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The effect of hydrocortisone treatment by bathing and daphnia enrichment on the salinity stress in Persian sturgeon *Acipenser persicus* juvenile

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Abstract

This study was investigated the effect of corticosteroid hormone treatments (hydrocortisone sodium phosphate) on induced salinity stress in Persian sturgeon (*Acipenser persicus*) juveniles (2 ± 0.6 g). The experiment was conducted using a 2 × 3 factorial experimental design with 2 hormonal treatment methods (daphnia enrichment and hormonal bathing) and three hormonal concentrations (3, 5, and 7 ppm). After the hormonal treatment, juvenile fish were encountered to salinity stress (7 ppt) for 24 h. Then blood cortisol and glucose levels, hematocrit value and mortality rate were measured. The hematocrit value was significantly ($P < 0.05$) higher in fish treated with the enrichment method. However, cortisol and glucose levels and mortality rate were similarly affected by two treatment methods. Increased hormonal concentration significantly lowered the glucose level, while this phenomenon led to a significant increase in the hematocrit value ($P < 0.05$). An interaction observed between hormonal treatment method and hormonal concentration for glucose, hematocrit and mortality indices ($P < 0.05$). After inducing the stress, the mortality rate was significantly lower ($P < 0.05$) in juveniles treated with daphnia enrichment method. Treatment 3 (daphnia enrichment with 7 ppm hormanoal concentration) led to a significantly higher ($P < 0.05$) blood cortisol level and hematocrit value after the hormonal treatment and just before the stress. Meanwhile, no mortality was observed in treatment 3. The results showed that treatment 3 was the best treating method for lowering the salinity stress in juveniles.

Keywords: Salinity stress, Hydrocortisone, Daphnia enrichment, Hormonal bathing, Persian sturgeon

Introduction

Persian sturgeon *Acipenser persicus* is one of the endemic fish species of Caspian sea. This species migrates into the Caspian rivers such as Volga and Sefidrood over March-June for spawning (Vosoughi and Mostajir 2003). Each year since early March, the broodstocks are captured from the sea and transferred to propagation centers. Following breeding and nursery periods, the juveniles are released in the Caspian rivers near the costal area. Meanwhile, the salinity in estuaries waters is higher than the freshwaters in which juveniles are produced in. This can lead to higher mortality due to the osmotic stress (Abdollahi and Baradaran Tahori 2005; Kazemi et al. 2003). It is clear that employing any strategy to reduce the level of stress could be useful in terms of ecological and economical aspects.

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Generally, there are some methods to reduce the osmotic stress. Each fish species shows a special adaptation response to stress (Evans and Claiborne 2005). Stress is caused by a stimulating hormone which eventually produces corticosteroid hormones such as cortisol (Adams 1993; Evans and Claiborne 2005). This hormone causes some biological changes in different body tissues and prepares the body to deal with unfavorable conditions. Blood cortisol level rises in stressful conditions in fish (Evans and Claiborne 2005). Other signs of stress include increased hematocrit value and heart rate, changes in chloride and sodium ion levels in blood and the elevation of blood glucose. Rising cortisol level affect the general physiology of fish and causes various responses in different tissues (Evans and Claiborne 2005). Given these issues, it can be concluded that corticosteroids hormones prepare fish to deal with unfavorable conditions such as osmotic stress. It seems that the hormonal treatments with corticosteroid hormones may make the fish ready to deal with stress. This means that increased cortisol levels using hormonal treatments may prepare the fish to face with stress.

There are different ways of hormonal treatments by corticosteroids including bathing and injections which are mostly used in large fish and is especially suitable for broodstocks (Madsen and Korsgaard 2004). Stewart et al. (2001) showed that artemia enrichment is an effective way to deal with stress induced by steroids in fish. Essential fatty acids (Sui et al. 2007), various enrichment media (Seoka et al. 2007) and different storage conditions (Davis et al. 2000) have been used to study the effect of hormonal treatment via enrichment. Meanwhile, the effect of using protein and steroids for reducing the salinity stress have already been investigated (Harel et al. 2001).

Applying a suitable method for hormonal treatments has not yet been explained in fish. In this study, the effects of corticosteroid (hydrocortisone sodium phosphate) treatment on osmotic stress reduction have been investigated. The aim of this study was to evaluate the effect of two hormonal treatment methods (daphnia enrichment or hormonal bathing) and three concentrations of corticosteroid on Persian sturgeon (*Acipenser persicus*) juveniles dealt with induced salinity stress.

Materials and methods

Fish Juvenile

Research was conducted using a 2 × 3 factorial experimental design (Table 1). Juvenile (2 ± 0.6 g) of Persian sturgeon (*A. Persicus*) was obtained from the Shahid Beheshti Breeding Center (Rasht, Iran). Fish were then transferred to the University of Tehran and kept in a recirculating system with the average temperature of 19 °C and the minimum oxygen level of 6 ppm and a daily water change of 30%. After 72 h adaptation period, fish were transferred to the experimental tanks.

Daphnia

Daphnia magna were fed with the emulsion of sheep manure with the daily rate of 10 ml per liter (the emulsion were prepared by adding 1 kg of clean sheep manure in 4 liter fresh water and then filtered) in a 400 liter tank. The temperature varied between 24 to 27 °C and the photoperiod was set at 14 h light/10 h dark. Rearing tanks were well aerated over the culture period (with a dissolved oxygen rate of 5 ± 0.50 mg/liter). After blooming of daphnia in the rearing tanks, 12 polyethylene containers (1.5 liter) were used for enrichment procedure. Then 50 adult daphnia were cultured in each well aerated enrichment container for 24 h. In order to do so, 10 ml of manure emulsion was mixed with the total amount of hormone (Hydro cortisone sodium phosphate/ MERC) needed for the final enrichment concentrations. The daphnia were fed with the enrichment media four times per day within a six hours intervals. Unenriched daphnia were only fed with sheep manure emulsion, without hormonal treatment.

Treating fingerlings with hormonal treatments

At the first series of tanks, bathing method was employed using three concentrations (3, 5 and 7 ppm) of resolved hydrocortisone hormone. In the second series of tanks, enriched daphnia with 3 enrichment hormone concentration of (3, 5 and 7 ppm) were employed. Then fish were daily fed by enriched daphnia up to 1.5-2% of their body weight. At the same time in the bath treatment tanks, unenriched daphnia were used to feed the fish with the same rate explained for the enriched daphnia treatment. At the end, the treated fish faced salinity stress (with concentration of 7 ppt) for 24 h and then the physiological responses and mortality rate of fish were measured.

Table 1. Different treatments employed in the study

Treatment	Treatment method	Hormonal concentration (ppm)
1	Daphnia enrichment	3
2	Daphnia enrichment	5
3	Daphnia enrichment	7
4	Hormonal bathing	3
5	Hormonal bathing	5
6	Hormonal bathing	7

Sampling procedure

Three fish per tank were anesthetized using clove oil (1 ml diluted in 40 liters of water). Blood samples were taken via tail fin and a drop of EDTA solution was added to each sample to prevent coagulation. Blood sampling was taken as follows: before transferring fish to the experiment tanks, right after the hormonal treatments, 30 min after salinity stress, 60 min after salinity stress, and at the end of the experiment (24 h after the salinity stress). The mortality rate of fish was evaluated: exactly after stress, 12 h after stress, and 24 h after stress.

Biochemical indices

Blood samples were centrifuged (3000 rpm, 20 minutes) to prepare serum samples. ELISA method (Diagnostics Biochem Canada, Ontario, sensitivity: 0.4 μg/dl kits) was used to determine the cortisol level in the samples. The glucose concentration was quantified using an electronic device (Digital Glucometri Method). The hematocrit value was determined using the capillary tubes.

Statistical analysis

Data were analyzed in two stages. At the first stage the effects of stress inducing method and hormonal concentrations was separately investigated and then the possible interactions between two main factors (type of hormonal treatment and hormonal concentration) were studied using two-way ANOVA. Duncan's test was employed to compare significant differences among treatments ($P < 0.05$). In the second stage, each combination of hormonal treatment and hormonal concentration were considered as an individual treatment and then their mean were statistically considered through employing one-way ANOVA. SAS statistical program (version 9.0) was used for statistical analysis and EXCELL software was employed for making the graphs ($P < 0.05$).

Results

The value of each parameter regarding the hormonal treatment method and concentration used for hormonal treatment is shown in Table 2.

Table 2. Comparison (mean ± SD) of the parameters investigated over the experiment

Parameter	Daphnia enrichment method	Cortisol bathing method	3 ppm concentration	5 ppm concentration	7 ppm concentration	Interaction
Cortisol (ng/ml)	18.50 ± 16.23^{a}	17.55 ± 12.23^{a}	20.01 ± 11.8^{a}	16.58 ± 9.80^{b}	17.57 ± 12^{b}	$P > 0.05$
Glucose (mmol/l)	3.21 ± 0.24^{a}	3.07 ± 0.53^{a}	3.30 ± 0.3^{a}	3.07 ± 0.43^{b}	3.05 ± 0.30^{b}	$P < 0.05$
Hematocrit (%)	0.65 ± 0.02^{a}	0.53 ± 0.01^{b}	0.52 ± 0.01^{a}	0.53 ± 0.02^{b}	0.54 ± 0.02^{b}	$P < 0.05$
Mortality (%)	0.17 ± 0.28^{a}	0.33 ± 0.32^{a}	0.37 ± 0.2^{a}	0.02 ± 0.02^{b}	0.37 ± 0.32^{a}	$P < 0.05$

* Values in the same row with different superscript letters were significantly different ($P < 0.05$).

Cortisol

As shown in Table 2, there was no significant difference between daphnia enrichment and bathing methods, but the cortisol concentration was significantly higher ($P < 0.05$) in 3 ppm hormonal treatment (regardless the hormonal treatment method employed) as compared to the other treatments. In other words, significant differences were observed between different concentrations of hormonal treatments. No interaction was observed between the main factors (hormonal treatment method and concentration of hormone) in this regard.

Blood cortisol level variations over the experiment are shown in Figures 1-4. As shown in Figure 1, there was no significant difference between treatments 1 to 3 and treatments 4 to 6 after hormonal treatment. However, the highest cortisol level was observed in treatment 4. Blood cortisol level 30 min after hormonal treatment (Figure 2) was similar in treatments 1 to 3, however, the cortisol level in treatments 5 and 6 was significantly higher ($P < 0.05$) than treatment 4. In overall, the cortisol level in enrichment method was significantly higher than bathing method in the similar concentration of hormone used. 60 min after the stress, cortisol level in treatments 1 to 3 was similar (Fig. 3), while the cortisl level in treatment 4 was significantly higher ($P < 0.05$) than treatments 5 and 6. In overall, the highest cortisol value among all treatments was observed in treatment 4. 24 h after the stress (Fig. 4), cortisol level in treatments 1 to 3 was similar, however the cortisol value in treatments 4 to 6 was different and treatment 5 was significantly higher ($P < 0.05$) than treatment 4 regarding this parameter.

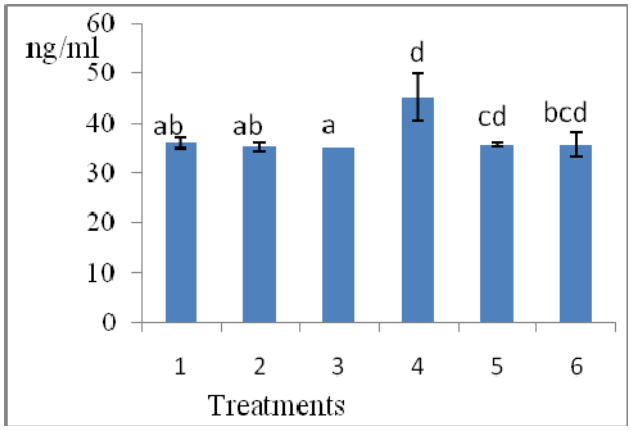

Fig. 1. Blood cortisol level after hormonal treatment (Treatments specifications are shown in Table 1)

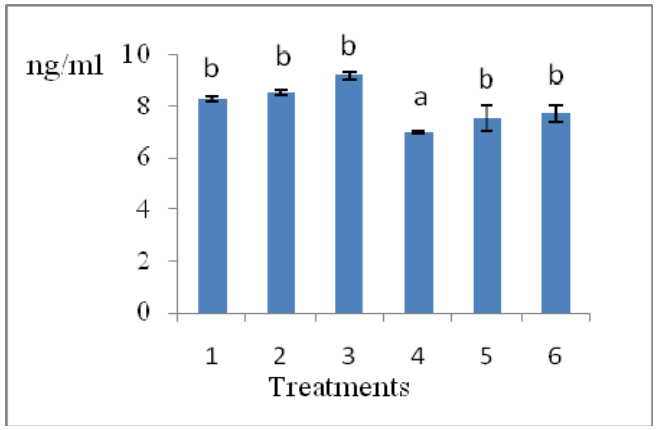

Fig. 2. Blood cortisol level 30 min after the stress (Treatments specifications are shown in Table 1)

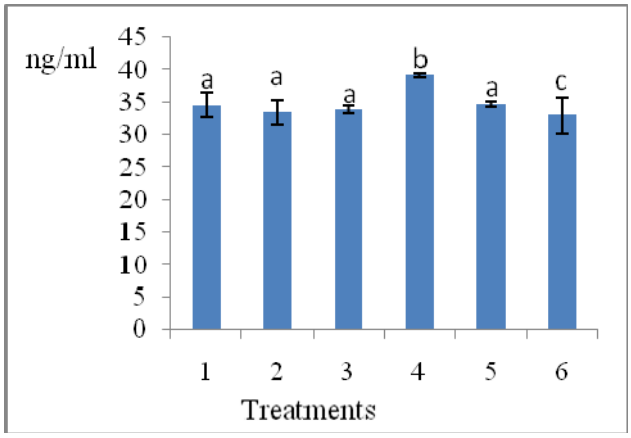

Fig. 3. Blood cortisol level 60 min after the stress (Treatments specifications are shown in Table 1)

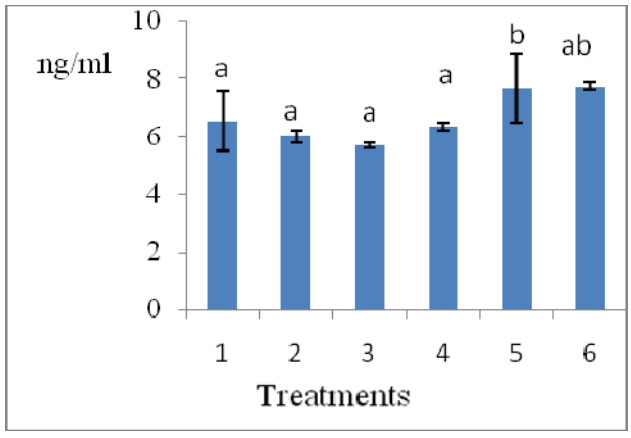

Fig. 4. Blood cortisol level 24 h after the stress (at the end of study) (Treatments specifications are shown in Table 1)

Glucose

As shown in Table 2, there was no significant difference between daphnia enrichment and bathing methods, but the glucose level was significantly higher ($P < 0.05$) in 3 ppm treatment compared to other treatments (regardless the hormonal treatment method used). Meanwhile, there was an interaction between the two main factors (treatment method and concentration of hormone). Results (Fig. 5) showed that the glucose level in treatment 5 was significantly higher ($P < 0.05$) than treatment 4 at the end of the experiment. There was no sample available due to the high mortality rate observed in treatment 6 at the end of the experiment.

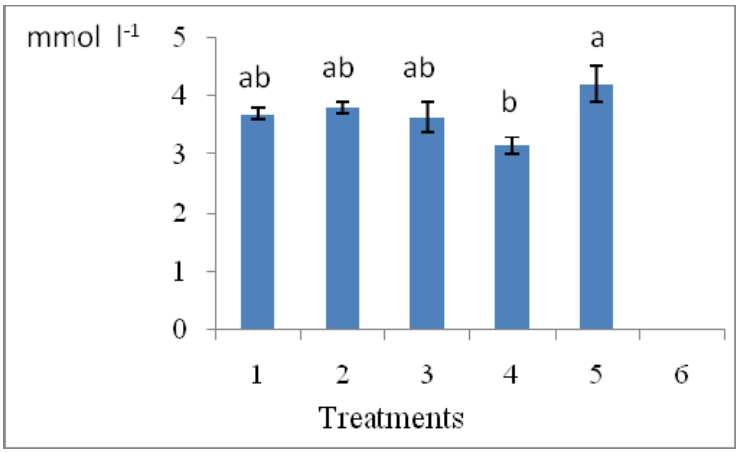

Fig. 5. Blood glucose level in different treatments at the end of the experiment (24 h after the stress)
(Treatments specifications are shown in Table 1)

Hematocrit

The hematocrit values in different treatments are shown in Table 2. There was a significant difference between hormonal treatment methods and the level of hematocrit was significantly higher ($P < 0.05$) in fish treated with daphnia enrichment method. The hematocrit value was significantly lower ($P < 0.05$) in 3 ppm treatment compared to other treatments (regardless the hormonal treatment method used). Meanwhile, there was an interaction between the main factors (treatment method and concentration of hormone). As shown in Figure 6 the hematocrit value in treatment 3 was significantly higher ($P < 0.05$) than other treatments at the end of the experiment. No hematocrit value could be reported due to the high mortality observed in treatment 6 at the end of the experiment.

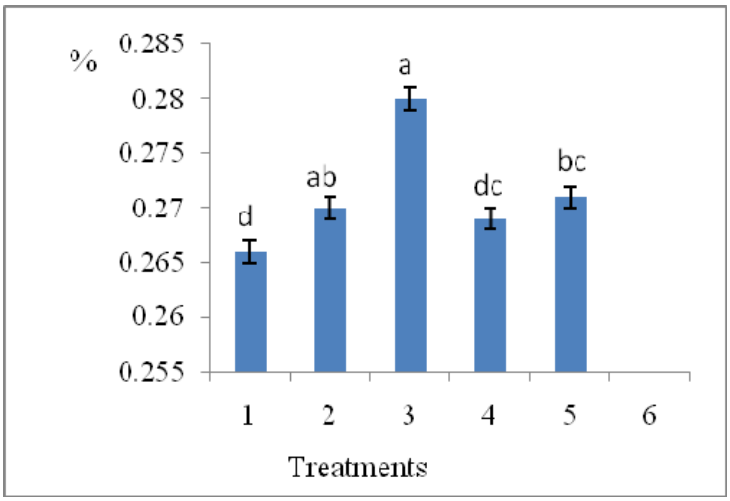

Fig. 6. Hematocrit value in different treatments at the end of the experiment (24 h after the stress)
(Treatments specifications are shown in Table 1)

Mortality

As shown in Table 2 there was no significant difference between daphnia enrichment and bathing methods in terms of fish mortality, but the mortality rate was significantly lower ($P < 0.05$) in treatment 5 (regardless the hormonal treatment method employed) as compared to other treatments. Meanwhile, there was an interaction between the two main factors (treatment method and concentration of hormone).

The mortality rates over the experiment are shown in Figures 7-9. According to Figure 7, the mortality rate after hormonal treatment in treatment 1 was significantly higher ($P < 0.05$) than treatments 2 and 3. Meanwhile, the morality rate in treatment 6 was significantly higher ($P < 0.05$) than treatment 5. After the salnity stress (Fig. 8) no mortality was observed in treatments 1 to 5. However, mortality rate in treatment 6 was significantly higher than other treatments. Mortality rate in treatment 1 was significantly higher than treatments 2 and 3, 24 h after the stress (Fig. 9). Meanwhile, the mortality rate in treatment 6 was significantly higher than ($P < 0.05$) treatment 4.

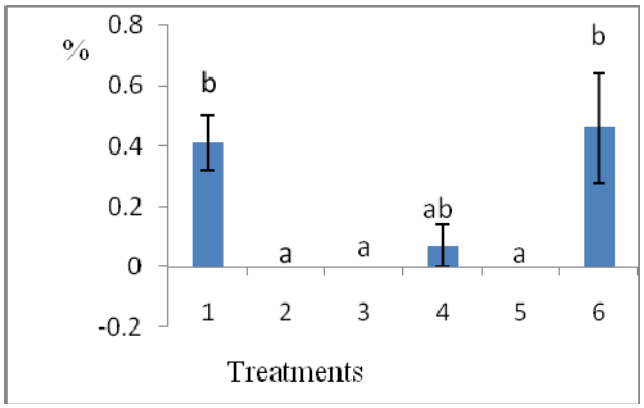

Fig. 7. Mortality rate after hormonal treatment
(Treatments specifications are shown in Table 1)

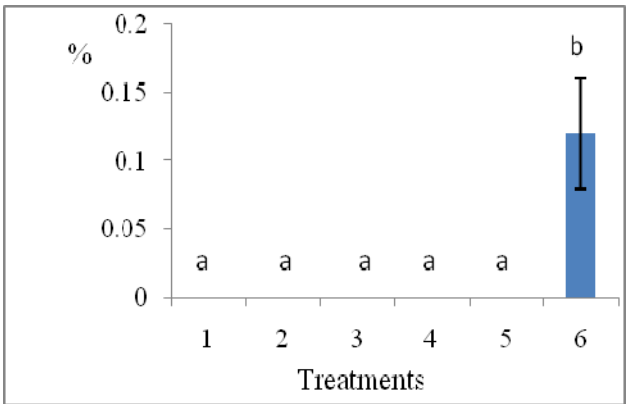

Fig. 8. Mortality rate 12 h after the salinity stress
(Treatments specifications are shown in Table 1)

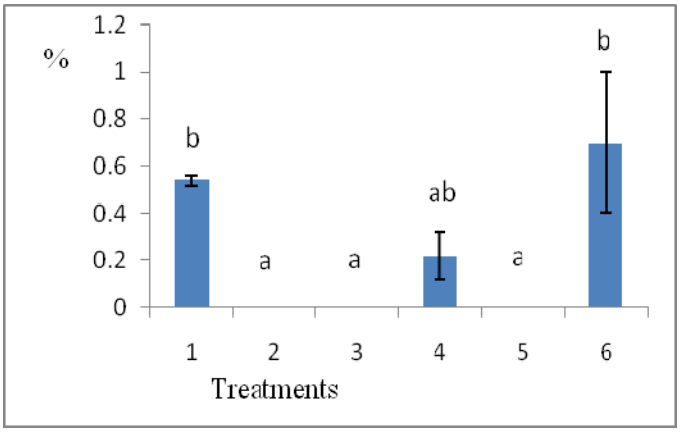

Fig. 9. Mortality rate 24 h after the stress
(Treatments specifications are shown in Table 1)

Discussion

Cortisol

Cortisol, as the main body corticosteroid, plays many important roles in fish. The main function of this hormone is to control stress especially in the initial phases of the stress induction (Evans 1993). The hormone also affect the reproduction (Huang et al. 2007), osmotic adjustment (Khodabandeh et al. 2007), growth (Cataldi et al. 1998) and migration (Iwata 1995). Cortisol is an osmo-regulatory hormone. The potential of the fish to deal with stress

increased by cortisol treatment (Khodabandeh et al. 2007). Meanwhile, blood cortisol level increased during Parr-smolt time in salmon (Ojima and Iwata 2007), also plasma cortisol increased in osmo-regulation process (Swallow and Fleming 1970). Cortisol acts in osmo regulatory process through increasing of the chloride cells (size and number) and activity of Na/K ATPase is the gill (Khodabandeh et al. 2007). Recent studies have shown that the cortisol receptor activity in the gills of fish significantly increases over the adaptation period in salt water (Khoshnood et al. 2008).

Results of this study showed that the treatment of fish with cortisol could improve the potential of the fingerlings to more appropriately deal with osmotic stress. However, there was no difference between the two employed hormonal treatment methods in terms of changes in blood cortisol levels. This observation is in agreement with the data that was already reported by other researchers (Madsen 1990; Khodabandeh et al. 2007). Due to the fact that the overall amount of the hormone used through enrichment daphnia method was significantly lower than the amount of hormone used via hormonal bathing method and also because of real concerns regarding the impact of cortisol on the environment, it seems that the daphnia enrichment method is more appropriate compared to bathing method in terms of economical and environmental point of view. Blood cortisol level was directly affected by cortisol treatment. The amount of cortisol significantly increased after the hormonal treatment. The blood hormone level was also affected by the concentration of the treatment and increased at two stages. The second step was after the stress. Regarding the rapid changes of blood cortisol, it can be concluded that blood cortisol level is more affected by the concentration of hormonal treatment rather than the stress.

Glucose

It has been shown that blood glucose level is affected by increased blood cortisol (Evans and Claiborne 2005). Some studies have shown that the cortisol treatment could increase the blood glucose concentration (Vijayan et al. 2003). Elevation of blood glucose happens to provide energy needed to deal with stress through gluconeogenesis in the liver (Swallow and Fleming 1970).

The blood glucose level were not different when two hormonal treatment were compared (Table 2). However, its level in the group treated with 3 ppm hormone was significantly higher than other treatments. Glucose is the main energy producer in the cells (Evans and Claiborne 2005). Therefore, the higher glucose concentration in the blood means that the body energy storage is decreased and this condition is not suitable for fish. Glucose increased under stressful conditions has already been reported, however increased blood glucose in the current study might be due to the lower concentration of hormonal treatment and the effect of cortisone on glyconeogenesis (Ctaldi et al. 1998). There was an interaction between two main factors. This means that for each of the two factors there is an appropriate combination. This phenomenon has not been already studied by other researchers.

Hematocrit

Increased hematocrit and glucose levels at the second phase of stress occur due to the effect of corticosteroids on the hematopoietic tissues (Evans and Claiborne 2005). Increase of hematocrit value improves the potential of blood to carry oxygen and increases aerobic activities at the cellular level. This phenomenon has been observed in various conditions such as stress, growth, migration and reproduction.

The hematocrit value in fish treated with enriched daphnia was significantly higher than hormonal bathing method. In addition, hematocrit value was increased along with the increasing cortisol concentration. Data obtained from measurements of blood cortisol levels confirmed this issue. As cortisol value in fish treated by enrichment method was higher, the increase in the hematocrit level was not unexpected. This is in agreement with the data reported by other researchers (Pottinger and Pickering 1990; Davis et al. 2001). The hematocrit value in treatments with 5 and 7 ppm of the hormonal treatment was significantly higher than 3 ppm hormonal treatment. This means that using of higher concentrations of corticosteroid hormones could improve the potential of fingerlings to more appropriately cope with stressful conditions. This is in agreements with the findings of Evans and Claiborne (2005).

Mortality

Body uses the energy to overcome stress under certain conditions. Decreased energy level in the body may lead to dysfunctions in other parts of the body. Furthermore, stress may cause mortality. In other words, there are threshold for stress tolerance (Evans 1993). In this study, there was no significant difference between two treatment methods. This was because of the high mortality in treatment 6 (7 ppm bathing) and tratment 1 (3 ppm enriched daphnia), which led to no difference between 2 methods. As it was observed between the concentrations of 3 and 7, there was no significant difference in mortality. As noted, the mortality rate for 5 ppm concentration treated was lower than

other concentrations. In other words, the most appropriate concentration for the hormonal treatment was 5 ppm. Previous studies have also shown that this concentration is more suitable for hormonal treatments (Khodabandeh et al. 2007).

After the treatment and before the stress, the mortality in treatment 6 was high. This loss can be caused by direct impact of hormone or high concentrations of hormone on fish tissues. Van Der Salm et al. (2003) have expressed that cortisol has a direct effect on the target tissue beside its indirect effects. They directly placed skin tissue in cortisol. Cortisol had high effects on different tissues of fish and caused some physiological changes (Evans and Claiborne 2005). After induction of stress, the mortality in treatment 6 was significantly higher than other tratments, but there was no difference between treatments 6 and 1. This happened due to the indirect effect of the hormone on the tissue and energy depletion in fish (Van der salm et al. 2002). High mortality in 2 treatment showed that treatment by enrichment (3 ppm concentration) and bathing methods (7 ppm concentration) were not suitable for the juveniles. In treatment 3 (enriched daphnia, 7 ppm) after the hormonal treatment, blood cortisol concentration was increased, but after induction of stress, the concentration of blood cortisol was similar to the other treatments. However, the blood glucose level and hematocrit value were increased after the cortisol increment. There was no mortality in this treatment which shows that this treatment was the best way of hormonal treatment in our study.

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