Mediterranean Aquaculture Journal 2025 12 (1): 28-39

Mediterranean Aquaculture Journal

https://maj.journals.ekb.eg

Print ISSN: 1687-7616 Online ISSN: 1687-7187

*Original Article*

# Investigating the changes of some blood parameters of cultured Persian sturgeon (*Acipenser persicus*)

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## ABSTRACT

In this research, a comprehensive seasonal analysis was conducted on various hematological parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), alongside fluctuations in calcium, cholesterol, total protein, triglycerides, creatinine, and phosphorus levels in six-year-old Persian sturgeon (*Acipenser persicus*) with an average weight of 5000 ± 350 grams. Thirteen specimens were collected at the conclusion of each season, with blood samples obtained from the caudal peduncle and subsequently transported to the laboratory under ice-cold conditions for analysis. The results revealed that the spring season exhibited the highest enzyme activities for ALP (179.38 ± 39.6 IU/L) and LDH (643.1 ± 236.53 IU/L). In contrast, the autumn season recorded peak levels of AST (174.41 ± 35.01 IU/L) and ALT (3.46 ± 3.58 IU/L), while winter showed elevated concentrations of creatinine (0.23 ± 0.03 mg/dL), total protein (4.91 ± 0.55 mg/dL), and phosphorus ions (14.24 ± 1.41 mg/dL), all of which were statistically significant when compared to other seasons ($p<0.05$). Furthermore, winter also demonstrated significant differences in cholesterol (126.19 ± 34.33 mg/dL) and triglyceride levels (385.50 ± 134.03 mg/dL) relative to other seasons ($p<0.05$). These findings are essential for establishing baseline blood parameter ranges that can aid in the effective management of breeding practices and the monitoring of the physiological health of this native species.

**Keywords**: Persian sturgeon (*Acipenser persicus*), Liver enzymes activity, Blood parameters, Sistan.

## 1. INTRODUCTION

Aquaculture has experienced the most significant growth among all sectors of food production over the last twenty years. According to a report by the FAO, of the more than 70 livestock farming systems that contribute to the global food supply, aquaculture stands out as the only sector with a pronounced potential for alleviating poverty (FAO, 2024). The importance of this issue will be further highlighted by analyzing the role of fish consumption in promoting public health and comparing its relatively low per capita consumption in developed countries.

The advancement of aquaculture is influenced by a variety of factors, each presenting unique challenges to its progress (FAO, 2024). Sturgeon, often referred to as "living fossils," represent a valuable group of ancient cartilaginous fish, with 28 species currently recognized, six of which are found in the Caspian Sea. Among these, Acipenser persicus is the most prevalent species in the Caspian region, historically accounting for over 60% of sturgeon catches along the Iranian coastline (Billard and Lecointre, 2001; Akrami *et al.*, 2015).

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*Received: Mars 4, 2025 Revised: April 10, 2025 Accepted: April 16, 2025*

The decline of this species is unfortunately attributed to irresponsible and illegal hunting practices, along with increasing urban and agricultural pollution, river damming, and habitat destruction (Moghim *et al.,* 2002). Although the Persian sturgeon currently contributes to more than fifty percent of Iran's caviar output, projections indicate a likely significant decrease in its population in the foreseeable future. This situation underscores the urgent need for comprehensive research into Persian sturgeon aquaculture for both meat and caviar production (Morshedi et al., 2013). Blood biochemical parameters are crucial indicators of the physiological processes within an animal, providing essential insights into its overall health status (Chatzifotis *et al.,* 2011). In aquaculture, fish health during breeding is often assessed through morphological traits, with any indications of disease or malnutrition inferred from observable symptoms alone. However, research has shown that factors such as nutritional status, environmental stressors, and diseases can also affect the blood composition of fish (Peres *et al.*, 2014). Despite this, the application of blood biochemistry as a diagnostic tool in fish health assessment remains underutilized, leading to a scarcity of reliable data on the importance of blood parameters in evaluating nutritional status and overall fish health. Therefore, blood biochemistry analysis presents a rapid, non-invasive, and cost-effective approach for diagnosing malnutrition, stress, or diseases that adversely affect aquaculture productivity (Guo *et al.*, 2023).

Different diseases may emerge in aquaculture settings across various seasons, which can have a direct impact on blood characteristics. By promptly acquiring the essential parameters, it is possible to differentiate between normal and abnormal physiological data. Most studies examining the biochemical parameters of Persian sturgeon and other fish species have primarily concentrated on wild populations and have been limited to specific seasons, leading to a lack of extensive and applicable information regarding Persian sturgeon in diverse seasonal contexts within rearing environments. Consequently, this study was conducted to evaluate the biochemical and enzymatic markers of Persian sturgeon across different seasons under regulated cultural conditions.

## 2. MATERIALS AND METHODS

To investigate the growth dynamics of Persian sturgeon in ponds within the climatic context of Sistan, a total of 200 specimens, each averaging 10 ± 2 grams, were relocated from the Marjani Caviar Fishery Rehabilitation Center in Gorgan to the Zahak Native Fishery Rehabilitation Center. These sturgeons were subsequently housed in a terrestrial pond covering an area of half a hectare. From 2017 to 2021, they were fed a standard concentrated diet specifically formulated for sturgeon breeding, while the pond's water depth was consistently maintained at 2 meters. The feeding strategy was established at 2% of the total body weight of the fish.

For the purpose of blood sampling, the water level in the pond was lowered using a trawl method, which is designed to minimize stress and injury to the fish. Each season, 13 fish, aged 6 years and weighing approximately 5000 ± 350 grams, were randomly selected and transferred to a tank containing an anesthetic solution made from clove flower powder at a concentration of 200 mg/l. Following the anesthesia, blood samples were collected from the caudal peduncle. It is noteworthy that blood sampling was conducted on a seasonal basis, beginning in the middle of each season from autumn 2022 and concluding in summer 2023.

Samples were placed in microtubes without heparin for serum isolation. Following clotting, the blood samples underwent centrifugation at 3000×g for around 10 minutes. The serum-containing tubes were then stored at -20°C for further analysis (Gharaei *et al.*, 2020). Physicochemical parameters of the fish farm water, including temperature, dissolved oxygen, sulfur, ammonium, ammonia, nitrate, sulfate, phosphate, iron, hardness, and pH, were measured using WTW and Palintest model 8000 portable field devices.

The serological factors were prepared and assessed at the Hamoun International Wetland Research Institute and Viromed laboratory in Rasht, Iran.

The sera samples were tested for Cr (creatinine), Ca (Calcium), P (phosphor), TPro (total protein), Chol (Cholesterol), and TGs (Triglycerides) (Gharaei *et al.*, 2010). LDH and ALP activities in serum were determined using the DGKC method with commercial testing kits from Pars Azmoon Inc., Tehran, Iran. AST and ALT activities in serum were evaluated by the IFCC method (without pyridoxal phosphate activation) with commercial testing kits from Pars Azmoon Inc., Tehran, Iran. Creatinine was measured using the JAFFE method (Foster-Swanson *et al.*, 1994), cholesterol using the enzymatic cholesterol oxidase method, triglycerides using the Bruno method (1986), and total protein according to (Tietz method, 1986). Prior to measuring the study parameters, the biochemical analyzer device was calibrated using serum calibrator Trucal U (number 3516), and then TruLab P and TruLab N control serums (numbers 4338 and 4339 respectively) from Pars Azmoon Company. These controls were utilized for monitoring and evaluation purposes before and during the experiments. Upon gathering the data, our initial step involved assessing their normality through the Kolmogorov-Smirnov test and examining the homogeneity of variances using the Levene test. Once we established confidence in the normality and homogeneity of the data, we proceeded with the analysis utilizing the one-way ANOVA test. To compare the means, we employed the Tukey test with a 95% confidence level. Furthermore, we utilized Pearson correlation to investigate the relationship between factors. All statistical analyses were conducted using SPSS software (Version 18).

## 3.RESULTS

The data presented in Table (1) illustrates the average fluctuations in environmental parameters across various seasons. During the spring season, the highest phosphate concentration was observed at 2.4 ± 0.5 mg/l. The hardness levels ranged from 151.66 ± 1.5 mg/l to 18 ± 1 mg/l, with a noticeable impact from the changing seasons. Winter exhibited the highest average sulfate concentration at 0.91 ± 0.2 mg/l, while spring had the lowest at 0.12 ± 0.01 mg/l. Nitrate levels varied between 1.6 ± 0.15 and 5.1 ± 0.01 mg/l, reflecting seasonal influences. Ammonia, sulfur, phosphor, and pH levels remained relatively stable across different sampling periods. Turbidity notably increased in autumn compared to other seasons. The minimum and maximum average temperatures were recorded in autumn (17.6 ± 0.06 ℃) and summer (26.51 ± 0.16 ℃) respectively. Dissolved oxygen levels also displayed significant seasonal variations, with the lowest in autumn (5.27 ± 0.25 mg/l) and the highest in summer (7.66 ± 0.05 mg/l). The mean values of blood biochemical parameters of cultured Persian sturgeon during various seasons can be found in Table (2). The findings reveal notable variations in the levels of calcium, phosphorus, and total protein between summer and autumn ($p<0.05$). In winter, there was a significant rise in serum cholesterol levels compared to spring and summer ($p<0.05$). Moreover, the highest levels of triglycerides and ALT were observed in winter (385.50 ± 134; 3.46 ± 0.58 mg/dl), showing significant differences from other seasons ($p<0.05$). Creatinine levels in fish serum were measured across all seasons, with the highest value noted in spring ($p<0.05$) compared to other seasons. ALP values exhibited significant variations across seasons ($p<0.05$), with the peak value recorded in winter and spring. LDH concentration also displayed a significant difference in spring (643.1 ±236.53 mg/dl) ($p<0.05$) compared to other seasons. Statistical analysis confirmed a significant increase in AST concentration during autumn compared to other seasons. The blood biochemical parameters of treated fish were analyzed in different seasons (spring, summer, autumn, and winter) as shown in tables 4, 5, 6, and 7 respectively. The annual relationship between these parameters was then presented in table 3. In the spring season, a positive correlation was found between creatinine and AST, ALP; calcium and phosphor; total protein and phosphor; and phosphor and ALP values ($p<0.05$). However, no such correlation was observed among other parameters.

**Table (1)**. Average changes of physical and chemical parameters in the breeding pond of Persian sturgeon in different seasons of the year.

| Parameter | Summer | Spring | Winter | Fall |
|---|---|---|---|---|
| Fe (mg/l) | 0.02 ± 0.005 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.33 ± 0.01 |
| $PO_4$ (mg/l) | 0.75 ± 0.03$^{b}$ | 1.93 ± 0.05$^{a}$ | 0.27 ± 0.02$^{c}$ | 0.2 ± 0.04$^{c}$ |
| Hardness (mg/l) | 181 ± 1$^{a}$ | 176 ± 2$^{b}$ | 165.6 ± 1.1$^{c}$ | 151.6 ± 1.5$^{d}$ |
| $SO_4$ (mg/l) | 0.63 ± 0.01$^{b}$ | 0.12 ± 0.01$^{c}$ | 0.91 ± 0.02$^{a}$ | 0.61 ± 0.05$^{b}$ |
| $NO_3$ (mg/l) | 2.7 ± 0.02$^{c}$ | 5.1 ± 0.01$^{a}$ | 4.19 ± 0.03$^{b}$ | 1.6 ± 0.15$^{d}$ |
| $NH_3$ (mg/l) | 0.01 ± 0.005$^{c}$ | 0.03 ± 0.01$^{b}$ | 0.01 ± 0.005$^{c}$ | 0.01 ± 0.005$^{c}$ |
| S (mg/l) | 64.3 ± 1.15 | 63 ± 2.64 | 62.6 ± 2.5 | 61.66 ± 2.08 |
| TP (mg/l) | 0.55 ± 0.08 | 0.64 ± 0.03 | 0.52 ± 0.09 | 0.66 ± 0.1 |
| Turbidity (FTU) | 3 ± 1.1$^{b}$ | 3 ± 1$^{b}$ | 1.6 ± 0.5$^{c}$ | 6 ± 1$^{a}$ |
| pH | 8.7 ± 0.1 | 8.6 ± 0.2 | 8.6 ± 0.1 | 8.16 ± 0.15 |
| $O_2$ (mg/l) | 7.66 ± 0.05$^{a}$ | 6.6 ± 0.02$^{b}$ | 5.87 ± 0.2$^{c}$ | 5.27 ± 0.25$^{c}$ |
| Temperature (C°) | 26.51 ± 0.16$^{a}$ | 22.35 ± 0.09$^{b}$ | 10.3 ± 0.16$^{d}$ | 17.6 ± 0.06$^{c}$ |

Data shown with different letters in each row have significant differences (*P<0.05*).

**Table (2)**. Seasonal fluctuations of biochemical indices of blood of Persian sturgeon in breeding pond in different seasons of the year and annual (Mean ± SD; n=13)

| Parameter | Season | | | | Annual average |
|---|---|---|---|---|---|
| | Summer | Spring | Winter | Fall | |
| Calcium (mg/dL) | 8.68 ± 1.34$^{b}$ | 10.18 ± 0.66$^{a}$ | 8.25 ± 0.56$^{b}$ | 8.66 ± 0.47$^{b}$ | 8.85 ± 1.01 |
| Cholesterol (mg/dL) | 81.87 ± 10.5$^{c}$ | 101.92 ± 23.1$^{b}$ | 126.19 ± 21.4$^{a}$ | 115.69 ± 22.4$^{b}$ | 104.62 ± 26.4 |
| Total Protein (g/dL) | 3.65 ± 0.4$^{c}$ | 4.91 ± 0.55$^{a}$ | 3.81 ± 0.81$^{c}$ | 4.24 ± 0.51$^{b}$ | 4.18 ± 0.76 |
| Triglyceride (mg/dL) | 287.13 ± 43$^{b}$ | 291.82 ± 54.2$^{b}$ | 385.5 ± 58.4$^{a}$ | 315.28 ± 61.9$^{b}$ | 313.97 ± 59.6 |
| Creatinine (mg/dL) | 0.19 ± 0.06$^{b}$ | 0.23 ± 0.03$^{a}$ | 0.18 ± 0.02$^{b}$ | 0.18 ± 0.04$^{b}$ | 0.2 ± 0.04 |
| LDH (IU/L) | 421.64 ± 92$^{b}$ | 643.1 ± 103.4$^{a}$ | 316.9 ± 86.8$^{c}$ | 635.18 ± 110.6$^{a}$ | 494.25 ± 74.9 |
| ALP (IU/L) | 147.8 ± 32.55$^{b}$ | 179.38 ± 39.6$^{a}$ | 182 ± 31.46$^{a}$ | 148.74 ± 40.2$^{b}$ | 162.9 ± 53.6 |
| ALT (IU/L) | 0.99 ± 0.12$^{c}$ | 0.69 ± 0.09$^{d}$ | 3.58 ± 0.75$^{a}$ | 2.39 ± 0.81$^{b}$ | 2.34 ± 0.53 |
| AST (IU/L) | 118.56 ± 24$^{b}$ | 77.92 ± 18.9$^{c}$ | 122.15 ± 23.7$^{b}$ | 174.41 ± 31.2$^{a}$ | 123.36 ± 25.3 |
| Total Phosphor (mg/dL) | 8.86 ± 1.23$^{c}$ | 14.24 ± 1.41$^{a}$ | 9.00 ± 1.52$^{c}$ | 10.75 ± 2.23$^{b}$ | 10.82 ± 1.89 |

Data shown with different letters in each row have significant differences ($p$<0.05)

**Table (3).** The correlation value among biochemical parameters of the cultured Persian sturgeon blood in breeding pond in the year (Mean ± standard deviation; n=13).

| | Cr[1] | Chol[2] | TGs[3] | Ca[4] | Phos[5] | AST | ALT | ALP | LDH | TPro[6] |
|---|---|---|---|---|---|---|---|---|---|---|
| Cr | 1 | - 0.002 | **-*0.202** | ****0.414** | ****0.35** | - 0.104 | - 0.134 | ****0.22** | ****0.448** | ****0.386** |
| Chol | | 1 | ***0.526** | ***0.181** | ***0.166** | ****0.257** | ****0.251** | ****0.442** | - 0.052 | ****0.3** |
| TGs | | | 1 | 0.013 | - 0.57 | - 0.069 | ****0.316** | 0.11 | - 0.063 | 0.133 |
| Ca | | | | **1** | ****0.855** | ****- 0.244** | ***- 0.195** | ****0.305** | ****0.264** | ****0.75** |
| Phos | | | | | 1 | - 0.137 | - 0.163 | ****0.263** | ****0.315** | ****0.679** |
| AST | | | | | | 1 | 0.146 | 0.031 | 0.084 | - 0.14 |
| ALT | | | | | | | 1 | 0.163 | ***- 0.18** | - 0.089 |
| ALP | | | | | | | | 1 | 0.092 | ****0.227** |
| LDH | | | | | | | | | 1 | ****0.377** |
| TPro | | | | | | | | | | 1 |

(*) The correlation is significant at the 0.05 level, (**) The correlation is significant at the 0.01 level

.1. Ceratinine, 2.Cholestrol, 3. Triglyceride, 4.Calcium, 5.Phosphor, 6. Total protein

Similarly, in the summer season, a significant relationship was noted between creatinine and cholesterol, calcium values, as well as a positive correlation between AST and LDH level. The autumn season results indicated a negative correlation between creatinine and triglycerides values, and a significant correlation between cholesterol and total protein, AST and ALT values (*p<0.05*). Additionally, a negative correlation was found between creatinine and triglycerides levels (*p<0.05*), and a significant relationship between triglycerides and phosphor, total protein, phosphor, and AST in the winter (p<0.05). The annual Pearson correlation test (table 3) revealed a significant correlation between creatinine and triglycerides, calcium and ALT, and ALT and LDH values *(p<0.05*).

Furthermore, positive relationships were observed between cholesterol and hardness, triglycerides and LDH with sulfate, AST with phosphate and iron, ALP with nitrate, total protein with oxygen, sulfur, ammonia, sulfate, and phosphate values, as well as between calcium and ammonia, nitrate, sulfate, and phosphate values. Additionally, a positive relationship was found between phosphor and pH, ammonia, sulfate, and phosphate values, as well as between creatinine and ammonia, nitrate, and sulfate values.

**Table (4)**. The correlation value among biochemical parameters of the cultured Persian sturgeon blood in breeding pond in the spring (Mean ± standard deviation; n=13).

| | Cr[1] | Chol[2] | TGs[3] | Ca[4] | Phos[5] | AST | ALT | ALP | LDH | TPro[6] |
|---|---|---|---|---|---|---|---|---|---|---|
| Cr | 1 | 0.256 | -0.163 | -0.151 | 0.031 | **0.372**$^{*}$ | **0.485**$^{**}$ | **0.364**$^{*}$ | 0.275 | 0.066 |
| Chol | | 1 | **0.469**** | 0.140 | 0.0142 | **0.613**$^{**}$ | **0.412**$^{**}$ | **0.645**$^{**}$ | **-0.516**$^{**}$ | -0.003 |
| TGs | | | 1 | **0.445**$^{**}$ | 0.033 | 0.081 | -0.135 | 0.164 | **-0.365**$^{*}$ | 0.137 |
| Ca | | | | 1 | **0.385**$^{*}$ | 0.135 | 0.018 | 0.288 | -0.044 | **0.394**$^{*}$ |
| Phos | | | | | 1 | 0.192 | 0.102 | **0.365**$^{*}$ | **0.419**$^{**}$ | **0.417**$^{**}$ |
| AST | | | | | | 1 | **0.676**$^{**}$ | **0.829**$^{**}$ | 0.111 | -0.062 |
| ALT | | | | | | | 1 | **0.558**$^{**}$ | 0.232 | -0.004 |
| ALP | | | | | | | | 1 | 0.061 | 0.216 |
| LDH | | | | | | | | | 1 | 0.042 |
| TPro | | | | | | | | | | 1 |

(*) The correlation is significant at the 0.05 level, (**) The correlation is significant at the 0.01 level
.1.Ceratinine, 2.Cholestrol, 3. Triglyceride, 4.Calcium, 5.Phosphor, 6. Total protein

**Table (5).** The correlation value among biochemical parameters of the cultured Persian sturgeon blood in breeding pond in the summer (Mean ± standard deviation; n=13).

| | Cr[1] | Chol[2] | TGs[3] | Ca[4] | Phos[5] | AST | ALT | ALP | LDH | TPro[6] |
|---|---|---|---|---|---|---|---|---|---|---|
| Cr | 1 | **0.325**$^{*}$ | 0.054 | 0.311 | 0.308 | **0.522**$^{**}$ | 0.130 | 0.276 | **0.767**$^{**}$ | **0.475**$^{**}$ |
| Chol | | 1 | **0.517**$^{**}$ | **0.450**$^{**}$ | **0.444**$^{**}$ | 0.236 | -0.053 | **0.638**$^{**}$ | **0.509**$^{**}$ | **0.590**$^{**}$ |
| TGs | | | 1 | **0.358**$^{*}$ | **0.534**$^{**}$ | 0.028 | -0.003 | -0.003 | **0.331**$^{*}$ | **0.377**$^{*}$ |
| Ca | | | | 1 | **0.746**$^{**}$ | **0.552**$^{**}$ | **0.618**$^{**}$ | 0.404 | 0.244 | **0.541**$^{**}$ |
| Phos | | | | | 1 | **0.548**$^{**}$ | **0.442**$^{**}$ | 0.183 | **0.444**$^{**}$ | 0.312 |
| AST | | | | | | 1 | **0.415**$^{**}$ | **0.514**$^{**}$ | **0.402**$^{*}$ | **0.628**$^{**}$ |
| ALT | | | | | | | 1 | 0.135 | -0.051 | 0.264 |
| ALP | | | | | | | | 1 | 0.284 | **0.753**$^{**}$ |
| LDH | | | | | | | | | 1 | **0.377**** |
| TPro | | | | | | | | | | 1 |

(*) The correlation is significant at the 0.05 level, (**) The correlation is significant at the 0.01 level.
1.Ceratinine, 2.Cholestrol, 3. Triglyceride, 4.Calcium, 5.Phosphor, 6. Total protein

**Table (6).** The correlation value among biochemical parameters of the cultured Persian sturgeon blood in breeding pond in the autumn (Mean ± standard deviation; n=13).

| | Cr[1] | Chol[2] | TGs[3] | Ca[4] | Phos[5] | AST | ALT | ALP | LDH | TPro[6] |
|---|---|---|---|---|---|---|---|---|---|---|
| Cr | 1 | -0.157 | **-0.396**$^{*}$ | 0.045 | -0.239 | 0.010 | -0.078 | -0.260 | **0.466**$^{**}$ | 0.133 |
| Chol | | 1 | 0.132 | **0.570**$^{**}$ | **0.502**$^{**}$ | 0.147 | **0.436**$^{**}$ | **0.463**$^{**}$ | -0.206 | **0.3**$^{*}$ |
| TGs | | | 1 | 0.047 | 0.250 | -0.225 | **0.397**$^{*}$ | -0.087 | -0.043 | 0.155 |
| Ca | | | | 1 | **0.786**$^{**}$ | 0.311 | **0.668**$^{**}$ | 0.213 | -0.127 | **0.747**$^{**}$ |
| Phos | | | | | 1 | **0.283**$^{*}$ | **0.42**$^{**}$ | 0.238 | -0.231 | **0.464**$^{**}$ |
| AST | | | | | | 1 | **0.322**$^{*}$ | 0.185 | -0.092 | 0.149 |
| ALT | | | | | | | 1 | 0.147 | 0.092 | **0.826**$^{**}$ |
| ALP | | | | | | | | 1 | 0.288 | 0.233 |
| LDH | | | | | | | | | 1 | 0.263 |
| TPro | | | | | | | | | | 1 |

(*) The correlation is significant at the 0.05 level, (**) The correlation is significant at the 0.01 level.
1.Ceratinine, 2.Cholestrol, 3. Triglyceride, 4.Calcium, 5.Phosphor, 6. Total protein

**Table (7).** The correlation value among biochemical parameters of the cultured Persian sturgeon blood in breeding pond in the winter (Mean ± standard deviation; n=13).

| | Cr[1] | Chol[2] | TGs[3] | Ca[4] | Phos[5] | AST | ALT | ALP | LDH | TPro[6] |
|---|---|---|---|---|---|---|---|---|---|---|
| Cr | 1 | -0.247 | **-0.472**$^{*}$ | 0.029 | 0.258 | 0.304 | -0.332 | 0.135 | -0.034 | 0.084 |
| Chol | | 1 | **0.727**$^{**}$ | 0.328 | -0.013 | -0.077 | -0.339 | 0.103 | 0.375 | **0.560**$^{**}$ |
| TGs | | | 1 | 0.043 | **0.454**$^{*}$ | **0.546**$^{**}$ | 0.033 | 0.171 | 0.330 | **0.426**$^{*}$ |
| Ca | | | | 1 | **0.738**$^{**}$ | 0.276 | **-0.579**$^{**}$ | -0.093 | -0.314 | **0.524**$^{**}$ |
| Phos | | | | | 1 | **0.487**$^{*}$ | **-0.552**$^{**}$ | -0.282 | -0.351 | 0.307 |
| AST | | | | | | 1 | -0.215 | -0.133 | 0.068 | -0.082 |
| ALT | | | | | | | 1 | -0.137 | -0.276 | -0296 |
| ALP | | | | | | | | 1 | 0.134 | -0.166 |
| LDH | | | | | | | | | 1 | 0.043 |
| TPro | | | | | | | | | | 1 |

(*) The correlation is significant at the 0.05 level, (**) The correlation is significant at the 0.01 level.
1.Ceratinine, 2.Cholestrol, 3. Triglyceride, 4.Calcium, 5.Phosphor, 6. Total protein

## Discussion

Numerous elements affect the blood composition of fish, including environmental conditions such as temperature and seasonal changes, stress induced by fishing and sampling activities, dietary influences, and breeding environments characterized by factors like oxygen availability and salinity. Additionally, variations in genetics, age, developmental stage, sex, and activity levels have been shown to affect blood parameters (Nabi *et al.*, 2022). At present, there is a lack of comprehensive data regarding the interplay between water chemical parameters and blood indices in fish. However, it is reasonable to suggest that this relationship may be linked to the types of fertilizers—whether organic or inorganic—applied to improve the aquaculture environment during warmer months. Such fertilizers could lead to elevated nutrient concentrations, changes in water quality, increased stress levels, and, consequently, alterations in the blood composition of the fish.

Calcium ions are a critical element in blood serum, significantly influencing various enzymatic metabolic processes. Their presence is essential for several physiological functions, including muscle contraction, cardiac activity, nerve signal transmission, and the coagulation of blood. The assessment of serum calcium levels provides a direct measurement of the total calcium content in the bloodstream, which is instrumental in evaluating thyroid function and calcium metabolism (Gharaei *et al.*, 2013).

In the study conducted, the serum calcium concentration was noted to be 8.85 mg/dl during the spring season, which was notably higher compared to other times of the year. This increase in serum calcium levels in the fish studied during spring can be linked to the maturation process of the fish, highlighting the influence of seasonal changes. Research has shown that in bony fish, a rise in intracellular calcium concentration occurs after the final maturation of eggs, which is crucial for activating and commencing fertilization. This surge in calcium ions not only initiates the fertilization process but also plays a vital role in the subsequent growth and development of the embryos (Machaty, 2016).

Holmes and Donaldsons (1969) undertook an investigation into the calcium concentrations in the blood of Atlantic sturgeon, finding a level of 6.48 mg/dl, which was notably lower than that observed in Persian sturgeon.

In a different study, the typical blood parameter values for both male and female Persian sturgeon in their natural environment were documented (Table 4-10), revealing inconsistencies with the findings of our research.

Furthermore, Potts and Rudy (1972) reported that the calcium concentration in green sturgeon (*Acipenser medirostris*) inhabiting freshwater was also measured at 6.48 mg/dl, which is lower than the levels identified in our study of Persian sturgeon. Additionally, Natochin *et al.* (1975) tracked the calcium levels in Russian sturgeon over a two-decade period (1933-1974), recording values ranging from 6.66 to 6.30 mg/dl, which stand in contrast to the results obtained in our current investigation.

Creatinine serves as a widely recognized marker for assessing kidney function (Rehulka *et al.*, 2005). Research has demonstrated that variations in creatinine concentrations in fish blood can result from factors such as renal impairment, temperature changes, and reproductive behaviors (Gharaei et al., 2020). The results of the current study reveal that, with the exception of spring, there were no notable seasonal differences in creatinine levels. The spring season recorded the highest creatinine levels, likely due to the increased availability of food and the dietary habits of the fish, which include both concentrated and live food sources. This dietary abundance leads to elevated creatinine levels in the fish's body, particularly accumulating in reproductive tissues. Thus, the heightened creatinine levels observed in fish may primarily reflect their reproductive maturity. In a study focusing on Esox lucius L. in the Danube River, it was noted that creatinine levels in males prior to the breeding season (December, January, and February) were nearly double those found in females (Lenhard, 1992). Likewise, research by Hosseinzadeh *et al.* (2013) on the serum creatinine levels of male and female Persian sturgeon in the southeastern Caspian Sea during spring indicated an increase in creatinine levels for both sexes.

The current investigation demonstrated that phosphor levels were elevated during the spring season in comparison to other times of the year. Analysis using Pearson's correlation coefficient revealed a positive relationship between calcium and phosphor ions, likely linked to the role of gonads in the treated fish. The regulation of calcium and phosphor levels in blood serum is maintained by intrinsic bodily mechanisms, with these minerals predominantly stored in the skeletal system. Additionally, the hormones that facilitate the release or reabsorption of these ions into the bloodstream are largely similar. Notably, research has shown that phosphorus absorption is entirely contingent upon calcium absorption (Yasaghi *et al.*, 2008).

In fish, plasma phosphorus concentrations rise in tandem with increases in the gonadosomatic index or ovarian development. Although research on the interplay between phosphates and calcium fluctuations in fish is limited, it is recognized that phosphates are vital for the formation of vitellogenin or yolk (Mommsen and Walsh, 1998). Consequently, female fish undergoing yolk formation tend to exhibit elevated levels of both calcium and phosphorus.

It has been noted that in sturgeon, the concentration of certain elements rises as they reach sexual maturity, which corresponds with their role in the process of yolk formation (Meknatkhah *et al.*, 2015).

Cholesterol, the predominant sterol in fish muscle, plays a crucial role in the construction and maintenance of cell membranes, as well as in the regulation of membrane fluidity across various temperatures (Akon, 2006). The levels of cholesterol in fish blood can fluctuate based on several factors, including dietary habits, physical activity, and the stage of sexual maturation. Furthermore, cholesterol acts as a precursor for steroid hormone synthesis, and its concentration in the bloodstream tends to increase under stress, which may result in heightened cortisol production (Hosseini *et al.*, 2012). Our research indicates that cholesterol levels vary significantly with the seasons, showing the lowest concentrations in summer and the highest in winter when compared to other times of the year (Table 5). In this regard, two potential explanations can be considered: the fluctuations in cholesterol levels may be linked to the consumption of fatty concentrated and live foods in the pond during autumn, as well as to a reduction in metabolic activity during winter due to cooler water temperatures and decreased fat intake, which collectively contribute to an increase in cholesterol levels in the bloodstream.

In contrast, during the summer months, the consumption of both live and artificial food leads to an increase in fat intake, which correlates with the gradual rise in water temperatures. Research by Bao *et al.* (2022) indicates that both species and dietary choices can have a profound effect on serum cholesterol levels.

Additionally, since cholesterol is a precursor for steroid hormones, the observed fluctuations in cholesterol levels—rising in winter and decreasing in summer—may also be linked to the sexual maturation processes of sturgeon. Seasonal studies on the serum biochemical parameters of Common carp have demonstrated that fat and cholesterol levels increase from autumn to spring before declining, which is consistent with the findings of the present study (Yeganeh, 2011). The elevated serum lipid levels observed during the final maturation phase may result from fat accumulation surrounding the developing eggs. This pattern of increased serum lipid levels during yolk formation, followed by a decrease after spawning, has been documented in various species, including sea bass (Dicentrarchus labrax) (Jensen and Taylor, 2002). The triglyceride composition in fish fat reflects their dietary intake, typically characterized by long-chain unsaturated fatty acids and polyunsaturated fatty acids, especially omega-3 fatty acids (De Castro et al., 2007). Fluctuations in triglyceride levels are regarded as important indicators of bone health and are closely associated with various factors, including diet, metabolic processes, water temperature, and species differences (Zhou *et al.*, 2011; Babin and Virner, 1989).

Triglycerides are capable of transferring readily from the bloodstream to various tissues and organs, with their concentrations being significantly affected by dietary habits (Peres *et al.*, 2012). The observed increase in triglyceride levels during winter, contrasted with their decrease in other seasons, indicates that fish likely consume a greater quantity of concentrated and live food in warmer months within ponds. In contrast, the reduction in water temperature during winter results in a lowered metabolic rate, which contributes to fat accumulation in their bodies (Babin and Virner, 1989). A diet high in fat, particularly one rich in cholesterol and triglycerides, can elevate triglyceride levels in both adipose tissue and blood, often leading to alterations in cholesterol levels as well (Yasaghi *et al.*, 2008). Total protein serves as a reliable measure of hunger intensity, as it is a vital energy source for the body, and its concentration in the bloodstream diminishes significantly during periods of hunger (Jeong *et al.*, 2012).

In this study, fish exhibited a notable increase in total blood protein levels during the spring compared to other seasons. It is crucial to highlight that the fish were provided with a protein-sufficient diet year-round, indicating that the decline in blood protein levels among the experimental samples cannot be attributed to malnutrition. Rather, it is likely a consequence of seasonal variations. The pronounced increase in spring is thought to be associated with the accumulation of lipoproteins surrounding the eggs (Yeganeh, 2011).

Research has demonstrated that serum protein levels are affected by a variety of factors, including

age, stress, illness, and dietary protein intake (Patriche *et al.*, 2009; Gharaei *et al.*, 2020). Furthermore, Yeganeh (2011) observed that the serum protein concentration in common carp rises during the spring, a period that coincides with egg maturation and the accumulation of protein in the form of lipoproteins, which supports the findings of the current study. Enzymes such as AST, ALT, ALP, and LDH are frequently employed in diagnosing liver diseases, as they are normally found within the cytoplasmic membrane and mitochondria of cells. When cellular membranes are compromised or when necrosis occurs, these enzymes are released into the bloodstream, resulting in elevated serum levels (Gharaei *et al.*, 2020).

The release of these enzymes into the blood is facilitated by increased membrane permeability due to tissue damage. Additionally, during fasting periods, their activity may diminish as a result of reduced metabolic rates (Peres *et al.*, 2012). In the present study, it was observed that AST enzyme activity was significantly higher in the autumn compared to other seasons, a change likely linked to stressors associated with seasonal transitions, environmental conditions, transportation activities, and fishing practices (Lemarie *et al.*, 2012).

In situations characterized by heightened metabolic activity, the body experiences an increased demand for energy (Kominami et al., 1985). Among various enzymes, aspartate aminotransferase (AST) is frequently employed as a biomarker for tissue damage and serves as an indicator of stress within aquatic ecosystems (Jung *et al.*, 2003).

The necrosis or damage of liver cells leads to a rise in the secretion of this enzyme, which subsequently enters the bloodstream. Therefore, elevated levels of AST activity are indicative of tissue damage, particularly in hepatic tissues. Research conducted by Lemarie *et al.* (2012) revealed that AST activity is subject to seasonal variations. Their findings indicated that both male and female shortnose sturgeon (*Acipenser brevirostrum*) exhibit the lowest AST concentrations in autumn, while the highest levels are recorded in spring. This observation challenges earlier studies that reported peak activity during autumn, suggesting a possible seasonal effect.

Additionally, lactate dehydrogenase (LDH) is found in nearly all body cells and serves as a reliable marker for tissue and cellular injury (Gharaei *et al.*, 2020). LDH functions as a lipoprotein that facilitates the transport of cholesterol in the bloodstream, comprising a mixture of protein and a small quantity of cholesterol. It plays a crucial role in removing excess cholesterol from tissues and delivering it to the liver for excretion, which is why LDH cholesterol is often termed "good cholesterol" (Harper, 2009). In the present study, the lowest LDH levels were recorded during winter, a significant variation that may be linked to the lower temperatures experienced during colder days, resulting in thermal stress.

In the context of protein metabolism, a seasonal investigation was conducted to assess the influence of stress on lactate dehydrogenase (LDH) enzyme activity in common carp, revealing that LDH activity reaches its nadir as temperatures transition from summer to winter (Md *et al.*, 2017).

Melloti *et al.* (2007) explored the seasonal variations in serum and muscle enzyme activities in both catfish and common carp. The alkaline phosphatase (ALP) enzyme, which plays a vital role in glycogen metabolism, is known to inhibit phosphorylase enzymes while facilitating glycogen synthesis. An increase in ALP production within the liver is associated with the mobilization of glycogen for energy during periods of stress. As a hydrolytic enzyme, alkaline phosphatase is responsible for cleaving phosphate groups from various molecules, including nucleotides and proteins. Research has indicated that ovarian hormones modulate the presence of these enzymes, with fluctuations in activity correlating with levels of estrogen, progesterone, and gonadotropic hormones following ovarian stimulation.

A study on mature sturgeon in their natural environment reported elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and LDH activity compared to the current findings, although ALP activity was found to be

lower. The observed gradual increase in ALP activity during the winter and spring months may be attributed to the progression of sexual maturation in the treated sturgeon. Furthermore, Yasaghi *et al.* (2008) examined reproductive indicators in silver carp, noting a concurrent rise in ALP activity in both genders as they approached sexual maturity, with activity peaking at stage IV.

The research indicates that the physicochemical characteristics of the aquatic environment in Persian sturgeon aquaculture vary across different seasons.

Additionally, it has been shown that seasonal changes and stressors significantly impact the blood biochemical parameters of these fish. The results further emphasize the established natural range of certain blood biochemical indices in farmed Persian sturgeon, which can serve as a reference point for assessing health during disease outbreaks.

## CONCLUSION

In conclusion, it can be deduced that fish exhibit a range of behaviors in response to fluctuating environmental conditions and seasonal variations, with factors such as age, species, season, and stress from fishing playing a crucial role in influencing their blood and enzyme profiles.

**Acknowledgment**

The research project was funded by University of Zabol (Grant cod: IR-UOZ-GR-7925).

**Ethics Statement**

Authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. All procedures were carried out in accordance with the Animal Care and Use Committee guidelines at the Faculty of Sciences of the University of Zabol.

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