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ORIGINAL ARTICLE

Determination of some normal serum parameters in starry sturgeon (*Acipenser stellatus* Pallas, 1771) during spring season

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Abstract Hematology can be a useful tool for monitoring health status, detecting illnesses, and following the progress of diseases and responses to therapy. Despite advances in fish medicine in recent years, interpretation of fish hematology is often hampered by lack of meaningful reference values and the bewildering diversity of fish species. Serum samples of 40 *Acipenser stellatus* fish were analyzed (20 male and 20 female) and their serum parameter values were measured in both sexes. Serum biochemical values were determined (mean±SEM) for sodium (Na; 149.2±1.917 mmol/l), potassium (K; 2.75±0.097 mmol/l), calcium (Ca; 8.293±0.282 mg/dl), phosphorus (P; 12.39±0.267 mg/dl), glucose (Glc; 166.40±8.264 mg/dl), triglyceride (trig; 699.6±22.94 mg/dl), bilirubin (bilirubin; 0.616±0.0234 mg/dl), total protein (TOP; 2.988±0.0842 g/dl), albumin (Alb; 1.218±0.0415 g/dl), cholesterol (CHO; 238.2±11.24 mg/dl), creatinine (CREA; 0.1085±0.0048 mg/dl), and blood urea nitrogen (BUN; 15.32±0.5104 mg/dl). The serum values for bilirubin, Na, P, and CREA were significantly higher in females, whereas BUN and Alb were significantly higher in males. The correlations of coefficients between measured parameters were also determined.

Keywords Blood · Electrolytes · Serum · Sturgeon

Abbreviations

(Alb)	albumin
(Bilirubin)	billirubin
(BUN)	blood urea nitrogen
(Ca)	calcium
(CHO)	cholesterol
(CREA)	creatinine
(Glc)	glucose
(K)	potassium
(Na)	sodium
(P)	phosphorus
(TOP)	total protein
(trig)	triglyceride

Introduction

Sturgeons are considered to be "living fossils" (Bemis et al. 1997). Primitive characteristics, such as a heterocercal tail and cartilaginous skeleton, have been maintained over approximately 100–200 million years, despite major environmental changes (Baker et al. 2005; Asadi et al. 2006). Sturgeons have undergone multiple genome duplications during their evolution, which may account for their resistance to deleterious mutations, since there are probably several functional copies of every gene (Blacklidge and Bidwell 1993). Their primitive characteristics make sturgeons intriguing animals for study, since their biochemical hematological profile may differ substantially from the

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teleost profile. All sturgeon species worldwide are covered under the provisions of the Convention on International Trade in Endangered Species of Wild Fauna and Fauna. Several species are considered threatened with extinction as a result of overfishing, poaching, water pollution, damming, and destruction of natural water courses and habitats (Anonymous 2002). While sturgeons, the most economically important fish of the Caspian Sea, are in danger of extinction, their hematological parameters are unknown.

Blood parameters are increasingly used as indicators of the physiological or sublethal stress response in fish to endogenous changes. The possibility of evaluation depends on the availability of reference values as close as possible to normal values of the various blood components considered as reliable descriptors of healthy fish under natural conditions (Cataldi et al. 1998).

Blood parameters are used in diagnoses as biomarkers. This can be used to study alterations in blood parameters revealing lesions in other organs or tissues (Garcia-Navarro 1994), or to help breeding, given that changes in the hematological profile may indicate infestations and infections or even environmental changes (Ranzani-Paiva et al. 1999). According to Serpunin and Likhatchyova (1998), the blood parameters of a fish species are true indicators of the state of health of the organism.

The objective of this study was to generate hematologic and biochemistry reference intervals for healthy stellate sturgeon.

Materials and method

Source of fish

Fish were captured during Apr to May 2008 from Bandar Torkaman, the southern east of the Caspian Sea, Iran. In this study, 40 mature fish (20 males and 20 females) with average size of 120±20 cm and weights of 13–20 kg were sampled.

Sampling and analytical methods

To reduce stress by a manual blow to the head, the head was covered with a wet cloth (Smith et al. 1952). Samples were collected from behind the anal fin using a 20 ml plastic syringe and sent to laboratory on ice. The fishes were subjected to an external examination to ensure that they were healthy. Following removal of caviar, the ventral cavity was also checked. After separation of sera by centrifugation (10 min at 3,000 rpm), the serum samples were immediately analyzed using an autoanalyzer (Biotechnicon, Targa 3000, Rome, Italy). A standard serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy. Biochemical measurements were carried out for sodium (Na), potassium (K), calcium (Ca), phosphorus

Table 1 Serum parameters in mature male ($n=20$) and female ($n=20$) of *A. stellatus* (mean±SEM)

Parameters	Na (mmol/l)	Alb (g/dl)	TOP (g/dl)	Bilirubin (mg/dl)	Tri (mg/dl)	Chlo (mg/dl)	CREA (ng/dl)	BUN (mg/dl)	Glc (mg/dl)	P (mg/dl)	Ca (mg/dl)	K (mmol/l)
Male	145.0±3.25	1.26±0.059	3.07±0.132	0.564±0.031	691±32.1	236±14.3	98.5±0.7.16	16.4±0.631	162±9.54	11.50±0.33	7.9±0.0.38	2.75±0.15
Female	154.0±1.5	1.18±0.058	2.91±0.106	0.669±0.032	7.8±33.5	240±17.7	119±5.86	14.2±0.737	171±13.7	13.30±0.33	8.68±0.041	2.76±0.12
Total sample	149.2±1.92	2.75±0.097	2.99±0.084	0.616±0.023	699.6±22.9	238.2±11.24	108.5±4.8	15.32±0.510	166.4±8.26	12.39±0.26	8.29±0.28	2.75±0.1

Table 2 The Pearson's correlations of different serum parameters in female sturgeons

	Parameter	K	Pho	Ca	Na	BUN	Chol	Gluc	CREA	Bilirubin	Alb	Trig
P	*P* value	0.336										
	r^2	0.052										
Ca	*P* value	0.195	0.801									
	r^2	0.092	0.004									
Na	*P* value	0.445	0.457	0.314								
	r^2	0.033	0.031	0.056								
BUN	*P* value	0.141	0.159	0.659	0.15							
	r^2	0.117	0.107	0.011	0.111							
Chol	*P* value	0.362	0.073	0.764	0.885	0.583						
	r^2	0.046	0.168	0.005	0.001	0.017						
Glu	*P* value	0.191	0.38	0.893	0.459	0.438	0.518					
	r^2	0.093	0.043	0.001	0.031	0.034	0.024					
CREA	*P* value	0.041*	0.289	0.875	0.288	0.875	0.487	0.571				
	r^2	0.213	0.062	0.001	0.062	0.001	0.027	0.018				
Bilirubin	*P* value	0.623	0.895	0.475	0.587	0.63	0.165	0.238	0.922			
	r^2	0.014	0.001	0.029	0.017	0.013	0.105	0.076	0.001			
Alb	*P* value	0.197	0.358	0.744	0.019*	0.568	0.721	0.305	0.143	0.145		
	r^2	0.091	0.047	0.006	0.271	0.018	0.007	0.058	0.116	0.114		
Trig	*P* value	0.581	0.607	0.47	0.095	0.407	0.923	0.32	0.048*	0.03*	0.923	
	r^2	0.017	0.015	0.029	0.147	0.038	0.001	0.055	0.2	0.235	0.001	
TOP	*P* value	0.128	0.711	0.056	0.035*	0.861	0.884	0.384	0.222	0.246	0.001**	0.915
	r^2	0.124	0.008	0.189	0.223	0.002	0.001	0.042	0.082	0.074	0.492	0.001

* values are significant at $P<0.05$

** values are significant at $P<0.001$

(P), glucose (Glc), triglyceride (trig), bilirubin (Bilirubin), total protein (TOP), albumin (Alb), creatinine (CREA), cholesterol (CHO), and blood urea nitrogen (BUN).Ca values were measured using arsenazo method, P by phosphorus molybdate method, K and Na by flue photometry. TOP levels were measured based on the biuret method, formation of a violet complex between cupric ions and protein. Cholesterol levels were measured by (CHOD-PAP) cholesterol oxidase. The amounts of serum triglyceride were measured by diazo with sulphanilic acid method. Alb was determined by a dye binding technique between Alb and bromocresol green that results in a colored complex. Urea was measured by means of diacetyl monoxime reagent and converted thereafter to BUN (Dawson 1990). CREA values were measured based on the Jaffe reaction, a colorimetric reaction between creatinine and alkaline picrate (Palm and Lundblad 2005). Glc values were determined using glucose oxidase method and bilirubin values were measured by means of diazo with method.

Data analysis

Data were statistically analyzed using the GraphPad Prism V 4.0 (GraphPad software, USA). The data were first tested for Gaussian distribution. To determine statistically significant differences between males and females, the data were analyzed using *t* test. The Pearson's correlation test was used to determine any correlation among measured values. In all cases $P<0.05$ was considered as significant. The values are presented as mean±SEM.

Results

The serum levels of measured parameters, in mature female and male of *A. stellatus* are shown (mean±SEM) in Table 1.

The serum levels of Na, BUN, P, CREA, Bilirubin, and Alb showed significant differences between males and females ($P<0.05$). The amounts of serum BUN and Alb in male sturgeons were significantly higher compared to those of the females (Table 1). The levels of serum P, CREA, Bilirubin, and Na in female sturgeons were significantly higher than those of males (Table 1).

There was a significant Pearson`s correlation for serum levels of Na with those of Alb ($r^2=0.270$, $P<0.05$) and TOP ($r^2=0.223$, $P<0.05$) in females (Table 2). They also

Table 3 The Pearson's correlations of different serum parameters in male sturgeons

	Parameter	K	Pho	Ca	Na	BUN	Chol	Gluc	CREA	Bilirubin	Alb	Trig
P	*P* value	0.307										
	r^2	0.058										
Ca	*P* value	0.468	0.875									
	r^2	0.030	0.001									
Na	*P* value	0.199	0.947	0.505								
	r^2	0.090	0.000	0.025								
BUN	*P* value	0.431	0.778	0.374	0.331							
	r^2	0.035	0.005	0.044	0.053							
Chol	*P* value	0.494	0.663	0.331	0.385	0.891						
	r^2	0.026	0.011	0.053	0.042	0.001						
Glu	*P* value	0.679	0.832	0.735	0.000**	0.813	0.127					
	r^2	0.010	0.003	0.007	0.512	0.003	0.124					
CREA	*P* value	0.021*	0.254	0.806	0.945	0.947	0.277	0.615				
	r^2	0.262	0.072	0.003	0.000	0.000	0.065	0.014				
Bilirubin	*P* value	0.535	0.027*	0.500	0.612	0.417	0.314	0.777	0.627			
	r^2	0.022	0.245	0.026	0.015	0.037	0.056	0.005	0.013			
Alb	*P* value	0.888	0.446	0.033*	0.711	0.054	0.765	0.864	0.954	0.764		
	r^2	0.001	0.033	0.229	0.008	0.191	0.005	0.002	0.000	0.005		
Trig	*P* value	0.085	0.630	0.721	0.057	0.612	0.151	0.279	0.071	0.564	0.378	
	r^2	0.156	0.013	0.007	0.187	0.015	0.111	0.065	0.170	0.019	0.043	
TOP	*P* value	0.485	0.758	0.474	0.852	0.189	0.906	0.579	0.724	0.582	0.687	0.561
	r^2	0.028	0.005	0.029	0.002	0.094	0.001	0.017	0.007	0.017	0.009	0.019

* values are significant at $P<0.05$

** values are significant at $P<0.001$

showed a positive correlation between bilirubin and Trig (Table 2) and a negative correlation between CREA and Trig ($r^2=0.199$, $P<0.05$). In male sturgeons, a significant positive correlation was observed between Na and Glu ($r^2=511$, $P<0.001$; Table 3). Ca and Alb were also positively correlated ($r^2=288$; Table 3). However, the correlation between P and bilirubin was negative ($r^2=0.245$, $P<0.05$; Table 3). In both sexes, a positive correlation existed between K and CREA (female: $r^2=0.213$, male: $r^2=0.262$, $P<0.05$; Tables 2, 3).

Discussion

Electrolyte (Na, K, P, and Ca) levels indicate the operation of a variety of homeostatic mechanisms in the body (Clarke 1998). Alb and globulin are two important parts of TOP, and changes in these parameters affect the level of TOP. The correlation between Alb and Top is, therefore, expected. Goos et al. (1995) showed serum total protein and albumin levels displayed a significant positive correlation with serum sodium levels in human hemolytic uremic syndrome.

Alb is an important protein for transportation of steroid hormones. On the other hand, an increase in serum steroid hormone level has an indirect effect on serum ACTH level, and consequently, increases blood Na, K, P, and N_2 levels (Cunha et al. 2000). The increase in serum Alb level (due to increase in ACTH secretion) in *A. stellatus* may account for the observed increase in blood sodium. The significant correlation between Na and Gluc may be due to an increase in ACTH level that causes increased Gluc and Na retention, and consequently causes high blood pressure.

In surgical patients, hypoalbuminemia may occur as a component of acute-phase response (APR) syndrome. The higher percentage of hyponatremia among APR-positive patients has been attributed to decreased serum albumin levels associated with APR (Cunha et al. 1999). Butler et al. 1984 showed that patients from two hospitals with no obvious disturbances of calcium homeostasis and with total serum calcium concentrations that were normal after adjustment for albumin concentration had low serum ionized calcium concentrations.

Here is a comparison of reference biochemical serum parameters between *A. stellatus* and some other fish species. Potts and Rudy (1972) measured some serum parameters in

green sturgeon (*A. medirostris*), in which Na (114 mmol/l), K (1.5 mmol/l), and Ca (2.6 mmol/l) were lower than values we found in *A.stellatus*. Holmes and Donaldson (1969) measured some parameters in *Acipenser oxyrinchus* (Na: 151 mmol/l, K: 2.7 mmol/l, and Ca: 1.9 mmol/l).In the latter study, the Na and K levels were the same as our study, but the level of serum Ca was lower than that of *A.stellatus*. Natochin et al. (2000) monitored the biochemical parameters of blood serum in Russian sturgeon, *Acipenser gueldenstaedti,* from 1974 through 1993. The reported values for Na (115.40–165.00 mmol/l) and K (2.20–4.60 mmol/l) levels were similar, but the serum Ca level (1.70–3.70 mmol/l) was lower than that observed in *A. stellatus*. According to the latter research, the Ca/Na ratio can be used as an indicator of the condition of fish when muscle damaged pathology is present. Serum total proten of *Acipenser naccarii* was 1.9–2.6gr/dl according to Cataldi et al. (1998) that is similar to that measured in the current study. Asadi et al. (2006) measured some biochemical values in *Huso huso*, including Ca (2.13±0.69–2.37±0.38 mmol/l), TOP (4.51±1–5.50± 0.94 mg/dl), BUN (1.32±0.23–1.35±0.31 mmol/l), Alb (0.88±0.18–1.26±0.29 mg/dl), globulin (3.63±0.84–4.5± 0.69 mg/dl), uric acid (1.66±0.18–1.79±0.27 mmol/l), CREA (30.48±4.31–30.48±6.10 mmol/l), P (2.18±0.38–2.91±0.67 mmol/l), Mg(1.15±0.26–1.51±0.35 mmol/l), Glc (3.42±0.84–6.69±1.54 mmol/l), and alkaline phosphatase (4.48±1.82–5±1.04 IU/l). In this research, male fish had higher Glc, P, and Alb compared to the females. Knowles et al. (2006) measured reference intervals for cultured shortnose sturgeon. Accordingly the total protein (2.7–5.3 g/dl), calcium (6.6–12.1 mg/dl), creatinine (0–1.4 mg/dl), and potassium (2.9–3.7 mmol/l) were higher than those in *A.stellatus*. However sodium (124–141 mmol/L), phosphorus (5.1–8.1 mg/dl), glucose (37–74 mg/dl), total bilirubin (0–0.1 mg/dl), cholesterol (42–133 mg/dl), albumin (0.8–1.7 g/dl) were lower than those in *A.stellatus.*

Blood chemistry parameters among fish species may be affected by sampling technique, analyses methods, age, habitat, and diet (Sakamoto et al. 2001). Therefore, reference values reported here will be useful for the early detection, identification, and monitoring of diseases and sublethal conditions in this endangered species. However further work is still necessary to understand different factors that may affect these parameters.

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