all groups. ALP levels in beluga reported between 279-320 IU L⁻¹ in this research. Normal ALP levels reported 83 to 330 IU L⁻¹, with most of value 100-150 IU L⁻¹ in different species. Plasma ALP activity is influenced by many factors including water chemistry, food intake, temperature and life stage (Sknoberg *et al.*, 1997). Based on present results, it appears that different light regimes can not affect the serum ALP levels and this aspect will need more investigation.

According to the results, beluga seems susceptible to light regime alternations and the mean values of cortisol, glucose, cholesterol, osmolality and ion concentration were changed in this species. The different individual susceptibility to stressors, as indicated by the broad range of values detected, may be an important consideration in the evaluation of certain situations.

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