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# Consideration of Blood Serum Biochemical Parameters of Yellow Fin Sea Bream (*Acantopagrus latus* Houttuyn, 1782) and Orange-Spotted Grouper (*Epinephelus coioides* Hamilton, 1822)

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## Abstract

**Serum biochemical parameters are important aspects in the management of endangered species. The values of these parameters can be used for confirming the maturity and for monitoring any changes in the quality of waters and related soils. The aim of this research was to determine the reference values of biochemical factors in *Acantopagrus latus* and *Epinephelus coioides*. Blood samples were collected from captured fish from coastal waters of Chabahar (Ramin waterfront). Serum levels of glucose, Blood Urea Nitrogen (BUN), cholesterol (CHO), triglyceride, total protein, albumin, calcium ($Ca^{2+}$), phosphorus (P), sodium ($Na^{+}$), bilirubin and potassium ($K^{+}$) were measured. The results showed that the levels of albumin and bilirubin in *E. coioides* were significantly higher than *A. latus* and there were significant differences in all of the serum values (except P and glucose) between *A. latus* and *E. coioides* ($P < 0.05$). It can be concluded that the values of blood biochemical parameters may be affected by physiological factors such as the species of fish.**

## Keywords

**Serum, *Acantopagrus latus*, *Epinephelus coioides*, Electrolyte, Non Electrolyte**

## 1. Introduction

As the aquaculture industry expands, there is an increasing need for improved diagnostic methods. There are

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few tools available to diagnose and monitor diseases on fishes. The analysis of blood indices has proven to be a valuable approach for analysing the health status of aquatic animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting [1]. Exogenous factors, such as management [2], diseases [3] and stress [4], always induce major changes in blood composition. For example, significant fluctuations were detected in the concentrations of cortisol, glucose, cholesterol and other basic components in response to handling and hypoxic stress [2] [5] [6].

One of the difficulties in assessing the state of health of natural fish population has been the paucity of reliable references of the normal condition. In pursuit to this goal, many fish physiologists have turned to studies of hematology. Blood serum biochemical data are used routinely in health care of humans and domestic animals. Blood biochemical evaluations are also gradually becoming a routine practice for determining health status in fish [7]-[9], and only a few normal values for small number biochemical parameters have been established for some teleosts [10].

Yellow fin sea bream (*Acantopagrus latus* Houttuyn, 1782) and Orange-spotted grouper (*Epinephelus coioides* Hamilton, 1822) are protandrous and protogeny hermaphroditic from family percidae and serranidae respectively and being distributed off southern Japan, southeastern China, Taiwan, southeastern Asia, Persian Gulf and Australia and in the Indian Ocean to southeastern Africa [11]-[14]. They are considered to be two of the most commercially important marine fish in Iran, due to their consumer preference and are newly successful cultured in the coastal area of Iran.

These economically important fish are under threat because of their continuous exposure to toxic chemical-rich industrial effluents that are discharged into the Pacific Gulf and illegal fishing practices. There is an urgent need to revive them through processes of conservation, management and mariculture, so knowledge of the blood serum biochemical factors in order to detect the health of fish is a basic prerequisite for successful management.

This present study would form a baseline data for assessment of health status of *A. latus* and *E. coioides* as well as reference point for future comparative study effects of different endogenous and exogenous factors on blood biochemical of these two fish species.

## 2. Materials and Methods

### 2.1. Experiment Design

*A. latus* and *E. coioides* fishes were captured by set net, 20 - 30 m deep on the sea bottom, from coastal waters of Chabahar (Ramin waterfront) in February 2013. In this study totally 20 fish (n = 10 *A. latus* (1.101 ± 23.78 g); n = 10 *E. coioides* (1.250 ± 34.5 g)) captured. Blood samples collected from each individual and body weight was measured.

### 2.2. Blood Collection

Blood sampling was performed immediately after fish were captured. Samples were collected from behind the anal fin using a 5 ml 22 no, plastic syringe. The blood samples about 2.5 ml were placed into vacutaineers tubes.

### 2.3. Biochemical Analyses

Biochemical analyses were conducted at state Sadaf Medical Laboratory (Chabahar, Iran). Blood samples were centrifuged at 3000 rpm for 10 min and extracted serum samples were stored at −20˚C prior to further analysis. A suite of Biochemical analyses for parameters, such as glucose, cholesterol, triglyceride, total protein, blood urea nitrogen, bilirubin, albumin, $Ca^{2+}$, phosphorus were carried out using an automated analyzer (Auto Analyzer Hitachi 917, Japan ), with a Pars Azmoon kit (Co, Pars Azmoon, Tehran, Iran). $Na^{+}$ and $K^{+}$ were assessed by flame photometer (PFP 7, UK) [15].

### 2.4. Statistical Analysis

All statistical analyses were performed using the computer program SPSS ver16.0 (SPSS, Chicago, IL). Significant differences of parameters between two fish species were determined with independent t-test. The correlation coefficients between parameters were calculated by Pearson correlation coefficients. Liner regressions were used to determine the relationship between serum biochemical parameters showing significant differences significant parameters are reported as means ± standard deviation.

## 3. Results

**Table 1** shows the mean and standard deviation values for each blood biochemical parameters in *A. latus* and *E. coioides*. The results showed that that the serum levels of albumin and bilirubin in *E. coioides* were significantly higher than *A. latus* and there were significant differences in all of the serum values (except P and glucose) between *A. latus* and *E. coioides* ($P < 0.05$).

**Table 2** and **Table 3** show correlation coefficient of blood serum biochemical parameters of *A. latus* and *E. coioides* respectively. Significant positive correlation of glucose on $Ca^{2+}$ ($r = 0.695$, $P < 0.05$), protein on $Ca^{2+}$ ($r = 0.704$, $P < 0.05$) and CHO on P ($r = 0.860$, $P < 0.01$) in *A. latus* and protein on BUN ($r = 0.721$, $P < 0.05$) and $Ca^{2+}$ and $K^{+}$ (0.840, $P < 0.01$) in *E. coioides* were observed. There were significant negative correlations of CHO on $Ca^{2+}$ ($r = -0.641$, $P < 0.05$) and BUN on $Ca^{2+}$ ($r = -0.695$, $P < 0.05$) in *A. latus* and P on $Ca^{2+}$ ($r = -0.735$, $P < 0.05$) in *E. coioides*.

Significant negative and positive regressions of parameters in both *A. latus* and *E. coioides* have been shown in **Table 4**.

**Table 1.** Changes of blood serum biochemical parameters (Means ± SD) of *Acantopagrus latus* and *Epinephelus coioides*.

| Species / Parameters | *Acantopagrus latus* | *Epinephelus coioides* |
|---|---|---|
| $Na^{+}$ (mmol/l) | 328.50 ± 32.03 a | 271.90 ± 7.78 b |
| $K^{+}$ (mmol/l) | 2.12 ± 0.42 b | 3.43 ± 0.39 a |
| $Ca^{2+}$ (mmol/l) | 19.80 ± 2.97 a | 16.80 ± 2.34 b |
| Phosphorus (mmol/l) | 2.35 ± 0.40 a | 2.40 ± 0.57 a |
| Cholesterol (CHO) (mg/dl) | 257.30 ± 35.51 a | 137.30 ± 42.11 b |
| Triglyceride (mg/dl) | 102.02 ± 11.82 a | 68.10 ± 15.34 b |
| Blood Urea Nitrogen (BUN) | 3.82 ± 0.55 a | 2.66 ± 0.45 b |
| Bilirubin | 0.24 ± 0.04 b | 0.44 ± 0.13 a |
| Glucose | 44.25 ± 16.18 a | 43.70 ± 10.11 a |
| Total Protein | 4.31 ± 0.32 a | 3.92 ± 0.36 b |
| Albumin | 0.15 ± 0.03 b | 0.36 ± 0.05 a |

The differences between two species were tested by using independent t-test. Different letters of each row indicate a significant difference ($P < 0.05$).

**Table 2.** Correlation of blood serum biochemical parameters in *Acantopagrus latus*.

| Parameters | $Na^{+}$ | $K^{+}$ | $Ca^{2+}$ | Phosphorus | Cholesterol | Triglyceride | Blood Urea Nitrogen | Bilirubin | Glucose | Total Protein |
|---|---|---|---|---|---|---|---|---|---|---|
| $K^{+}$ | 0.084 | | | | | | | | | |
| $Ca^{2+}$ | −0.311 | −0.200 | | | | | | | | |
| Phosphurus | −0.353 | −0.206 | −0.457 | | | | | | | |
| Cholesterol | −0.253 | −0.167 | −0.641* | 0.860** | | | | | | |
| Triglyceride | 0.196 | 0.015 | 0.179 | −0.356 | −0.402 | | | | | |
| Blood Urea Nitrogen | 0.406 | −0.132 | −0.659* | 0.168 | 0.404 | 0.444 | | | | |
| Bilirubin | 0.122 | −0.049 | 0.113 | −0.350 | −0.065 | −0.088 | 0.355 | | | |
| Glucose | 0.194 | 0.399 | 0.695* | 0.291 | 0.319 | −0.251 | 0.139 | −0.548 | | |
| Total Protein | 0.096 | −0.400 | 0.704* | 0.392 | 0.569 | 0.025 | 0.441 | −0.281 | 0.472 | |
| Albumin | 0.234 | −0.072 | −0.385 | 0.305 | 0.223 | 0.008 | −0.086 | −0.554 | −0.496 | 0.476 |

Correlation coefficient of serum cholesterol was determined by spearman and the other parameters was determined pearson. *Correlation is significant at the 0.05. **Correlation is significant at the 0.01 level.

**Table 3.** Correlation of blood serum biochemical parameters in *Epinephelus coioides*.

| Parameters | $Na^+$ | $K^+$ | $Ca^{2+}$ | Phosphorus | Cholesterol | Triglyceride | Blood Urea Nitrogen | Bilirubin | Glucose | Total Protein |
|---|---|---|---|---|---|---|---|---|---|---|
| $K^+$ | 0.202 | | | | | | | | | |
| $Ca^{2+}$ | 0.147 | 0.840** | | | | | | | | |
| Phosphurus | −0.499 | −0.596 | −0.735* | | | | | | | |
| Cholesterol | 0.180 | 0.417 | 0.543 | −0.251 | | | | | | |
| Triglyceride | 0.126 | −0.575 | −0.360 | −0.290 | −0.284 | | | | | |
| Blood Urea Nitrogen | −0.363 | 0.375 | 0.161 | 0.010 | 0.280 | −0.365 | | | | |
| Bilirubin | −0.435 | 0.202 | −0.082 | 0.341 | −0.153 | −0.221 | 0.331 | | | |
| Glucose | −0.058 | −0.125 | 0.184 | −0.131 | 0.325 | −0.030 | −0.339 | −0.186 | | |
| Total Protein | −0.142 | 0.051 | −0.294 | 0.428 | 0.189 | −0.028 | 0.721* | 0.242 | −0.313 | |
| Albumin | 0.340 | 0.377 | 0.099 | 0.305 | 0.00 | 0.008 | −0.264 | 0.270 | 0.032 | −0.102 |

Correlation coefficient of serum cholesterol was determined by spearman and the other parameters was determined pearson. *Correlation is significant at the 0.05. **Correlation is significant at the 0.01 level.

**Table 4.** Regression equations between significant parameters in both *Acantopagrus latus* and *Epinephelus coioides*.

| Independent Factor (X) | Dependent Factor (Y) | Equation | R Square ($R^2$) | Pearson Correlation |
|---|---|---|---|---|
| Protein (*E. coioides*) | Blood Urea Nitrogen | Y = 0.892X − 0.837 | 0.519 | 0.721 |
| Phosphorus (*E. coioides*) | $Ca^{2+}$ | Y = −2.98X + 23.95 | 0.541 | −0.735 |
| $Ca^{2+}$ (*E. coioides*) | $K^+$ | Y = 0.142X + 1.051 | 0.706 | 0.840 |
| Cholesterol (*A. latus*) | $Ca^{2+}$ | Y = −0.054X + 33.61 | 0.441 | −0.641 |
| Cholesterol (*A. latus*) | phosphorus | Y = 0.010X − 0.198 | 0.740 | 0.860 |
| Protein (*A. latus*) | $Ca^{2+}$ | Y = 6.38X + 47.32 | 0.496 | 0.704 |
| Blood Urea Nitrogen (*A. latus*) | $Ca^{2+}$ | Y = −3.50 X + 33.22 | 0.439 | −0.659 |
| Glucose (*A. latus*) | $Ca^{2+}$ | Y = −0.128X + 25.44 | 0.483 | −0.695 |

## 4. Discussion

The wild populations of *A. latus* and *E. coioides* have almost collapsed in recent years to intensive fishing and water pollution, prompting the development of aquaculture facilities for the commercial culture of these species [11]-[14]. A rearing system for the maximization of fish productivity is the aim of fishery management programmers, and an evaluation of the physiological status of the fish during rearing is necessary to achieve this. Serum biochemical data are of immense importance in monitoring the health status of aquatic organisms, especially in fisheries management programs [7]-[9].

The serum levels of different biochemical values in *A. latus* and *E. coioides* were as follows: $Na^+$ (328.50 ± 32.03, 271.90 ± 27.88), $K^+$ (2.12 ± 0.42, 3.43 ± 0.39), $Ca^{2+}$ (19.80 ± 2.97, 16.80 ± 2.34), P (2.35 ± 0.40, 2.40 ± 0.57) (mmol/l) and CHO (257.30 ± 35.51, 137.30 ± 42.11), triglyceride (102.02 ± 11.82, 68.10 ± 15.34), BUN (3.82 ± 0.55, 2.66 ± 0.45), bilirubin (0.24 ± 0.04, 0.44 ± 0.13), glucose (44.25 ± 16.18, 43.70 ± 10.11) (mg/dl), protein (4.31 ± 0.32, 3.92 ± 0.36) and albumin (0.15 ± 0.03, 0.36 ± 0.05) (g/dl) respectively (as shown **Table 1**). The results showed that the serum levels of albumin and bilirubin in *E. coioides* were significantly higher than *A. latus* and there were significant differences in all of the serum values (except P and glucose) between *A. latus* and *E. coioides* ($P < 0.05$). It can be concluded that perhaps further confounding these values are variables such as age, sex, dietary state and stress all of which may alter blood values [16] [17].

Electrolyte ($Na^+$, $K^+$, $Ca^{2+}$ and P) levels indicate the operation of a variety of homeostatic mechanisms in the body [18]. In this study, both in *A. latus* and *E. coioides* $Na^+$ levels were higher than the values in *Scorpaena porcus* (195.07 ± 1.70 mmol/l) [17], *Scophthalmus aquosus* (157 ± 0.3 - 186 ± 0.4 mmol/l) [19] and *Salmo salar* (137 ± 1.1 - 196 ± 18.6 mmol/l) [20] but were lower than those of *Rutilus frissi* (387.1 ± 11.5 - 420.7 ± 7.1

mmol/l) [21]. $K^+$ levels were lower than the values in *Scophthalmus aquosus* (4.10 ± 0.18 - 5.48 ± 0.15 mmol/l) [19] and *Rutilus frissi* (37.9 ± 7.4 - 39.9 ± 10.6 mmol/l) [21] but were higher than those of *Salmo salar* (1.3 ± 0.4 - 4.5 ± 0.1 mmol/l) [20]. At the same time, the densities of $K^+$ were similar to those reported in *Scorpaena porcus* (3.81 ± 0.18 mmol/l) [17]. $Ca^{2+}$ activities were higher than values reported in *Scorpaena porcus* (3.66 ± 0.07 mmol/l) [17], *Salmo salar* (3.3 ± 0.1 - 4.7 ± 1.4 mmol/l) [22] and *Scophthalmus aquosus* (3.49 ± 0.11 - 4.43 ± 0.10 mmol/l) [19] but were similar to those reported in *Rutilus frissi* (15.9 ± 1.5 - 21.6 ± 1 mmol/l) [21]. P values were lower than those from *Scorpaena porcus* (15.12 ± 0.26 mmol/l) [17] and *Rutilus frissi* (19.2 ± 1 - 28.9 ± 2.8 mmol/l) [21].

The levels of glucose and cortisol are considered to be specific indicators of sympathetic activation during stress conditions [6] [21]. This study showed that there were significant differences in all of the serum values (except P and glucose) between *A. latus* and *E. coioides* ($P < 0.05$). Glucose levels were lower than those reported in *Rutilus frissi* (83.8 ± 11.3 - 209.8 ± 23.2 mg/dl) [21]. In the present study, the albumin and bilirubin levels from *E. coioides* were significantly higher than *A. latus*. Albumin, BUN, Triglyceride and CHO levels were lower than those reported in *Rutilus frissi* [21] but bilirubin and protein levels were similar to *Rutilus frissi* [21]. The ranges of serum biochemistry vary from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age and sex of the fish [23]. The increased plasma protein concentration can be caused by structural liver alternations that reduce aminotransferase activity, with concurrent reduction in deamination capacity [24]. Hrubec *et al*. [24] stated that protein level in striped bass increased with age.

Hill [25] reported that cholesterol concentrations increase as the fish size increased. A high blood urea concentration recorded in *M. cephalus* is likely to be a sign of stress associated with the increase in the cortisol level [26].

In this study, significant positive correlation of glucose on $Ca^{2+}$ ($r = 0.695$, $P < 0.05$), protein on $Ca^{2+}$ ($r = 0.704$, $P < 0.05$) and CHO on P ($r = 0.860$, $P < 0.01$) in *A. latus* and protein on BUN ($r = 0.721$, $P < 0.05$) and $Ca^{2+}$ and $K^+$ (0.840, $P < 0.01$) in *E. coioides* were observed. There were significant negative correlations of CHO on $Ca^{2+}$ ($r = -0.641$, $P < 0.05$) and BUN on $Ca^{2+}$ ($r = -0.695$, $P < 0.05$) in *A. latus* and P on $Ca^{2+}$ ($r = -0.735$, $P < 0.05$) in *E. coioides*. Also significant positive correlation of CHO on P was observed in *Rutilus frissi* [21].

## 5. Conclusion

In conclusion, variations between blood biochemical parameters among fish species depend on the sampling technique, analyses methods, age, habitat and diet [16] [17].

Finally, the results showed that the levels of albumin and bilirubin in *E. coioides* were significantly higher than *A. latus* and there were significant differences in all of the serum values (except P and glucose) between *A. latus* and *E. coioides*. It can be concluded that the values of blood biochemical parameters may be affected by physiological factors such as the species of fish. Also, the results of present study may also be helpful in obtaining standard values of blood parameters. Furthermore, examining blood parameters may allow us understand biological and ecological characteristics of these species.

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