

Serum Biochemical Parameter of Male, Immature and Female Persian Sturgeon (*Acipenser persicus*)

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Abstract: The serum biochemical parameters of *Acipenser persicus*, one of the most valuable, endangered and ancient fish of the world were evaluated in this study. The study was carried out with 20 Persian sturgeons from Iranian fishing area of the Southern part of the Caspian Sea caught in summer and winter 2008. Serum sample of *A. Persicus* were analysed and their serum parameter value determined as mean \pm SD in male, female and immature fishes. According to the present results, some parameters were significantly ($P < 0.05$) higher in males than immature and females sturgeons ($P < 0.05$), including; Albumin (0.89 ± 0.1 , 0.65 ± 0.3 , 0.64 ± 0.2 mg/dl), Phosphorous (2.9 ± 0.4 , 2.5 ± 0.5 , 2.4 ± 0.3 mg/dl), Magnesium (1.6 ± 0.1 , 1.4 ± 0.2 , 1.2 ± 0.1 mg/dl), Total protein (5.6 ± 1.4 , 4.5 ± 0.2 , 4.42 ± 1.7 g/dl), Glucose (6.3 ± 0.7 , 2.7 ± 0.2 , 3.5 ± 0.4 mg/dl). Blood biochemical of male, immature and female values of Alkaline phosphatase (30.2 ± 6.3 , 35.1 ± 7.54 , 25.47 ± 8.1 IU/dl) Aspartate aminotransferase (27.6 ± 4.8 , 47.8 ± 24.3 , 33.9 ± 9.9 IU/dl), Alanine aminotransferase (8.15 ± 0.96 , 17.7 ± 1.06 , 11.5 ± 5.2 IU/dl), Creatine Phosphokinase (263.7 ± 19.6 , 283.8 ± 39.7 , 256.4 ± 61.9 IU/dl) and amylase (163.5 ± 22.1 , 189.8 ± 22.9 , 154.2 ± 15.4 IU/dl) were higher in immature fish than males and females respectively. There were no differences in case of the C3, C4, Cratinin, IgM, Iron, Calcium, BUN, lysozyme, Cholesterol and blood Urea nitrogen between sex and immature fishes. Serum biochemical values reported here will be used as reference for the early detection, identification, and monitoring of disease and sublethal conditions in cultured Persian sturgeon.

Key words: Persian sturgeon, biochemical parameters, serum, *Acipenser persicus*

INTRODUCTION

The sturgeons (Acipenseridae) are an ancient group of chondrosteian fishes which are considered to be "living fossils" (Bemis *et al.* 1997), appeared approximately 150-200 million years ago and have retained various life-history traits, such as longevity and migratory patterns, despite major environmental changes (Bemis *et al.* 1997). But in recent years threatening agents like water pollution, over fishing, destruction of natural spawning beds and etc cause Persian sturgeon, *Acipenser persicus*, has listed as threatened, vulnerable and endangered throughout their ranges (Moghim *et al.* 2002).

There are few tools available to diagnose and monitor condition of this fish. The analysis of blood can reveal for us nutritional situation, physiological condition, situations of living habitat of fish, for example in response to stress, pollutants, and nutrition as well as ecological and physiological conditions, major changes occur in the fish blood compositions, such as fluctuations in the hormones levels of proteins, sugar, cholesterol and other basic components (Bahmani *et al.*, 2001). Caspian basin is dwelling place of 6 species of sturgeons. Persian sturgeon is one of these six species that lives in the southern margin of the Caspian basin (Bahmani *et al.* 2001). While most population of in Caspian sea have diminished, the stocks of *Acipenser persicus* in south part of Caspian sea are self-sustaining and provide a unique population. Interest in the *A. persicus* as a potentially endangered species, its valuable caviar, special spawning, late maturity and in order to establish normal and base line values of this species, we measured a number of biochemical parameters in the blood of Persian sturgeon. These parameters can provide information on the nutritional status, digestive function and routine metabolic levels of fishes. These data can be used to monitor the physiological status of individuals in future assessment of wild fish populations and fish in aquaculture.

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MATERIAL AND METHOD

Persian sturgeons were collected in summer and winter 2008 by fishery independent trawler operating in the Iranian fishing area of the Southern part of the Caspian Sea, in water 10-100m deep. Water temperature, Oxygen, Salinity and pH ranged from 27.5-32.2°C, 2.8-7.2 mg/l, 9.2-14ppt, 8.2-8.5 respectively. Trawlers used bottom trawl with mesh of 37mm tie to tie. Trawl duration was limited to 30 minute. Blood was collected from sturgeons within 3-5 minutes of being brought on board. Sturgeons were counted, weighed (to the nearest 0.1 kg), and measured (straight length from tip of nose to tip of tail, to the nearest 1 cm). Sex and maturity stage of the samples were determined by necropsy. The females, had distinct eggs and males, had lobulated testes, but sexual identification for immature group was impossible. Persian sturgeons were manually restrained, and blood was collected from the caudal vein using 5 ml syringes. Serum was stored and maintained at -20°C on shore until processed in the laboratory.

Analysis methods:

but maximum of IgM level
Before analysis, the frozen samples were left to stand at room temperature to thaw, and then inverted several times to mix. The serum samples for each specimen were analyzed together in one batch, to avoid run-to-run variability, for the following analyses: Blood urea nitrogen (BUN), creatinine(CREA), albumin(Alb), Glucose(Glu), total protein (TP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Creatine phosphokinase (CPK), Cholesterol (CHOL), Complement C3, Complement C4, Immunoglobulin (IgM), Lysozyme (LYS), Amylase, Calcium (Ca), Iron (Fe), phosphorus (P) and Magnesium (Mg). All analyses were performed using a blood chemistry Auto analyser (Model Eurolyser).

Statistical Analysis:

Blood biochemical values of the *A.persicus* were statistically evaluated using an analysis of variance procedure using one-way ANOVA, followed by using the Duncan. Differences in $P < 0.05$ were considered to be significant, and all results in the text were stated as mean \pm standard error (SE).

Results:

Male, immature and female Persian surgeon's weight, total length and fork length were shown in table 1.

Table 1: Male, immature and female Persian surgeon's weight, total length and fork length

Parameters	Number of sampling	Weight	Total length	Fork length
Male	6	3958.3 \pm 1437.5	96 \pm 10.1	83.6 \pm 8.8
Immature	6	765.8 \pm 612.2	60.6 \pm 11	52 \pm 10.8
Female	8	2858.8 \pm 1435.9	94.5 \pm 35.5	71.6 \pm 12.6

Blood biochemical parameters of all groups are given and all parameter levels of male, immature and female Persian sturgeons are compared with each other (table 2). We have found that there was no difference among the groups in terms of the C3, C4, Cratinin, IgM, Iron, Calcium, BUN, lysozyme, Cholesterol and blood Urea nitrogen between sex and immature fishes ($P > 0.05$). However our analysis has revealed significant differences in the levels of some other parameters between groups. For example, immature Persian sturgeon have significantly higher Alkaline phosphatase, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Creatine Phosphokinase and amylase in comparison with male and females ($P < 0.05$). Also they have significant difference ($P < 0.05$) in Albumin, Phosphorous, Magnesium, Total protein and Glucose in male Persian sturgeon are higher than other groups.

Discussion:

Performing blood chemistry analyze determinations in a clinical laboratory can provide vital information to aid in the diagnosis and management of infected individuals or in health assessment (Pincus, 1996) but differences in sampling technique and test methodology may generate variable results. This may cause limitation in aquatic veterinary medicine because of the lack of standard techniques and reliable reference values for most fish species (Hrubec & Smith, 2000). Additionally, numerous external factors can affect the blood values of fishes, including environmental conditions (e.g., temperature and seasonality), stress from capture and sample collection, diet, and culture conditions (e.g., oxygen levels and salinity). In individual fish, genetic variation, age, stage of development, reproductive state, sex, and activity level have been documented to affect hematologic values (Knowles *et al*, 2006). However, only a few serum biochemical parameters have

Table 2: Blood biochemical parameters of male, immature and female Persian sturgeons Mean \pm S.D. of three replicates. Numbers within the same row with different superscripts are significantly different ($p < 0.05$).

Parameters	Male	immature	Female
Glucose(mg/dl)	6.3 \pm 0.7a	2.7 \pm 0.2b	3.5 \pm 0.4c
Cratinin(mg/dl)	0.31 \pm 0.25	0.32 \pm 0.34	0.30 \pm 0.22
ALT(IU/L)	8.2 \pm 0.9b	17.7 \pm 1.1b	11.5 \pm 1.2a
AST(IU/L)	27.6 \pm 4.8b	47.8 \pm 24.3a	33.9 \pm 9.9b
C3(mg/dl)	28.6 \pm 14.2	24.1 \pm 8.3	30.1 \pm 12.9
C4(mg/dl)	20.4 \pm 6.4	10.01 \pm 6.4	18.4 \pm 18.2
IgM(mg/dl)	52.6 \pm 22.8	131.1 \pm 51.9	110.5 \pm 88.9
TP(g/dl)	5.6 \pm 1.4a	4.5 \pm 0.2b	4.4 \pm 1.7b
Albumin(mg/dl)	0.89 \pm 0.1a	0.65 \pm 0.3b	0.64 \pm 0.2b
ALP(IU/ml)	30.2 \pm 6.3b	35.1 \pm 7.5a	25.8 \pm 8.2b
LYS(mg/ml)	0.25 \pm 0.00	0.25 \pm 0.01	0.25 \pm 0.1
CHOL(mg/dl)	87.3 \pm 9.3	91 \pm 2.7	81.49 \pm 7.5
CPK(IU/L)	263.7 \pm 19.6	283.8 \pm 39.7	256.4 \pm 61.9
BUN(mg/dl)	1.4 \pm 0.2	1.3 \pm 0.1	1.3 \pm 0.2
Amylase(IU/L)	163.5 \pm 22.1b	189.8 \pm 22.9a	154.2 \pm 15.4b
Ca(mg/dl)	2.5 \pm 0.1	2.5 \pm 0.2	2.2 \pm 0.2
Fe(μ g/dl)	23.4 \pm 0.2	21.2 \pm 0.2	24.5 \pm 0.4
P(mg/dl)	2.9 \pm 0.4a	2.5 \pm 0.5a	2.4 \pm 0.3b
Mg(mg/dl)	1.6 \pm 0.1a	1.4 \pm 0.2b	1.2 \pm 0.1c

been determined in Persian sturgeon in studies. Although these studies are useful in determining effects of environmental factors or stress on Persian sturgeon. Moreover the collection method employed in this study, i.e., trawling followed by physical restraint of *A.persicus* out of water, would be expected to cause some alterations in blood chemistry values from those of *A.persicus* in a normal swimming condition. However, for collecting blood from wild *A.persicus*, some capture stress, either by net or by hook and line, is unavoidable. Blood collection of captive fish is not much different, usually involving net capture and out-of-water sampling. In captivity, the options exist for chemical sedation prior to removal from water, or placement of indwelling catheters to permit blood collection while the fish remains in water (Craig *et al.*, 2002). However, chemical sedation with tricaine methanesulfonate causes increases in blood glucose and potassium concentrations, and urinary electrolyte loss in teleosts (Brown, 1993).

In our study total serum protein levels of male, immature and female Persian sturgeon were (5.6 \pm 1.4, 4.5 \pm 0.2, 4.4 \pm 1.7 g/dl) respectively. Total plasma protein concentration in fishes range from 2 to 8 g/dl when determined by refractometry (McDonald & Milligan, 1992) which is similar to our result. Plasma protein is mainly altered by changes in plasma volume, which in fish may be observed with prolonged starvation or stress (Knowles *et al.*, 2006). The concentration of total protein in blood plasma is used as a basic index for the health status of brood fish (Swain *et al.*, 2007). In this study total protein for Persian sturgeon were consistent with those of shortnose sturgeon (*Acipenser brevirostrum*) (Knowles *et al.*, 2006) and beluga (*Huso huso*) (Asadi *et al.*, 2006b). As we observed in Persian sturgeon protein levels in rainbow trout and hybrid striped bass increased with age both in juvenile and adult fish (Sano, 1960, Hrubec *et al.*, 2001). These increases were mainly due to an increase in the globulin fraction and to some extent the albumin fraction (Sano, 1960, Hrubec *et al.*, 2001).

Alkaline phosphatase is a cell-membrane-associated glycoprotein found in all tissues. The specific metabolic role of ALP is unknown, but it is believed to function in the transport of ions and absorption of water across cell membranes (Gasser & Kirschner, 1987; Moss, 1992). Liver is one of the main digestive glands in fish and can produce different enzymes such as alkaline phosphates (ALP) in hepatocyte. The level of this enzyme is an appropriate indicator for diagnosing hepatic and osteal problems (Sknobrg *et al.*, 1997). Plasma ALP activity is influenced by many factors, including water chemistry, food intake, temperature and life stage (Sknobrg *et al.*, 1997). As in mammals and hybrid striped bass ALP, activity was high in younger (immature) Persian sturgeon compared to male and female fish (Hrubec *et al.*, 2001). It is not known whether fish have the same ALP isozymes that are found in mammals; however the decrease in serum ALP activity with age may indicate a decrease in a bone-derived isozyme (Hrubec *et al.*, 2001).

Generally, glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in plasma. Glucose levels are maintained principally through the conversion of liver glycogen, with some being derived from non-carbohydrate source (hepatic gluconeogenesis). Increases in plasma glucose levels may be due to increased glucose production or release (Aengwanich, & Tanomtong, 2004). Glucose concentrations vary considerably between species and have been shown to increase with a fish stress response (Percin & Konyalioglu, 2008). Glucose concentration also varies because of size, age, and nutritional and reproductive status (McDonald & Milligan, 1992). In our study glucose levels of male,

immature and female Persian sturgeon were 6.3 ± 0.7 , 2.7 ± 0.2 , 3.5 ± 0.4 (mg/dl) respectively. As we observed in Persian sturgeon glucose concentration of male Bluga (*Huso huso*) and bluefin tuna (*Thunnus thynnus*) was higher than female (Percin & Konyalioglu, 2008, Asadi *et al.*, 2006b).

Total protein and glucose in male were higher than female and immature Persian sturgeon. These higher values for Glu and TP may reflect higher growth rates or higher conversion efficiency in males than females and immature fish. Baker *et al.* (2005), Giberson & Litvak (2003), and Hardy & Litvak (2004) compared two species of sturgeon in their studies and they attributed higher values for Glu and TP as reflection of higher growth rates or higher conversion efficiency in their comparisons.

Cholesterol, levels of the immature Persian sturgeons were found to be higher than those of the male and female but not significantly. Cholesterol concentration varies both among and within fish species because of variations in diet, activity, and sexual development (McDonald & Milligan, 1992). The cholesterol concentration in Persian sturgeon in this study was consistent with data reported for the shovelnose sturgeon, (Hunn & Christenson, 1977), and shortnose sturgeon (Knowles *et al.*, 2006). Cholesterol is essential for the growth and viability of cells in higher organisms. Also, cholesterol is a precursor of steroid hormones such as progesterone, testosterone, oestradiol and cortisol (Moerland 1995; Stacey & Sorensen 1995; Van Der Kraak, Chang & Janz 1998; Aengwanich & Tanomtong 2004), but was much lower than data for rainbow trout (Manera & Britti, 2006) and salmonids (Congleton & Wagner, 2006) and may reflect a species or dietary difference.

In the present study, Amylase levels of immature Persian sturgeons like ALP were found to be higher than those of male and female. The Amylase concentration in Persian sturgeon in this study was consistent with data reported for beluga (*Huso huso*) and like this study males had higher amylase levels (Asadi *et al.*, 2006b). Amylases in company with uric acid, creatinine, and serum urea concentration are parameters use as indicators of renal failure and renal function impairment (Tietz *et al.*, 1990; Pickering & Pottinger 1995) but we didn't observe any significant differences between data analyze for creatinine and serum urea.

In comparison with our study blood urea concentration between males and females in *A.persicus* and *Huso huso* weren't significant different (Asadi *et al.* a,b., 2006). Also blood urea levels in males were higher than females; in line with other study on *A.persicus* (Asadi *et al.*, 2006a) and *Huso huso* (Asadi *et al.*, 2006b).

Lysozyme plays an important role in non-specific immune response and it has been found in mucus, serum and ova of fish (Murray & Fletcher, 1976, Yousif *et al.*, 1991). Lysozyme is known to act as a potent non-specific immune factor against parasitic and bacterial infections (Alexander & Ingram, 1992). Among serum immune parameters we evaluate in this study data for Serum lysozyme levels of Persian sturgeon had not significant differences between the three groups such as the same results obtained between data for total IgM, C3 and C4. In case of cultural condition by using Probiotic or prebiotic we may have efficacious on these factors (for review See: Yousefian and Shikholeslami, 2009) but in natural condition the results related to the condition that the fish are exist.

In our study calcium levels of male, immature and female Persian sturgeon were 2.5 ± 0.1 , 2.5 ± 0.2 , 2.2 ± 0.2 mg/dl respectively, while calcium measured for other species were higher like 6.6-12.1 mg/dl for *Acipenser brevirostrum* (Knowles *et al.*, 2006), 14.03 for *A. medirostris* (Kieffer *et al.*, 2001) and 7.21 mg/dl for *A.transmontanus* (LeBreton & Beamish, 1998). Neither stress nor circadian fluctuations have negligible effects on calcium levels (McDonald & Milligan, 1992). Because about one-half of total plasma calcium is ionized and one-half is bound to plasma proteins (Andreasen, 1985; Bjornsson *et al.*, 1989), a decline in plasma proteins in fasting fishes should also lower plasma calcium concentrations also increased values can be seen with acute stress (Hrubec *et al.*, 1997).

Concentrations of total magnesium are lower than for total calcium in freshwater species and are tightly regulated (McDonald & Milligan, 1992). Magnesium concentrations for freshwater teleosts and *Acipenser brevirostrum*, *Scaphirhynchus platyrhynchus* are consistent with our study (Knowles *et al.*, 2006, Barton *et al.*, 2000a).

Most of the Mg in fish is located in the bone. The remainder is found within the cells of soft tissues. The red blood cells of fish contain significantly higher levels of Mg and lower levels of Ca than found in humans (Lall, 2002). Erythrocyte hemolysis can cause false increases in plasma magnesium concentration (McDonald & Milligan, 1992) however, hemolysis was not observed in samples in this study.

The electrolyte values (Ca and Mg) indicated the operation of a variety of homeostatic mechanisms in the body (Asadi *et al.*, 2006a). According to, Srivastava and Srivastava (1994) the Ca and Mg levels were subject to seasonal variations so that in the pre-spawning their concentrations increased while during spawning and post spawning they gradually decreased. In this study the blood serum Ca and Mg levels in males were higher than females; in line with other study on *A.persicus* (Asadi *et al.*, 2006a) and *Huso huso* (Asadi *et al.*, 2006b).

Little is known of the regulation or factors that affect plasma phosphate concentration; in the present study P levels of male *A. persicus* were higher than those of female *A. persicus* ($P < 0.05$). Similar to our study the P levels of male *Huso huso* were significantly higher than females (Asadi *et al*, 2006b).

It is evident that understanding the physiological indices of blood serum of *A. persicus* under natural condition (sea) is essential for aquaculture because it relevant normal indices for propagation, rearing and stocking of this species but fish are in close contact with their environment and, as a result, their physiology is influenced accordingly. Therefore, “normal” values for a group of fish in one environment may be “abnormal” or deviate outside the reference interval for another population (Hunn & Christenson, 1977), hence evaluation of environment beside other factors seems necessary. The current findings can provide a helpful reference for evaluating the health and vigor of wild Persian sturgeon.

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