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Effect of Salt Stress on ALT and AST Enzymes Activity and Cortisol Level in Adults of *Carassius auratus*

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Abstract: The gradual increment of salinity from 0.1g/l (control) to 4, 8 and 12g/l dealt with stress in adult goldfish *Carassius auratus* through determination of ALT and AST enzymes activity and cortisol concentration in blood plasma. ALT activity was increased to 56.42, 65.16 and 76.47 IU/l in 4, 8 and 12 g/l salt concentrations respectively, compared with tap water 0.1g/l (control) (48.64 IU/l). AST enzyme activity was also increased to 76.43, 97.58 and 114.48 IU/l in 4, 8 and 12g/l salt concentrations respectively, in comparison with control (54.08 IU/l). Cortisol concentrations in goldfish blood plasma were increased to 0.92, 1.84 and 2.86g/100ml when the salinity raised to 4, 8 and 12g/l, respectively, compared with control (0.73g/100ml). Fish were fed a test diet (31.30% crude protein) during the experiment.

Key words: Salt stress, ALT and AST enzymes, cortisol hormone, goldfish

INTRODUCTION

Living organisms, especially aquatic animals are facing many types of stress under natural and artificial conditions. Fish are sensitive to stress in their natural environment which are resulting from changing in abiotic environmental factors such as temperature, salinity and pollution; or biotic factors such as food and space competition, predation, migration and reproduction, whereas in laboratory (artificial environment) stress sources would be transportation, crowding and vaccinations (Iwama *et al*., 2006). Any stress factor may create an internal physiological imbalance in fish appears through disorder hormones and enzymes functions and changing in some blood picture characteristics, which need systematic physiological response by fish against stressors returning to homeostasis (Eddy, 2006).

Many physiological changes occur when the stenohaline freshwater fish are exposing to the salt stress, those are depending on the salinity levels range, time of exposure to salinity changes and the effects of other 2 ecological factors (Bailey *et al*., 2002) which leads to increase energy demands in order to maintain the internal balance (Evans, 2002).

The increment of Alanine transaminase (ALT) and Aspartate transaminase (AST) levels occurs during nervous shock, hypoxia and stress where their plasma standard levels would be affected by several factors such as pollution, ammonia and nitrite toxications and other environmental parameters like temperature and salinity (Das *et al*., 2004).

Rainbow trout plasma ALT concentration ranges between 17 and 136 IU/l, whereas plasma AST concentration ranges from 51- 445 IU/l (Gaudet *et al*., 1975).

Cortisol is one of the important fish hormones responsible in increasing the salinity tolerance during freshwater fish transferring to seawater through its effect on reducing body fluid concentration (hypo-osmotic) (Morgan and Iwama, 1991). Cortisol, regulates body fluid electrolytes of Teleosts through its direct effect on cellular membrane permeability, increases number and size of mitochondria and activating ATPase levels which control salt motion in gill chloride cells of teleosts (Uchida *et al*., 1997). The aim of this study is to investigate the stress effect of gradual salinity increase on the activity and concentrations of plasma ALT and AST levels and cortisol in goldfish *(Carassius auratus*).

MATERIALS AND METHODS

Acclimatization: Fish were transported from local fish farm located at southern part of Baghdad, Iraq. Fish were set randomly in 12 glass aquaria (capacity 60l) filled with dechlorinated tap-water (salinity = 0.1gm/l) at 8 individuals per aquarium and acclimated to experimental diet (Table 1) for two weeks before starting the trail. Initial mean weight of fish was 42.0+1.48 gm. Dried salt was brought from Fao city, south of Iraq and a known weight was added each 24 hours chronically at a rate of 2gm salt/l to the dechlorinated tap-water of experimental aquaria except the control treatment in order to adapt fish gradually to salt concentrations treatments with three replicates which were 4, 8 and 12gm/l. Experimental fish were fed the diet contained 31.3% crude protein at 3% of fish body weight for 14 days (Table 1). Aquaria were supplied with air pumps and 1/43 of their water has been exchanged daily with a constant salinity to each treatment. Dissolved oxygen concentration, temperature and pH of aquaria water were measured during the experiment period.

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Table 1: Food constituents ratio used in diet structure

Constituent	Fish meal	Soya bean	Wheat bran	Yellow corn	Local barley	Corn oils	Vitamins and minerals
Percentage	25	35	25	4	6	2	3 %

ALT, AST and cortisol activity determinations: ALT plasma enzyme activity was determined by using ALT kit supplied by Randox (French company) depending on the concentration of pyruvate hydrazone in 2, 4-dinitrophenyl-hydrazone formula. AST plasma enzyme activity was determined by using AST-kit depending on the concentration of oxaloacetate hydrazone in 2, 4-dinitrophenyl-hydrazine. Absorbance of ALT and AST were measured by spectrophotometer at 546nm wave length.

Cortisol of blood plasma level was measured by radioimmunoassay (RIA) using a manual kit (Cortisol RIA kit-IM1841). Experiment run under Completely Randomized Design (CRD), data were statistically analyzed and mean significant differences compared at 0.05% probability (Duncan, 1955).

RESULTS AND DISCUSSION

Water temperature of experimental aquaria ranged between 24°C to 26°C, dissolved oxygen 6.2mg/l to 6.8 mg/l and pH between 7.0 to 7.4, where their values were within normal levels of goldfish living (Hattingh *et al*., 1975) plasma ALT and AST concentrations increased together with gradual increase in salinity as shown in Table 2, with a significant differences among means of treatments 4.

Plasma ALT and AST concentrations are an obvious indicator of salinity exposure stress on goldfish comparably to natural environment, however, AST activity was increased more than that of ALT, where Brett (2009) reported that the AST activity increase in the cytoplasma and then ALT activity increase in mitochondria and cytoplasma since fish exposed to stress, because fish requires to compensate the destructed body cells as a result of increase in metabolic rates. Therefore, when the fish faced a liver tissue destruction, ALT and AST concentrations would increase because of their role in an initial amino acids compensation and formation that the body needs them, physiological changes are an increase in energy demand or malnutrition (Ebeid *et al*., 2005). Results of many literatures agree with the significant ALT and AST activity increments of this study, where Salaei (2006) reported that plasma AST and ALT activity increased in *Cyprinus carpio* during catching process from earthen pond comparably with those reared in aquaria and he believed this increment was due to catching stress which elevate the internal oxidizing effort towards membrane permeability that increase the fluxes of ALT and AST enzymes into the blood stream (Bahjat and Shaban, 1985). Roche *et al*. (1989) reported that an increase in transaminases activities in seabass (*Dicentrarchus labrax*) acclimatized to salinity (5g/l). Transferring of Tilapias from freshwater to seawater for two weeks leads to a significant increase in AST and ALT activities in liver because of increase in liver protein destruction and energy demand (Vijayan *et al*., 1996).

Table 2: ALT and AST activities (IU/l) in blood plasma of goldfish in different salt concentrations (mean+standard error)*

Salt concentration (g/l)	ALT (IU/l)	AST (IU/l)
0.1	48.64±0.54 d	54.08±0.38d
4	56.42±0.42 c	76.43±0.56 c
8	65.16±0.34 b	97.58±0.68 b
12	76.47±0.55 a	114.48±0.87 a

*Values with different letters within a column are significantly different from each other (Pb 0.05).

Table 3: Cortisol hormone levels (µg/l) of goldfish plasma in different salt concentrations (mean±standard error)*

Salt concentrations (g/l)	Cortisol hormone levels (g/l)
0.1	0.73±0.28 c
4	0.92±0.38 c
8	1.84±0.42 b
12	2.86±0.62 a

*Values with different letters within a column are significantly different from each other (Pb 0.05).

Sultan (2007) reported that plasma ALT and AST concentration of *Acanthopagrus latus* juveniles in 3g/l salt solution were 3.34 IU/l and 38.3 IU/l, respectively and these concentrations were increased to 7.43 IU/l and 68.28 IU/l when fish exposed to 23g/l salt solution and then increased to 8.70 and 74.79 IU/l when transferred to 30g/l salt solution, respectively. The investigator returned the reasons of these ALT and AST activities elevations to the infiltration of the enzymes to the blood stream.

Table 3, values show the gradual increase in cortisol hormone levels in blood plasma of goldfish (0.92, 1.84 and 2.86g/l) with gradual increase of salt concentrations (4, 8 and 12g/l) in comparison with control 0.1g/l salt (0.73g/l). No significant differences appeared in cortisol levels between control and second treatment (4g/l), whereas it is quite clear ($P<0.05$) between control and T3 (8g/l) and T4 (12g/l) and among treatments.

An increase of cortisol hormone concentrations in blood plasma of freshwater fish when exposed to gradual increase in salinity clarify fish adaptation towards salted water. The hormone decreases the body fluids concentration of freshwater fish in order to osmoregulate the physiological responses which locate under hormonal control (McCormick, 1995).

Similarly many literatures noted that cortisol hormone combined with freshwater fish during their acclimatization to seawater and thus increase salinity tolerance of fish (Deane *et al*., 2002). Cortisol hormone plays a conspicuous role in increasing number and size of gill chloride cells which are responsible to gill ion exchanges Na+/K+ATPase activity and increase size and number of chloride cells mitochondria (Uchida *et al*., 1997). Shirashi *et al*. (1997) explained the companion of the evolution of chloride cells and elevation of cortisol hormone levels in embryonic and larval growth stages of tilapia *(Oreochromis mossambicus*) during fish transferring to seawater. Madsen and Bern (1992) declared an increment in size and number of chloride cells and Na+/K+ATPase activity levels in salmonids when they have injected with cortisol hormone. It has been noted that decrease in thyroxine levels and an elevation of cortisol levels in plasma, beside an increment of chloride cells numbers and gills Na+/K+ATPase activity in salmonids species elevers during their migration to the sea, because of thyroxine might activated the cortisol receptors in gills (Ayson *et al*., 1995).

Cortisol plays an important role in increasing ions uptake and it acts with prolactin hormones during acclimatization to freshwater (Pelis and McCormick, 2001), whereas the hormone itself shares growth hormone in increasing fish capability to salinity stress (Hyde *et al*., 2004).

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