

Stress Response of *Carassius auratus* to Salt and Formaldehyde Exposure

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Abstract: The aim of the present work was to evaluate the effect of formaldehyde or salt exposure on stress response in *Carassius auratus*. Fish (90.3 ± 3.1 g) were subjected to either formaldehyde (250 ppm) or salt (10 ppt), over a 0.5-h period. Then they were placed in freshwater for 24-h to recover and serum cortisol and glucose levels were monitored at 0, 0.5, 3 and 24 h post exposure. Both formaldehyde and salt caused rapid increase in cortisol with the peak at 3 h, which stayed elevated until 24 h. Glucose showed similar patterns, however, returned to initial levels at 24 h. It is suggested that formaldehyde and salt at therapeutic concentrations cause rapid stress response in *C. auratus* which is eliminated after 24 h in freshwater, however, the fish might become stress responsiveness which show cortisol elevation in response to sampling.

Abbreviations: Formaldehyde-treated (FT) % Salt-treated (ST)

Key words: Blood Biochemistry % Glucose % Cortisol % Salt % Formaldehyde

INTRODUCTION

Carassius auratus is one of the most common ornamental fish in Iran. It is one of the fundamentals of ancient Nowruz Holiday and is sold by ornamental fish seller in large amount, near the Nowruz. Breeding and rearing of this species is performed by many local people in some regions of Iran [1].

Ecto-parasites are common in fish culture activities, including *C. auratus* culture, causing huge economic loss. Certain chemical compounds are used as therapeutic agents to control ecto-parasites infection. Formaldehyde and Salt are two effective therapeutics in ecto-parasites control. It was suggested that concentrations of 250 ppm and 10 ppt, over a 30-min period, is effective for removing ecto-parasites in fish [2].

Although therapeutics have the health benefit for fish, they might cause adverse effects on fish, as well. For instance, formaldehyde is a reducing agent forming methylene cross-link in proteins [3]. Likewise, pathological symptoms in fish gill are the widely-recognized consequences following formaldehyde treatment [4]. On the other hand, Salt exposure causes osmotic disturbance in freshwater fish which causes stress response. There are some studies evaluating the effect of formaldehyde treatment in certain fish species [4-7]. Effect of ambient Salt exposure has been described

in details for some freshwater fish by previous researches [8, 9, 10]. However, no study was conducted of *C. auratus* to evaluate the effect of therapeutic dose of formaldehyde and salt on stress response and serum characteristics. Thus, in the present work, adult *C. auratus* were subjected to 250 ppm formaldehyde or 10 ppt salt over a 30-min period [2] and changes in their serum characteristics were monitored.

MATERIALS AND METHODS

Fish and Maintenance Conditions: A total of 90 fish (90.5 ± 5.1 g) were randomly distributed in 9 glass aquaria ($1 \times 0.4 \times 0.5$ m) filled with 200 L well water. Fish were fed twice a day based on 2% of body weight. All aquaria were aerated continuously and total of 90% of the water was exchanged daily. Fish were maintained under these conditions for 1 week.

Treatment Test: One fish were captured from each aquarium (three samples per treatment) and blood-sampled. After sampling, the aquariums assigned as three triplicate groups: control, Formaldehyde treatment (FT) and Salt treatment (ST). FT and ST groups were exposed to 250 ppm formaldehyde or 10 g L^{-1} salt (Sodium Chloride) over a 0.5 h period (short-term treatment, [2]), respectively, whereas control remained untreated.

After 0.5 h, second blood samples were collected from all groups. Then, the remaining fish from all groups were gently captured and transferred to 9 new aquaria filled with clean and aerated water (3 aquaria per/ group). 3 and 24 h after exposure, further blood samples were collected from all groups.

Sampling and Analyses: Fish were anesthetized using 100 ppm MS-222 over less than 1 min. Thereafter blood samples were collected by caudal severance. Blood samples were poured in non-heparinized plastic tubes and centrifuged (5000 rpm for 6 min, 10 °C) to attain serum. All serum samples were stored at -20°C until further analyses. Sera were analyzed for cortisol using ELISA method [11] by commercial kite (IBL, Gesellschaft für Immunchemieund Immunbiologie, Germany). Glucose was determined according to Thomas (1998) using commercial available kits (Pars Azmun Co. Ltd, Tehran, Iran).

Statistical Analyses: All data were checked for normality and homogeneity of variances using Shapiro-Wilk's and Levene's test, respectively. Accordingly, cortisol and glucose values transformed to logarithmic scale before any analyses. All data were subjected to 2 way ANOVA test with treatment (control, FT and ST) and time (0, 0.5, 3 and 24 h) as factors. Significant difference was detected by Duncan test. Data are presented as treatment mean \pm SD. The values of $P < 0.05$ were considered significantly different.

RESULTS

Cortisol levels were significantly ($P < 0.0001$) affected by treatment, sampling point and interaction (Table 1). In the control group, cortisol slightly but significantly increased (~ two folds) at 0.5 h compared to 0 h, however, there was no significant difference between 0, 3 and 24 h (Table 1). In both FT and ST groups, cortisol showed significant elevation (~ 20 folds) at 0.5 h compared to 0 h and stayed elevated at 3 and 24 h, despite the significant decrease compared to 0.5 h (Table 1). Both FT and ST groups, showed significantly higher cortisol compared to control at 0.5, 3 and 24 h, but not 0 h (Table 1).

There was no significant difference in glucose values of control group at any sampling point (Table 1). Both FT and ST groups showed significant elevation in glucose levels at 0.5 and 3 h which returned to 0 h levels at 24 h. There was no significant difference in 0 and 24 h values between the studied groups. However, FT group showed

Table 1: Changes in serum cortisol and glucose after formaldehyde and salt exposure.

| | Time after exposure (h) | | | |
|----------|-------------------------|------------------|------------------|-------------------|
| | 0 | 0.5 | 3 | 24 |
| Cortisol | | | | |
| Control | 5.2 \pm 1.2 ab | 9.1 \pm 2.5 b | 9.5 \pm 3.6 b | 5 \pm 1.9 a |
| FT | 4.8 \pm 0.4 a | 210 \pm 32 d | 75 \pm 12.5 c | 59.3 \pm 16.6 c |
| ST | 5 \pm 1 a | 175.5 \pm 31 d | 75.2 \pm 12 c | 65.1 \pm 6 c |
| Glucose | | | | |
| Control | 24.9 \pm 3.2 a | 28.6 \pm 2.4 a | 28.2 \pm 3.5 a | 25.1 \pm 2.5 a |
| FT | 26 \pm 2.2 a | 102 \pm 18 c | 122 \pm 20 c | 26.1 \pm 1.9 a |
| ST | 25.5 \pm 2.5 a | 40.8 \pm 2.8 b | 65.9 \pm 3.6 b | 26.5 \pm 3 a |

Different letter in front of the values show significant difference ($P < 0.05$)

significantly higher glucose levels at 0.5 and 3 h compared to control, while, ST group showed significantly higher levels only at 3 h.

DISCUSSION

Fish exposed to chemicals can manifest a stress response and blood biochemical changes [1]. Cortisol has been known as a primary stress response which increases rapidly after stress and circulating level of cortisol is commonly used as an indicator of the degree of stress experienced by fish [12, 13]. Hyperglycemia is secondary stress response, which is stimulated by primary stress responses (release of catecholamines and corticosteroids) to supply demanded energy to cope stress [12, 13]. Formaldehyde found to damage gill tissue [4, 14] which could cause respiratory distress. Present results demonstrated that formaldehyde and Salt exposure over 0.5 h was stressful for *C. auratus*, since near 20 folds increase in circulating levels of cortisol was observed after treatment (0.5 h).

However, it seems that FT group experienced severe stress than ST, since showed higher glucose levels than ST group at 0.5 and 3 h. Likewise, results showed that FT and ST groups did not recover from stress at 3 h, as instead decrease in cortisol levels in both groups at this point, it did not reach the pre treatment levels. On the other hand, glucose levels remained still high, particularly FT group which showed peak of glucose. However, a higher cortisol level at 24 h in both FT and ST groups seems not to be attributed to treatments, since glucose levels reached pre treatment levels at this point. Glucose levels remain higher during stress [12, 13] to ensure energy supply to cope stress. On the other hand, it was found that glucose levels remained elevated for a while even after stress termination [11, 15]. Higher cortisol levels in FT and ST groups at 24 h might suggest fish became stress responsiveness as a result of formaldehyde

and salt treatments resulting stress response due to capture and blood sampling. Present results are in agreement with the previous works [5, 16, 17] which showed stress response as a result of formaldehyde treatment in rainbow trout *O. mykiss* (Walbaum) and common carp *Cyprinus carpio* L.

It is concluded that formaldehyde and salt treatment at therapeutic concentrations caused rapid stress response in *C. auratus* which was eliminated after 24 h recovery in freshwater; however, treated fish might become stress responsiveness at this point. Likewise, since formaldehyde causes more stress response (glucose levels), salt treatment is suggested as alternative.

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