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Effect of Citalopram on Reducing Transportation Stress in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

It is often necessary to transfer live fish between aquaculture facilities to permit on-growing or fishing, handling and the physical disturbances associated with loading, transport, and discharge could be regarded as the potential to cause distress and injury, and then leading to possible long term health impairment. Fish in different ways keep its stability (homeostasis) after stress responds. Stress response is involved in many physiological changes. One of the main reactions to stress is increasing the plasma cortisol level. Purpose of this study was to examine the effect of a selective serotonin reuptake inhibitor (citalopram) on plasma cortisol, and handling stress of rainbow trout. Immature rainbow trout (*Oncorhynchus mykiss*) with an average of 50 ± 7 g weight set in three treatments, control, acute (5 mg/l for 48 hours) and chronic (5 µg/l for 10 days). Plasma cortisol before transport in the control group was 22.11 ± 5.33 (ng/ml), chronic dose was 15.99 ± 5.85 (ng/ml) and in the acute treatment was 18.81 ± 7.42 (ng/ml). Mean plasma cortisol after transportation in the control, acute and chronic treatment respectively was 286.01 ± 54.26, 107.12 ± 25.53 and 239.89 ± 57.56 ng/ml. Based on the results, significant difference observed ($p<0.05$) between mean plasma cortisol before and after transportation in chronic treatment. Also in acute dose, there was a significant difference in mean plasma cortisol before and after transportation ($p<0.05$). As a conclusion it can be expressed that, chronic treatment was more effective than acute treatment in reducing handling stress in rainbow trout.

Keywords: Citalopram; Stress; Transport stress; Rainbow trout

Introduction

During transport, significant changes in water quality may occur and these may have numerous adverse effects on the vital physiological processes of fishes [1]. When fish are continuously exposed to management practices such as handling, transportation or confinement, which can elicit stress responses in intensive rearing facilities [2]. It was shown that fishes respond in different ways to maintain homeostasis after stress [3]. Many physiological changes are involved in such a stress response including hematology [4] osmoregulation [5], hormone release, and energetic metabolism [6,7].

Increasing plasma levels of cortisol as one of the most accepted primary response to stress has been previously reported [7-9]. Air exposure produced a 50-fold increase in plasma cortisol concentrations in gilthead sea bream (*Sparus aurata*) [10]. Two days of confinement procedures increased three-fold plasma cortisol in gilthead sea bream, *S. aurata* [11]. In another study turbot, (*Scopthalmus maximus*), net confinement procedures provoked a 10-fold increase in plasma cortisol [12]. The stress response is highly comparable in all vertebrates, and the secretion of both the glucocorticoid hormone corticosterone and cortisol is the main component [13]. In fish, the secretion of cortisol is mainly controlled by the hypothalamus-pituitary-interrenal (HPI) axis [14]. Corticosteroids affect behavior through genomic (slow) and non-genomic (fast) mechanisms in the central nervous system [15,16]. In mammals the immediate increase in corticosteroids leads to escalated aggression propagated by a fast feed forward mechanism [17]. Moreover, regulation of glucocorticoid levels is influenced by behavior [18]. Sloman et al. [19] observed that the basal levels of circulating cortisol in rainbow trout (*Oncorhynchus mykiss*) prior to social interaction were associated with the outcome of fights for social dominance. Schjolden et al. [18] perceived that there is relevance between aggressive behavior and cortisol content. Adding anesthetic and hypnotic drugs to the transport tank water may be significantly useful to reduce the physiological stress associated with handling [20]. Drugs are primarily used to slow down metabolism, thus this will lead to reducing oxygen consumption and ammonia and carbon dioxide production. Furthermore they will mitigate the stress response caused by excitement and handling, and reduce swimming [21,22]. Serotonin is an important neurotransmitter in the regulation of social interactions in many animals. Correlative studies in numerous vertebrate species, including fish, indicated that, aggressive males have lower relative serotonergic activity than less aggressive males [23]. In the last decade, it was established that selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine and sertraline could be effective tools for chronic elevating serotonin activity. In recent studies the mentioned drugs are used to study the role of serotonin in aggressive behavior [24, 25]. The relationship between the brain serotonergic systems, social status, and aggression has been established through a large number of correlative and experimental studies [26-28]. Thus, the aim of this study is to examine the effects of citalopram (a selective serotonin reuptake inhibitor) that caused reduced aggression in many species, on stress and plasma cortisol levels in rainbow trout.

Materials and Methods

Immature rainbow trout (*Oncorhynchus mykiss*) with 50 ± 7 g body weight were obtained from a fish farm in Iran (Golestan, Zarringol

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Farm). Then they were maintained in small raceways in the freshwater open system (not recirculating system). Experiments were conducted from July to August of 2011.

The fish randomly selected and placed in three separate tanks (200 L) and adaptation was carried out for one week. Fish were fed once a day with a commercial diet until 24 h before the experiments. Physical and chemical water parameters were measured at the beginning and during the experiments. Water temperature was 16°C, pH was about 7.2 and water hardness was 145-155 (DH).

The fish was set in three doses of citalopram including acute (5 mg/l for 48 hours), chronic (5 µg/l for 10 days) and control treatment. We set 45 fish for each treatment, due to a wide individual difference in fish, each blood sample collected from 3 fishes and then mixed together. For inducing stress to fish, individuals were netted and carried (air exposure 10-15 s (to a transport tanks (40l vol). Fish were transported for 2 hours with a small truck with constant oxygenation. After anesthesia with clove oil blood sample were immediately taken (with 1-ml heparinized syringes) from each fish without any anticoagulant material, The blood collection lasted less than 3 min in order to avoid the cortisol rise induced by the manipulation during sampling [29]. The extracted blood was left to clot at 4°C and centrifuged at 6000 rpm for 5 min at room temperature. The collected serum was stored at 80°C for cortisol assay and then samples were transported to laboratory for plasma cortisol measurements.

Statistical tests were done by use of a statistical program, SPSS 15.0 for Windows. A one-way ANOVA test was thereafter performed at each sampling time to test for differences among the groups. Data analysis performed with p-value=0.05.

Results

Mean plasma cortisol levels in the control group before transport was 22.11 ± 5.33 ng/ml and after the transport was 286.01 ± 54.26 ng/ml (Figure 1). In the acute dose mean plasma cortisol was 18.81 ± 7.42 ng/ml and after transport was 107.12 ± 25.53 ng/ml (Figure 2). Mean plasma cortisol in chronic treatment was 15.99 ± 5.85 ng/ml, before transport was 239.89 ± 57.56 ng/ml (Figure 3). Based on the results, the difference between mean plasma cortisol in chronic dose before and after the transport was significant ($p<0.05$). Also, there were significant differences ($p<0.05$) between the mean plasma cortisol before and after transportation in acute treatment (Figure 4).

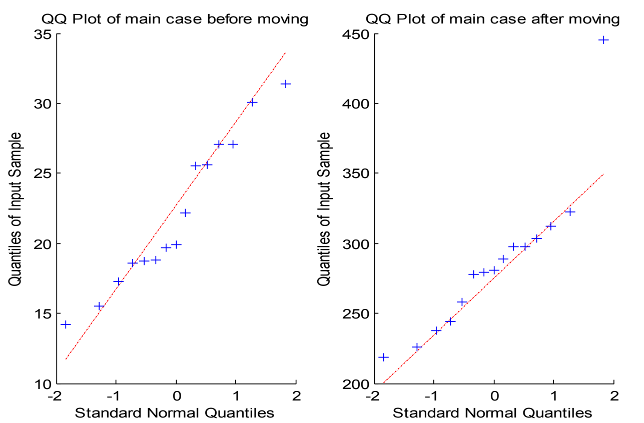

Figure 1: Overall relationship between plasma cortisol before and after moving in control group. Each point represents value per 3fish blood sample.

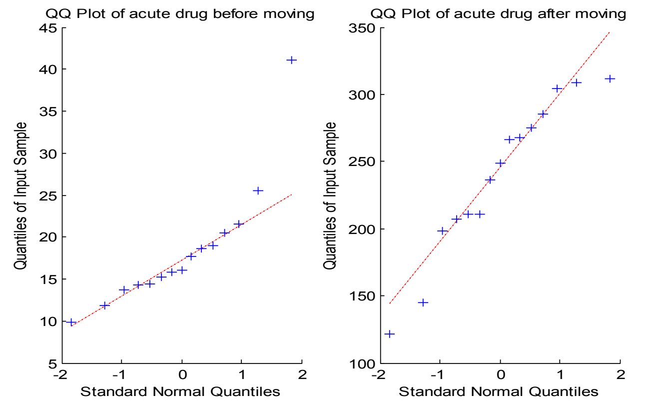

Figure 2: Overall relationship between plasma cortisol before and after moving in acute group. Each point represents value per 3fish blood sample.

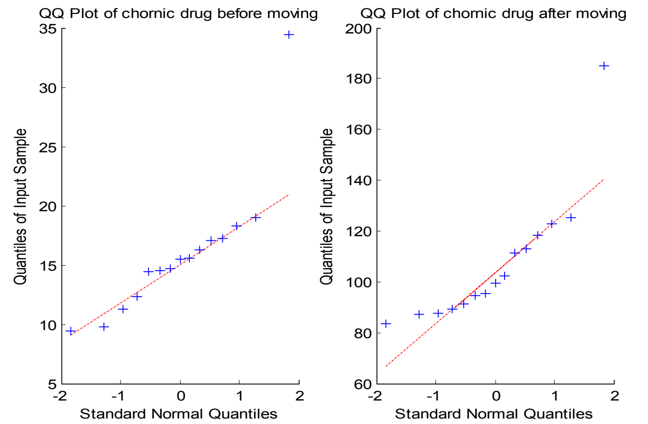

Figure 3: Overall relationship between plasma cortisol before and after moving in chronic group. Each point represents value per 3fish blood sample.

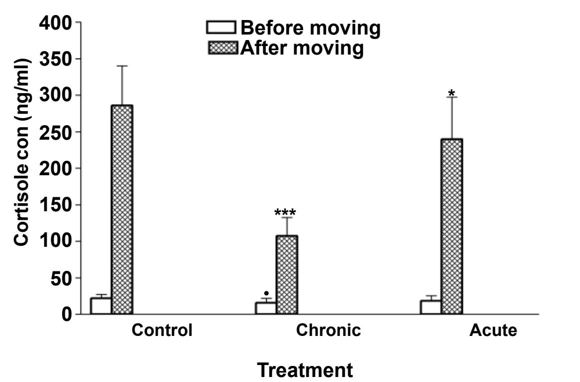

Figure 4: Comparison between all treatments.

Significant differences between treatments at the same time point are denoted by asterisk (*): *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Discussion

The relationship between brain serotonergic systems, social status, and aggression has been established through a large number of correlative and experimental researches or studies [23]; on the other hand, in other researches (e.g. Schjolden et al. [18]) the relevance between stress and aggression has been demonstrated. The results showed that artificial modification of the serotonin, which is an important neurotransmitter, can affect the stress behavior in rainbow trout. Because this drug is absorbed through the fish gills and its absorption is depending on temperature and fish metabolism, (e.g. in lower temperatures drug absorption is less), and because in the prolonged duration of exposure in chronic dose, the bioavailability of drug in the fish body will increase. As a conclusion it can be expressed that, chronic treatment was more effective than acute treatment in reducing handling stress in rainbow trout. On the other hand, various drug onset times may be the reason for variant results in the experiment. Notably, this study did not survey other effects of citalopram on various biological factors of (fish); therefore more research should be carried out in order to make perfect this study perfect.

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