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Changes in Some Serum Biochemical Values of Yellowfin Sea Bream (*Acanthopagrus latus*) in Mahshahr Creeks (Persian Gulf)

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Abstract: Biochemical indices are indicators measured in a biological system which can be related to exposure to a xenobiotics compound. In the current paper some serum biochemical parameters in five creeks of Mahshahr region in North West of Persian Gulf with different levels of pollutant were detected to characterize biochemical response of Yellowfin seabream to environmental pollutant and undesirable materials. The quantitative determination of serum glucose was carried out using commercially available diagnostic Experimental Protocols kits by the glucose oxidase method. Serum total protein levels were determined with bovine serum albumin serving as standard. Values recorded for activity of total protein show depletion in infected creeks with respect to clean creek, however, there was no significant variation. Glucose significantly increased in infected that pollutants have direct correlation with fish serum biochemical abnormalities and also indicated that the different in environmental condition of marine ecosystems may cause several changes in the serum biochemical parameters of the studied fish.

Key words: Fish · Serum biochemical · Persian Gulf · Yellowfin Sea Bream.

INTRODUCTION

Biochemical indices are indicators measured in a biological system which can be related to exposure to a xenobiotic compound. They are measures of the rates of chemical reactions or the amounts of biochemical products in cellular or subcellular systems, sublethal biological effects of contaminants [1].

Proteins are involved in major physiological events therefore the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of organism. Proteins are highly sensitive to heavy metal poisoning [2]. Total serum protein concentration is a measure of all of the different proteins in plasma with the exception of those that are consumed in clot formation such as fibrinogen and the clotting factors. For this reason, plasma protein concentration is generally about 0.3 to 0.5 g/dl higher than serum protein concentration [3].

Blood Glucose level in fish is known to be very useful as a criterion for diagnosis of liver and muscle tissue functions [4], Levels of glucose were measured as conventional stress markers to assess the reliability of stress response triggered under our experimental conditions. Smet and Blust [5] observed no significant changes in the levels of serum glucose at cadmium exposure. A weak or no change in plasma glucose may be attributed to a high energy demand so that glucose cannot be accumulated (acute experiments) or the organism be habituated (chronic experiments).

Serum glucose concentration depends on intestinal absorption, hepatic production and tissue uptake of glucose. The balance between hepatic production and tissue uptake is influenced by a variety of hormones including glucagons, corticosteroids, adrenocorticotropic hormone (ACTH), growth hormone and catecholamines. Corticosteroids, catecholamines and growth hormone. glucagons and glucocorticoids stimulate hepatic gluconeogenesis and glucagons and catecholamines glycogenolysis. These actions tend to increase serum glucose concentration. The practice of fasting animals prior to blood collection decreases the variability that accompanies postprandial intestinal absorption of glucose. Another procedural consideration for glucose analysis is prompt separation of the serum from clotted blood. erythrocyte and to a lesser degree leukocyte,

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glycolysis will reduce serum glucose concentration by approximately 7 to 10 mg/dl every hour that the blood cells remain in contact with the serum at room temperature [3].

Mahshahr creeks, located in the north west of Persian Gulf, faces this problem because it is being surrounded by polluting industries, which deposit pollutants directly into the creeks. Serum biochemical parameters are very sensitive to environmental pollutant and their response to undesirable materials is very fast, so in this study we detect different levels of biochemical parameters in five creeks of Khoor-Mousa in Mahshar region with different levels of pollutant to Characterized hematological response of Yellowfin Sea Bream to environmental pollutant and undesirable materials.

MATERIAL AND METHOD

***In vivo* Exposure:** According to our past data, from 26 creeks in Mahshahr region (northwest of Persian Gulf) we choose four more pollutant creeks (Jafari, Ghazaleh, Majidieh and Petroshimi) and one less pollutant as control treatment (Zangi). For every creek we choose three station and for every station two yellowfin sea bream with the same size (170 g) and same sexually (all immature male) were caught with hooks and transferred immediately in a fiberglass tank equipped with specific creek water and chargeable aeration (SOBO, China) to the Mariculture Research Station of the South Iranian Aquaculture Research Center, Mahshahr, Iran.

Serum Collecting: Blood was placed in non-heparinized tubes and left to clot at 4°C for 15 min, Afterwards, tubes were centrifuged at 3000 rpm using an Eppendorff centrifuge for 10 min to obtain serum. The serums were separated into aliquots and were frozen and stored at -80 °C until metabolite analyses. All samples were immediately immersed in liquid nitrogen and then transferred to a _80 C freezer until analysis.

Glucose Analysis: The quantitative determination of serum glucose was carried out using commercially available diagnostic Experimental Protocols kits Pars Azmoon, Iran (1 500 0178), at 546 nm and 37°C by the glucose oxidase method according to Trinder [6]. The limit of detection (LOD) of the procedure was 5 mg/dl. Intra-assay and Inter-assay Mean ± SD were 64.2 ± 1.12 and 92.5 ± 1.10 mg/dl, respectively.

Protein Analysis: Serum total protein levels were determined using Pars Azmoon, Iran (1 500 028) kit, with bovine serum albumin serving as standard at 546 nm and 37C. The limit of detection (LOD) of the procedure was 5 mg/dl. Intra-assay and Inter-assay coefficients of variation were of 0.91 and 1.06%, respectively. Intra-assay and Inter-assay Mean ± SD were 5.27 ± 0.05 and 5.24 ± 0.06 g/dl, respectively.

Statistical Analyses: One-way analysis of variance ANOVA with Duncan Post Hoc was used to determine significant differences. The differences between means were analyzed at the 5% probability level (p value of less than 0.05 was considered as statistically significant). Data are reported as means ± standard deviation ($\bar{x}$ ± SD). The software SPSS, version 11.5 (SPSS, Richmond, Virginia, USA) was used.

RESULTS

Five sampling stations (Zangi, Jafari, Ghazaleh, Majidieh and Petroshimi respectively) from Mahshahr coastal waters which were considered important for their commercial and recreational potential were selected to enumerate the presence of mercury and its synergetic action on the liver of A. latus. Station Zangi had lower levels of mercury contamination in all measurements and choose as clean station. Other Stations had higher levels of mercury contamination in all measurements and were choose as infected station. Stations Majidieh and Petroshimi were noticeably close to an area of industrial activities (oil and petrochemistry, respectively) and higher amount were predictable.

Results of *in vivo* biochemical analysis are presented in table 1. Values recorded for activity of total protein show depletion in infected creeks with respect to clean creek, however, there were no significant variations ($P<0.05$). Glucose significantly increased in contaminated creeks with respect to clean creek (Fig 1).

During *in vivo* results, the correlation between mercury with biochemical parameters was statistically tested by analyzing the data obtained during the five sampling creeks. Correlation coefficients between water mercury concentrations and biochemical indices wasn't significant ($P<0.05$), however, both correlation were negative in correlate with water mercury (Table 2). Result of sediment correlation showed that only glucose had significant positive correlate with sediment mercury whereas glucose parameter had negative correlation with sediment mercury (Table 3).

Table 1: *In vivo* biochemical activities of yellowfin seabream exposed to mercury.

	Zangi	Jafari	Ghazaleh	Majidieh	Petroshimi
Glucose (mg/dl)	195±26^{ab}	187±34^{ab}	177±42^{b}	124±26^{c}	223±27^{a}
Total protein (mg/dl)	5.27±0.4^{a}	4.13±0.23^{b}	4.85±0.55^{ab}	4.45±1^{ab}	4.67±0.76^{ab}

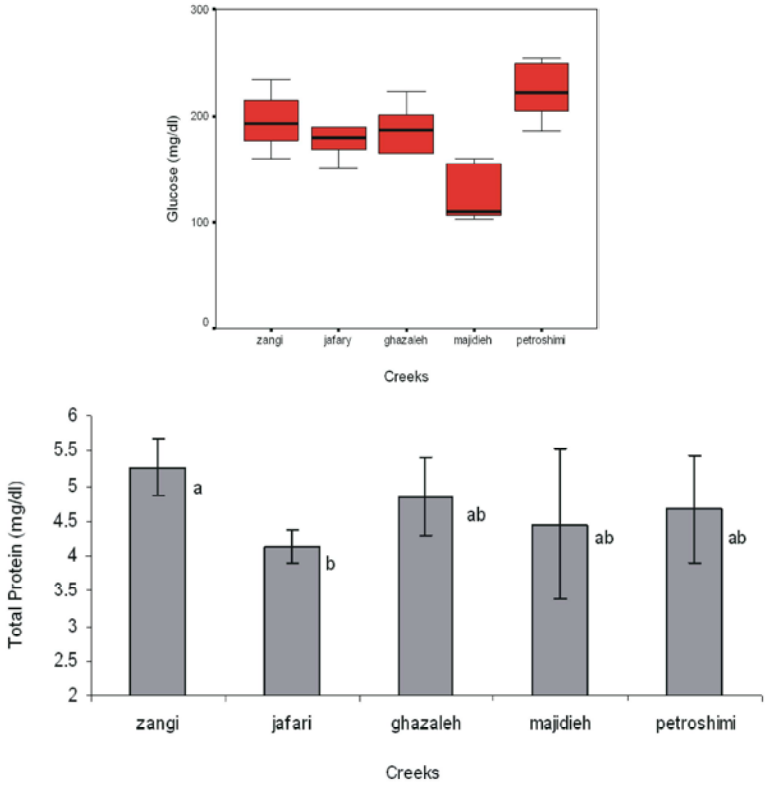

Fig. 1: Biochemical response (Glucose and Total protein) of the yellowfin seabream during in vivo exposed to different concentration of mercury chloride (box plots contain mean and standard deviation for glucose, beside line chart for total protein).

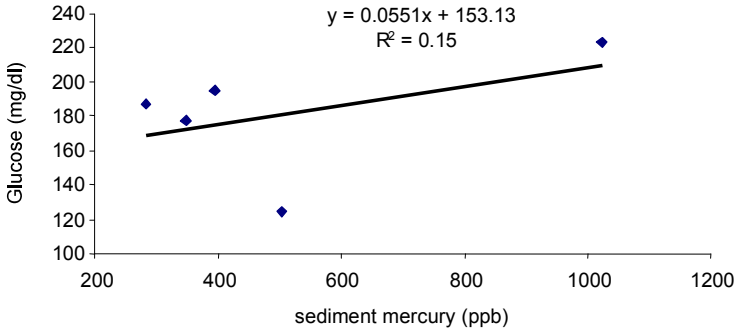

Fig. 2: Regressions model (Y = a ± bX) of Glucose of the yellowfin seabream during in vivo exposed to different concentration of sediment mercury.

In order to evaluate the response of A. latus to mercury concentrations in creeks, a linear model and a sigmoid model were tested and compared. The adjusted correlation coefficients (R2) were calculated between: (a) biochemical and water (Table 4), (b) biochemical and sediments (Table 5), in order to find the model with the best fit to our data. The variation of mercury concentration in the water and sediments was best fitted by a linear model equation, so a linear regression model was found to fit well the relations between the concentrations of mercury in the water and sediment of different creeks.

Table 2: In vivo correlation of enzyme activities of yellowfin seabream with water mercury

	Glucose	Total protein
Pearson correlation (r)	-0.29	-0.19
sig (p)	0.11	0.29

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Table 3: In vivo correlation of enzyme activities of yellowfin seabream with sediment mercury

	Glucose	Total protein
Pearson correlation (r)	0.39*	-0.06
sig (p)	0.03	0.71

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Table 4: In vivo curve fit linear regression of biochemical activities of yellowfin seabream with water mercury

	Glucose	Total protein
R square (r^2)	0.08	0.03
F	2.66	1.12
sig (p)	0.11	0.29

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Table 5: In vivo curve fit linear regression of biochemical activities of yellowfin seabream with sediment mercury.

	Glucose	Total protein
R square (r^2)	0.15	0.00
F	5.01	0.13
sig (p)	0.03*	0.71

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Curve estimation regressions data were used to determine the relationship between water mercury and glucose and protein content in different creeks. Any parameter did not show significant linear regression ($P<0.05$) with water mercury and *R square* wasn't more than 0.08 in biochemical indices.

Curve estimation regressions data were used to determine the relationship between sediment mercury and glucose and protein content in different creeks. Only glucose had statistically significant and protein did not show significant linear regression ($P<0.05$) with sediment mercury. Regressions model $Y = a \pm bX$ of glucose are in Fig 2.

DISCUSSION

Result of our study show that the amount of glucose in polluted creeks was lower than clean creek that means hypoglycemia was evident . Hypoglycemia can result from improper handling of the specimen, malnutrition, malabsorption, severe hepatic disease, endotoxemia and some tumors, in particular, insulinomas and hepatomas. Although the mechanism for this phenomenon is not clear, two possibilities are poor assimilation of the food and alteration of the body's "set-point" for serum glucose. Regardless of the cause, the reduction is probably of little biological importance and is simply a reflection of the overall process that has caused the animals to do poorly [3]

The liver is a glucose-utilizing, glucose-producing and glucose-storing organ. As such, it acts as a glucostat in the vertebrate organism, regulating glucose levels of blood. When glycemia is challenged, the liver elicits adaptive metabolic responses, thereby maintaining a level of blood glucose which is optimal for animal function. Glycemic levels and glucose turnover rates vary among fish species [7].

Hepatic removal of excess glucose from the blood is comparatively inefficient in teleost fish. Hexokinase, the enzyme responsible for glucose phosphorylation and the maintenance of the membrane gradient for glucose, is present at low activities in fish liver, whereas glucokinase that is responsible for increased hepatic glucose phosphorylation during high levels of plasma glucose appears to be absent from fish liver [8].

The conservation of plasma glucose during starvation of fish appears to be achieved by strongly decreased glucose turnover rates [9] and enhanced gluconeogenesis in the liver [10]. Glucose synthesis (gluconeogenesis) in the liver of food-deprived fish utilizes amino acids from protein breakdown or glycerol from lipid catabolism [11].

Some authors reported a weak rise of glucose [12], others found no change [13] and even a decrease [14]. Sometimes no significant changes in plasma glucose may be observed, because under stress the fish is rapidly consuming the energetic substrates generated (glucose) since the main function of the central nervous system (CNS) is to maintain homeostasis.

Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy [15]. In suboptimum or stressful conditions (internal or external) the chromaffin cells

release catecholamine hormones, adrenaline and noradrenaline toward blood circulation [16]. Those stress hormones in conjunction with cortisol mobilize and elevate glucose production in fish through glucogenesis and glycogenolysis pathways [17] to cope with the energy demand produced by the stressor for the "fight of flight" reaction. [18]. Glucose is then released (from liver and muscle) toward blood circulation and enters into cells through the insulin action [19].

Results of our study showed that amount of protein during polluted creeks were lower than clean creek that means occurrence of hypoproteinemia. Hypoproteinemia results from either decreased production or increased loss of protein. In dietary toxicity studies, decreased protein production can result from effects on food consumption, digestion, or absorption. Because of the reserve capacity of the liver, hepatic injury must be fairly severe before protein synthesis is notably diminished. However, in large studies, small differences between the control and treated groups might be apparent with mild to moderate hepatotoxicity [20]. Hypoproteinemia, like anemia, can be masked by dehydration. A small, statistically significant decrease in serum albumin concentration is one of the most frequent findings in toxicology studies. The exact mechanism is usually not apparent but a combination of factors, similar to those causing mildly lower glucose, are probably responsible [3].

Some studies indicated a decrease in total protein content during heavy metal exposure. Such decreases were, for example, found in the edible crab *Scylla serrata* exposed to cadmium or in the common carp exposed to mercury [21]. Depletion in the protein content of the *Catla catla* exposed to mercury chloride sub-lethal concentrations were estimated [22].

The rapid decrease in total protein content was associated with active degradation of proteins under stress. This fact is correlated to the development of resistance toward to toxic stress. Proteins being involved in the architecture and physiology of the cell, they seem to occupy a key role in cell metabolism. Catabolism of proteins makes a major contribution to the total energy production in fishes. Under stress situations may constitute a physiological mechanism with an important role in providing energy to cope with the stress situation. Therefore, depletion of total protein content might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery [23]. When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted [24]. The depletion of total protein content may be due to breakdown of protein into free amino acid under the effect of mercury chloride at the lower exposure period [4]. Reduction in protein content in liver of exposed fish might be due to either arrested metabolism in the liver or to use it to build up new cells or enzymes to reduce the stress [25].

Results of the present investigation showed that pollutants have direct correlation with fish serum biochemical abnormalities and also indicated that the different in environmental condition of marine ecosystems may cause several changes in the serum biochemical parameters of the studied fish.

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