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Secondary Stress Response of Nile Tilapia, *Oreochromis niloticus*, After Direct Transfer to Different Salinities*

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Abstract: The secondary stress response of Nile tilapia, *Oreochromis niloticus* after direct transfer to saline water was evaluated assessing the levels of hematocrit, plasma glucose, sodium, potassium, chloride and calcium. Fish were transferred directly from freshwater to two experimental salinity (9 and 18 ppt) for 72 hours. Plasma glucose, Na^+, K^+, Cl^- and Ca^{++} all increased however, hematocrit did not change throughout the 3 days exposure period. Considering the parameters measured in this study Nile tilapia appeared to exhibit a stress response to direct transfer to saline water, nevertheless, the magnitude of stress response was related to salinity level.

Key Words: Tilapia, *Oreochromis niloticus* , saline water, secondary stress

Tilapianın, *Oreochromis niloticus* Farklı Tuz Konsantrasyonlarındaki Sulara Direkt Transferinde Oluşan Sekonder Stres Yanıtı

Öz: Tilapianın, *Oreochromis niloticus* tuzlu suya direkt olarak transfer edilmesi durumunda gelişen sekonder stres yanıtı hematokrit, plazma glukoz, sodyum, potasyum, klorid ve kalsiyum ölçümleri ile değerlendirilmiştir. Balıklar doğrudan iki farklı deneysel tuzluluğa (9 ve 18 ppt) 72 saatlik bir periyotta maruz bırakılmışlardır. İncelenen parametrelerden plazma glukoz, plazma Na^+, K^+, Cl^- ve Ca^{++} deney süresince artış gösterirken hematokrit değerleri değişmemiştir. Bu çalışmada değerlendirilen parametreler gözönüne alındığında tilapianın tuzlu suya direkt transferinin strese neden olduğu, ancak stres yanıtının büyüklüğünün tululuk seviyesi ile ilgili olduğu belirlenmiştir

Anahtar Kelimeler: Tilapia, *Oreochromis niloticus* , tuzlu su, sekonder stres

Introduction

Fish belonging to the genus *Oreochromis* (formerly *Sarotherodon*) tolerate a wide variety of environmental conditions (acidity, salinity, temperature, poor quality water, etc.) and show high growth and reproductive rates that make them very suitable for culture (Avella et al. 1993). Increasing demands on the use of freshwater for agricultural, industrial and domestic purposes progressively limit freshwater-based aquaculture. The efficient uses of marine and brackish environments for aquaculture becomes a vital alternative (Suresh and Lin 1992). It has been widely suggested that the euryhaline tilapias could be cultured in higher salinity of brackishwater and marine systems, thereby enabling their exploitation in arid lands and coastal areas (Watanabe et al. 1985). Many species of tilapia are euryhaline, but the tolerance limits of species vary considerably (Suresh and Lin 1992). *T.nilotica* survived after direct transfer to 50% seawater (17,5 ppt), but not 75 % seawater (Stickney 1986).

Hormones, such as catecholamines, glucagon, prolactin, and cortisol are believed to regulate the entire acclimation process (Suresh and Lin 1992). Stress in fish has been shown to cause a primary response, involving neuro-hormonal stimulation, resulting in an increase in corticosteroid and catecholamine secretions. In turn, these primary effects cause a number of physiological changes known as 'secondary effects' (Foo and Lam 1993).

Studies involving tilapia in saline waters include basic research on the physiology of salinity stress in fish as well as more practical research on aquacultural practices. Stress response of tilapia due to saline water exposure has not received a great deal of attention.

The objective of this study was to evaluate the secondary stress response of Nile tilapia after direct transfer to different salinities (9 and 18 ppt) in a short-term.

Materials and Methods

One hundred and twenty Nile tilapia, *O. niloticus* with a mean body weight of 67.36±17.68 g obtained from Ankara University, Fisheries Unit of Aquaculture and Fisheries Department were used in this study. Fish (n= 40 for each treatment) were transferred directly from freshwater to two different experimental salinities; 9 and 18 ppt. Control fish were also transferred from freshwater to freshwater. Fish were maintained for 72 hours in experimental salinities and then sampled.

Fish were held in the fiberglass tanks containing 200 l water at a stocking density of 20 fish/a tank. Water of different salinities was prepared by mixing tap water and

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Instant Ocean artificial sea salts. Water temperature during the experiment was maintained at 24°C.

Blood samples were drawn by cardiac puncture into heparinized syringes. The blood was centrifuged for 7 min at 3300 rev./min. and the plasma was removed. Fish were not anaesthetized before bleeding. Plasma glucose, Ca^{++}, Cl^{-} were measured using assay kits prepared by Labkit (Barcelona, Spain) with a Boehringer 4010 spectrophotometer. Plasma Na^{+} and K^{+} were determined using Hitachi 911 instrument. Hematocrit measurements were made immediately by drawing samples of blood into heparinized capillary tubes and centrifuging at 12500 r.p.m. for 4 min (Siwicki and Anderson 1993).

One-way ANOVA was used to determine differences among the treatment groups for each blood analyte. When a significant ($p<0.05$) difference was detected, the means were compared by use of Duncan's multiple range test.

Results

Nile tilapia (*O. niloticus*) could tolerate direct transfer from freshwater to 9 and 18 ppt salinities for 72 hours. No fish in any of the treatment group died when placed in different salinities. However, Nile tilapia in general, could not maintain hydromineral balance during the experimental period. Mean values of blood parameters of fish transferred directly from freshwater to different salinities (9 and 18 ppt) of sea water for 72 hours are shown in Table 1. In fish transferred to 9 ppt salinity for 72 h hematocrit remained unchanged when compared to control group ($p>0.05$). Plasma glucose concentration exhibited only a small increase however, plasma Cl^{-}, Na^{+}, K^{+} and Ca^{++} increased significantly ($p<0.05$). Following transfer direct from freshwater to 18 ppt salinity at 72 hours all parameters except hematocrit were higher than those of control ($p<0.05$). The mean values of plasma parameters measured in the treatment groups, increased with increasing salinity, among the treatment groups only plasma K^{+} values did not vary significantly ($p>0.05$), however, plasma K^{+} values of fish from both groups were higher than that control of freshwater tilapia ($p<0.05$).

Table 1. Mean ± SE and the range values for selected secondary stress indices of Nile Tilapia after direct transfer to 9 and 18 ppt for 72 h

Parameters	Control	Salinity Level	
		9ppt	18 ppt
Hematocrit (%) (min-max)	10.19±1.55[a*] (7.54-22.00)	11.09±1.12[a] (7.84-19.60)	9.19±0.46[a] (7.50-11.66)
Plasma glucose (mg/dl) (min-max)	94.87±0.22[ab] (94-96)	97.87±0.69[b] (95-101)	120.37±0.26[a] (119-121)
Plasma Cl^{-} (mmol/l) (min-max)	136.62±0.18[c] (136-137)	152.00±0.26[b] (151-153)	188.25±0.411[a] (187-190)
Plasma Na^{+} (mmol/l) (min-max)	165.00±0.53[c] (163-167)	176.87±0.22[b] (176-178)	210.25±0.45[a] (209-212)
Plasma K^{+} (mmol/l) (min-max)	4.80±0.02[b] (4.7-4.9)	5.13±0.018[a] (5.1-5.2)	5.15±0.018[a] (5.1-5.2)
Plasma Ca^{++} (mg/dl) (min-max)	7.34±0.029[c] (7.34-7.36)	7.68±0.069[b] (7.65-7.70)	9.34±0.012[a] (9.30-9.40)

* Different letters in a row refers significant differences between the control and the treatment groups ($p<0.05$).

Discussion

The stress response of Nile tilapia to increased salinity was evaluated using the levels of hematocrit, plasma glucose, Na^{+}, K^{+}, Ca^{++} and Cl^{-} as indicators. Secondary stress might be indicated by a significant decrease or increase in some biochemical variables in the blood. Changes at this level can reflect loss of homeostasis (e.g. decreased blood electrolytes) and demonstrate a compensatory response (e.g. elevated blood glucose) as reported by Schreck (1990). Thus, primary stress response includes increased production of catecholamines and cortisol and a partial stress response emphasizing either catecholamines or cortisol. Responses to disruptions of homeostasis due to the effects of catecholamines and cortisol released during primary response are presented in the blood parameters as secondary responses (Smith 1991, Pickering 1992).

The salinity levels of 9 and 18 ppt did not influence hematocrit values. Hemoconcentration has been observed in some teleost fishes as a response to acute stress (Iwama et al. 1993). In the present study, there is no evidence of hemoconcentration occurring in the Nile tilapia exposed to 9 and 18 ppt salinity.

In this study the plasma glucose levels in the tilapia exposed to 18 ppt salinity for 72 hours were relatively high when compared to control and the fish exposed to 9 ppt salinity. The plasma glucose level of the fish exposed to 9 ppt saline water was similar to control. The high plasma glucose in seawater adapted tilapia, *Saratherodon melanotheron* was also observed by Lea Master et al. (1990). Hyperglycemia is an expected result of stress or exhaustive excercise in fishes (Barton and Iwama 1991; Hrubec et al. 1997). Blood glucose levels may elevated immediately by catecholamines thus; in the present study increases observed in the group of 18 ppt salinity may be the result of corticosteroids, which facilitate gluconeogenesis, as stated by Barton and Iwama (1991). It is known that the degree of hyperglycemia may change depending on the type of stress and the sampling times (Rotlland et al. 1997). In the fish exposed to 9 ppt salinity slight increase in plasma glucose level is possibly related to relatively lower salinity.

Stress leads to a hydromineral imbalance (Mazeaud et al. 1977) and rectification of the stress-related osmotic dysfunction places an energetic load on the fish. (Schreck, 1990). In addition, electrolytes serve as a general measure of osmoregulatory dysfunction (Robertson et al. 1987). In the present study, electrolytes of the treatment groups had a marked deviation from the control values, however, plasma K^{+} values did not vary between the experimental groups. Plasma Cl^{-} and Na^{+} values increased

with increasing salinity. Recovered or unstressed fish should have plasma Na^+ levels of rested, healthy animals. In this study plasma Na^+ values of the experimental groups may not be representative of unstressed state. Plasma Ca^{++} concentration of control was lower than the experimental groups. As well as that increases in plasma Na^+, K^+ and Cl^- values have been reported in tilapia, *S. melanotheron* that have adapted from freshwater to seawater (Lea Master et al. 1990).

Finally, it seems that Nile tilapia under direct transfer to saline water elicit stress response associated with changing blood characteristics. Considering the values of the stress indicators measured in this study the magnitude of stress response may be related to the salinity level.

Conclusion

Pre-acclimation of Nile tilapia at low salinity and gradual transfer to higher salinities may diminish the magnitude of stress response.

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