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Correlations Between Biochemical Factors of Blood with Biological Characteristics of Gonad and Some Reproductive Indices in Persian Sturgeon, *Acipenser persicus*

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Abstract: A number of biochemical factors of blood (calcium, magnesium, total protein, cholesterol and glucose) were determined with weight and fork length of female fishes, biological characteristics of gonad (e.g. the number of eggs/gram, the number of larvae/gram, fluid egg rate, relative fecundity, gonadosomatic index, hatching success and fertilization rate) in 16 female of the migratory population of Persian sturgeon (*Acipenser persicus*) in Spring of the 2012. There was an invert correlation between weight and blood Mg^{+2} ($P<0.05$). Also, relationship between blood glucose and fluid egg rate, relative fecundity as well as gonadosomatic index was reverse ($p<0.05$). These relationship was the same between glucose and the number of eggs/gram of gonad ($p<0.05$). Cholesterol was positively correlated with weight, fork length, the number of larvae/gram and hatching success but these correlations were not significant.

Key words: *Acipenser persicus* % Blood % Fertilization Rate % Gonadosomatic Index % Relative Fecundity

INTRODUCTION

The Caspian Sea is the largest continental water body on earth [1], surrounded by Russia, Kazakhstan, Turkmenistan, Iran and Azerbaijan [2]. *Acipenser persicus* is one of important species that live in the southern margin of the Caspian basin [3]. Unfortunately, nowadays this fish has become an endangered species [4] due to the damages of their natural spawning environments and elevation of international trade in caviar [5]. Early life stages of development are some of the most important phases of fish development, which include the replacement of embryonic adaptations and functions. Mortality of acipenserids during embryonic and larval development is of considerable importance [6]. There has been an increased demand for information on all aspects of sturgeon biology and physiology [2, 7]. Substantial efforts were focused on the early life developmental stages of this group of fishes in order to understand how to increase their survivorship and improve hatchery efficiency [6]. Egg quality traits could be helpful for fish farmers in order to assess fingerling production, hatching management, improving rearing techniques and evaluation of the quality of fish produced [8]. For increasing the survival rate at different stages of these fishes, it is necessary to study the potential factors affecting the growth and survival rate of incubating eggs, larvae and other larger stages [5]. In many fish species, larger females often produce larger eggs and egg size for each female decrease with each progressive batch through spawning [9-11]. A significant positive relationship between body size and egg production (fecundity and egg size) has been observed among Pacific salmon populations [12-17]. The seasonal decrease in egg size coincides with a seasonal decrease in the body size of spawners [15, 18]. Egg size and development may affect their quality, ability to produce viable larvae and to some extent directly determine growth and survival of young fish [6]. The gonadosomatic index (GSI) usually provides limited information of the internal changes occurring in the ovary, particularly with regard to gonadal development and composition [19]. In stock assessment and population density to predict the available catch several factors are considered. Population density has been found to be a strong predictor of fertilization success and has been used to explain differences among species in gamete traits such as sperm swimming speed, egg size and gamete longevity [20].

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The analysis of the blood parameters is one of the most valuable methods because it has been shown that their physiological values are species- specific and age-dependent. For example, variations in some serum parameters of sturgeons, including calcium, total protein and magnesium have been reported to be characteristic for spawning time [21].

Biochemical indicators provide more accurate information about gonadal performance, such as changes in size, frequency and composition of oocytes, as well as nutrient flow and usage during gametogenesis [19].

Accordingly, blood serum biochemistry data are of immense importance in monitoring the health status of aquatic organisms, especially in fisheries management programs [22].

Studies on the valuable species reproduction assist the aquaculture industry in inland rearing and restocking specially those endangered fishes such as sturgeon fish by improving protocols for higher efficiency of egg production and larval survival and In spite of the importance of blood on fertilization process of fish, this study with the objective of investigating the correlation of biochemical compounds of blood (e.g. calcium, magnesium, cholesterol, total protein and glucose) with weight and fork length of female fishes and number of biological characteristics of gonad (e.g. the number of egg/ gram, the number of larvae/gram, fluid egg rate, relative fecundity, gonadosomatic index, hatching success and fertilization rate) in Persian sturgeon (*Acipenser persicus*) was carried out.

MATERIALS AND METHODS

Broodstocks Preparation and Fertilization: In this study, female Persian sturgeon (*Acipenser persicus*) were captured at the end of winter from south-east of Caspian Sea, during their upstream migration and then transported to Shahid Margani sturgeon fish farm (Golestan, Iran). For fertilization experiment 16 mature female with the average weight of 21.5±15.65 (kg) and average fork length of 1.01±81.8 (m) was used. A group of males' breeder was captured and all broodstocks of both sexes were maintained in several separate circular tanks (8 m diameter, 1 m depth, 50 m^3 volume). They were injected intramuscularly with acetone-dried sturgeon pituitary at water temperature in the range of 17-20°C (45-50 mg for female and 35 mg for male). These broods showed the polarization index (PI) less than 7%. For measuring GV (germinal vesicle) position by polarization index (PI), a sample of 15-20 eggs for each female were boiled for 2 minutes and were cut along their animal-vegetal poles axis and observed under a dissection microscope with a micrometer eyepiece. The oocyte polarization index (PI) for GV position was calculated by the formula PI = a/A × 100, in which a: distance between GV and cell membrane and A: diameter of oocyte along animal-vegetal axis.

For establishing equal condition for fertilization and lowering the effect of male characteristics on the results we used the semen of those fishes with high motility (85-92%) and on the practical protocol of Shahid Margani fish propagation farm, sperm of different fishes were stripped separately, attaining ovum fertilized with mixed sperm. The dry fertilization method was used and the insemination dosage was 1 percent of egg volume for each fertilization experiment. Before each insemination, the excess of coelomic fluid was removed by pouring the eggs onto a screen suspended over a beaker.

Incubation and Fertility Examination: After eliminating eggs adhesiveness, eggs were placed in Yushchenko incubators in running freshwater system at17-20 °C and in the presence of dissolved oxygen more than 6 ppm.

In order to calculate the fertilization rate, three hours after fertilization and 100 eggs were randomly removed and preserved in formalin 10% solution, monospermic percentage was considered only for the eggs containing four cells.

In order to determine the hatching rate of each broodstock, the number of fertilized eggs which transferred to each incubator as well as the number of larvae from each incubator, belonging to each female, calculated using the f ollowing formula: number of egg (or larvae) = number of egg (or larvae) in gram × the weight of all attaining eggs in gram that this trials was done as 3 replicates in sterile Petri dishes.

Hatching success is calculated by dividing the number of larvae by the ovum number, according to the following formula: Hatching success= number of larvae / number of ovum×100.

Gonadosomatic index was calculated using the formula GSI = Wg/W × 100, where Wg: gonad weight, W: fish weight.

For determine the fluid egg rate, the egg mass left in the body cavity of the fish was also weighed. The relative fecundity was calculated by dividing the total egg number by the total body weight.

Biochemical Experiments of Blood: Blood samples (2 ml) were collected from the caudal vein using a syringe and kept in nonheparinized vials on ice until separated by centrifugation at the end of the day. After separation (5000g for 20 min.), all sera were stored at -80°C while awaiting transfer to the chemistry laboratory of Gorgan University of Agricultural Sciences and Natural Resources. Serum samples were analyzed for magnesium, calcium, total protein, cholesterol and glucose by colorimetric-spectrophotometric measurements.

Statistical Analysis: The correlation between biochemical factors blood and weight and fork length of female fishes, biological characteristics of the gonad (e.g. the number of egg in one gram, the number of larvae in one gram, fluid egg rate) relative fecundity and gonadosomatic index, hatching success and fertilization rate were analyzed using the bivariate correlation coefficients of Pearson (SPSS, ver. 16).

RESULTS

The mean values and standard deviation of the serum biochemical parameters and biological characteristics of the *Acipenser persicus* are summarized in Table 1.

Overall results are presented in Table 2. The relationship between weight and blood Mg^{+2} was reverse ($p<0.05$) and relationship between blood glucose and fluid egg rate, relative fecundity as well as gonadosomatic index was reverse ($p<0.05$). These relationship was the same between glucose and the number of eggs per one gram of gonad ($p<0.05$).

As shown in Table 2, cholesterol was positively correlated with four variables, but these correlates were not significant: weight, fork length, the number of larvae/gram and hatching success. Also relationship was the same between total protein with weight and fork length. In this work, weight with Ca^{+2} and blood glucose and also fork length with Ca^{+2}, glucose and Mg^{+2} of blood had a negative relation that was not significant. With increasing in blood cholesterol level, the number of larvae/gram and hatching success were increased but this increase was not significant. Regression equation and R^2 coefficient between some parameters are presented in Table 3.

Table 1: Mean ± SD of the evaluated parameters in the *Acipenser persicus*

Variables	Mean ± SD
Weight (kg)	21.50 ± 15.65
Fork length (m)	1.01 ± 81.8
Number of egg (g)	33.37 ± 27.2
Number of larvae (g)	36.68 ± 29.8
Fluid egg rate	2.46 ± 2.26
Hatching success	68.92 ± 55.45
Fertilization rate	39.18 ± 33.13
Relative fecundity	4.35 ± 4.05
Gonadosomatic index	8.04 ± 7.22
Glucose (mg/dl)	32.06 ± 8.76
Cholesterol (mg/dl)	1.07 ± 59.13
Magnesium (mg/dl)	1.50 ± 0.64
Total protein (g/dl)	1.76 ± 0.81
Calcium (mg/dl)	5.82 ± 2.27

Table 2: Reciprocal correlation between factors

	Ca^{+2}	TP	CH	G	Mg^{+2}
Weight (kg)	-0.141	0.209	0.238	-0.347	-0.502*
Fork length (m)	-0.104	0.056	0.181	-0.286	-0.340
The number of eggs (g)	-0.391	-0.155	-0.010	-0.499*	-0.309
The number of larvae (g)	-0.367	-0.128	0.008	-0.496	-0.325
Fluid egg rate	-0.475	-0.244	-0.132	-0.522*	-0.303
Relative fecundity	-0.431	-0.322	-0.148	-0.508*	-0.169
Gonadosomatic index	-0.439	-0.294	-0.134	-0.512*	-0.202
Hatching success	-0.353	-0.077	0.033	-0.486	-0.353
Fertilization rate	-0.396	-0.251	-0.037	-0.405	-0.299

*P<0.05. Ca^{+2}: Calcium, Mg^{+2}: Magnesium, TP: Total Protein, G: Glucose, CH: Cholesterol.

Table 3: Regression equation and R^2 between some parameters

Correlation	Regression equation	R^2
Weight and Mg^{+2}	Y= -12.202X+ 39.917	R^2= 0.25
The number of eggs (g) and Glu	Y= -1.547X+ 83.004	R^2= 0.24
Fluid egg rate and Glu	Y= -0.135X+ 6.793	R^2= 0.27
Relative fecundity and Glu	Y= -0.235X+ 11.893	R^2= 0.25
Gonadosomatic index and Glu	Y= -0.421X+ 21.566	R^2= 0.26

DISCUSSION

Biochemical analysis can provide valuable information for monitoring the health and condition of fishes. Biochemical indices changed depend on the fish species, age, the cycle of sexual maturity and health condition. Moreover, analysis of serum constituents has showed useful information in detection and diagnosis of metabolic disturbances and disease in fishes. Fish reproduction is one of the factors seriously affecting the internal milieu of the organism. Therefore, great attention is paid to the study of biochemical indices during the reproduction period [23].

Since glucose in serum is a major metabolite of carbohydrate metabolism [24], an increase in the plasma glucose of teleosts was believed to be caused by a wide range of environmental stressors (such as hypoxic environment, starvation and captivity) [22]. In the present

study, glucose had a negative relationship ($p<0.05$) with the number of egg/ gram, fluid egg rate, relative fecundity and gonadosomatic index that these negative results can be due to physical processes involved in transporting the fish, keeping them in tanks and administering anesthesia for blood sample collection caused physical stress and affected hormone and glucose levels in the blood serum that This finding is agreement with results obtained by Imanpoor and Bagheri [8].

Blood Ca^{+2} can be considered as a negative factor for successful fertilization [25-26]. Our results showed that Ca^{+2} and Mg^{+2} with all variables had a negative relation that was not significant, that could be related to role Ca^{+2} in inhibiting motility sperm [27]. The Ca^{+2} and Mg^{+2} values indicated the operation of a variety of homeostatic mechanisms in the body [28]. Srivastava and Srivastava [29] have previously demonstrated that the Ca^{+2} and Mg^{+2} levels were subject to seasonal variations so that in the pre-spawning their concentrations increased while during spawning and post spawning they gradually decreased.

The levels of total protein, cholesterol are considered to be major indices of the health status of teleosts [22] and as indicator of nutritional status [23]. In this work, total protein had a positive relationship only with weight and fork length and also showed that total protein with other parameters had a negative relationship.

Total protein level of blood serum can be a negative index for egg quality. However, finding of some previous researchers did not show this factor as a suitable index for determining egg maturation and quality. Plasma protein is mainly altered by changes in plasma volume, which in fish may be observed with prolonged starvation or stress [8].

In the case of cholesterol, Bartley [30] reported that cholesterol concentration is strictly regulated, showing only slight annual variation related to dietary changes. Diwan and Krishnan [31] stated a fluctuation of serum cholesterol in males and females of *Etroplus suratensis* as related to maturity. Cholesterol concentration in blood plasma of females was the lowest when the gonadosomatic index (GSI) was the highest and vice versa [32] This finding is agreement with our results. The concentration of cholesterol, in *Acipenser persicus* from the present study, were very similar to the values obtained from red (*Cervus elaphus*), fallow deer (*Dama dama*) [33]. In the present study, the concentration of total protein was 1.76 ± 0.81 g/dl, concentration of calcium was 5.82±2.27 mg/dl and concentration of magnesium was 1.50 ± 0.64 mg/dl that this is in agreement with the investigation by Asadi *et al*. [28].

Body size and/or weight have been traditionally considered key determinants of an organism's ecological and physiological properties [34]. In this study weight and fork length had a positive relationship only with total protein and cholesterol.

However, Sakomoto *et al.* [35] have proposed that variations in blood parameters among fish could be affected by other variables such as the sampling technique, the capturing method, the condition of captivity and the analysis techniques.

In other hand, factors such as photoperiod, temperature, salinity and pH of the water influence egg quality [36].

Fish are in close contact with their environment and, as a result, their physiology is influenced accordingly. It is evident that understanding the physiological indices of blood serum of *Acipenser persicus* is essential for aquaculture in Iran, because it reveals normal indices for propagation, rearing and stocking of this species and also every mentioned factor can be effective in broodstock selection programs and larvae growth and development.

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