Abbassa Int. J. Aqua., V0l.5 No.(1) 2012. *178-199*

IMPACT OF SALINITY ON THE CORTISOL, GLUCOSE, CIRCULATING IMMUNOGLOBULIN AND LYSOZYME LEVELS IN BLOOD OF TILAPIA AUREA, *OREOCHROMIS AUREUS*

Mohamed Wafeek, Mohamed Abdel Salam and Amany Abdel Aziz Gharib

Department of Hatchery and Fish Physiology, Central Laboratory for Aquaculture Research, Agriculture Research Center, Ministry of Agriculture, Egypt.

Corresponding author email: wafeek66@yahoo.com

Received 25/ 1/ 2012 *Accepted 4/ 3/ 2012*

Abstract

In this study, the effect of environmental factor (salinity) on the serum cortisol, glucose immunoglobulin M and lysozyme activities in serum tilapia, *Oreochromis aurea* were investigated. All treatments, fish was acclimatized in particular environmental conditions for 21 days according to each experiment. Fish reared at different salinities and pH, serum levels of IgM and lysozyme will be increased significantly with high salinity and low pH. The IgM and lysozyme concentrations were significant difference with different salinities levels from 2 up to 8ppt and ranged from 0.215±0.021 to 0.547±0.041 mg/ml and 0.502±0.014 to 0.569±0.018 µg/mg protein at 2 weeks exposure duration respectively. Serum IgM and lysozyme concentrations generally increased with increasing salinity after 2 weeks. These results suggest that the specific immune system of tilapia changes by certain factors in aquatic environment such as salinity and pH, whereas, the cortisol and glucose levels have no significant difference between the different times of the same salinity from the onset up to the end of the experiment, but there are a significant increased with increasing salinity levels.

Keywords: Environmental factor, salinity, pH, serum cortisol, glucose immunoglobulin M, lysozyme, *Oreochromis auria.*

INTRODUCTION

Fingerling *Tilapia aurea* were reared for 90 days in three 1.0 m^3 floating cages in seawater (36 ppt) at Lee Stocking Island, Bahamas. Fish stocking density (100, 200 and 400 fish/m^3) apparently did not affect growth rate but it appears salinity inhibited growth. Daily weight gain and specific growth rate (G) averaged 0.34 g/day and l.08%/day, respectively, for *Tilapia aurea* fingerlings. The relatively low growth rate and the high incidence of disease and mortality of *Tilapia aurea* in seawater indicate that it may not be a good candidate for cage culture in full-strength seawater (McGeachin *et al.* 1987). They added that commonly used names include *Tilapia aurea* and *Sarotherodon aurea*. Distinguishing characteristics, synonyms, photographs, keys, and discussion of hybrids were provided in Trewavas (1983a); for identification also see Page and Burr (1991), and Skelton (1993). Illustrations and diagnoses of larval and small juveniles of introduced populations were given by McGowan (1988). Color photographs were presented in Axelrod *et al.* (1985) and Axelrod (1993). Many or most accounts of "*Tilapia nilotica*" in U.S. ponds probably refer to *O. aureus*, likely imported from Israel, before the two species were shown to be distinct (Trewavas, 1983a).

The blue tilapia's local abundance and high densities in certain areas have resulted in marked changes. All species from the genus *Oreochromis* readily hybridize, potentially posing a threat to genetic diversity through introgression (D'Amato *et al.* 2007). *Oreochromis aureus* has been used widely in aquaculture and is able to live and reproduce in brackish waters. The origin of the U.S. stocks of *O. aureus*, imported as *Tilapia nilotica*, was Israel (Courtenay and Hensley 1979a).

Environmental salinity is a very important factor for aquatic organisms, and changes in salinity would seriously affect physiological processes (Cuesta *et al.,* 2005), they added stress is a complicated and

delicate response that animals experience in response to environmental stimulation such as salinity changes, exerting negative effects on their physiology, psychology, growth, and breeding. Environmental stimulation, such as sudden changes in temperature, can lead fish to become more sensitive to the environment (Crawshaw, 1979). Tilapia can avoid dehydration and adapt to the hyperosmolality of seawater (SW), making tilapia an ideal model for investigating osmoregulation by teleosts; tilapia can survive the direct transfer from freshwater (FW) to 25 parts per thousand (ppt) SW (Weng *et al.,* 2002). Most of the studies in tilapia were focused on its osmoregulation, but knowledge of the immunological responses of fish facing hyperosmolality is limited. Lysozyme, a bactericidal peptide, is an important component of the immune defenses of both freshwater and marine fish species (Lie *et al.*, 1989). Increase in lysozyme activity, phagocytosis, cell meditated cytotoxicity; and decrease in antibody production were found in salmon during FW to SW transfer (Marc *et al.,* 1995). Aquatic pollution often results in unexpected changes in the aquatic environment and occasionally affects the immune system. In general, it seems that each fish species has a specific tolerance range with respect to different environmental conditions. A few studies have been done on adaptive changes in the humoral defense system under various environmental conditions in tilapia (genus *Oreochromis*), which are native to Africa and have been introduced as economically important aquaculture finfish to many countries (Naylor *et al.,* 2000 and Almeida *et al.,* 2002). Lysozyme is an enzyme which catalyzes the hydrolysis of *â*-1, 4-glycosidic linkage between N-acetyl muramic acid and N-acetyl glucosamine of peptidoglycan in the bacterial cell wall, and is widely distributed among vertebrate and invertebrate animals (Jollès & Jollès, 1984). In teleost fish, lysozyme activity has been detected in serum, skin, mucus, and certain tissues (Sankaran & Gurnani, 1972 and Yousif *et al*., 1991) and is

considered to form part of an intrinsic defense system in many species against parasitic, bacterial and viral infections (Ingram, 1980).

Indeed, cortisol is a key hormone involved in enhancing the hypoosmoregulatory capacity of fish in seawater, including modifying the number and morphology of ionocytes and elevating gill Na^+/K^+ ATPase activity (Dange, 1986; McCormick, 1995; Evans et *al*., 2005). In addition to its role in ion regulation, cortisol plays an important role in the energy substrate repartitioning that is critical for seawater adaptation. For instance, gill biogenesis and activation of branchial ion transporters, which are essential for ionregulation upon seawater exposure, are energy demanding processes (Mommsen, 1984). Glucose is a preferred fuel for gill metabolism (Mommsen, 1984) and, indeed this metabolite is elevated upon seawater exposure of tilapia (Vijayan *et al*., 1996; Fiess *et al*., 2007). Cortisol enhances gluconeogenesis in tilapia leading to the proposal that cortisol elevation in seawater mobilizes glucose to fuel gill metabolism that is essential for hypoosmoregulation (Vijayan *et al*., 1996, 1997; Mommsen *et al*., 1999). To this end, prior cortisol treatment of fresh water acclimated Mozambique tilapia protected the fish against mortalities associated with seawater exposure (Assem and Hanke, 1981), while the mode of action of cortisol in adapting fish to osmotic shock is far from clear processes (Mommsen, 1984).

Environmental changes affect the circulating immunoglobulin M (IgM) which is the only component of the specific humoral defense system in teleost fishes and can be detected in the blood circulation (Geir and Heidrun 2000). Among different factors in aquatic environments, the effects of water temperature and salinity on the humoral defense system have been well studied. Production of humoral defense substances fluctuates with salinity (Yada and Azuma, 2002).

This study aimed to evaluate relationship between changes in various environmental conditions of salinity (0, 2, 4, 6 and 8ppt) and the

effects on the immune system of fish represented in cortisol, glucose, immunoglobulin M and lysozyme levels in serum at different duration of exposure.

MATERIALS AND METHODS

Fish:

Tilapia aurea, *Oreochromis aureus*, used in the present study was caught originally from the Central Laboratory for Aquaculture Research (CLAR) fish farm, Abbassa, Abo Hamad Sharkia, Egypt using a cast net. Their body mass ranged from 48.5g. They were kept in tanks (1-metric ton capacity) supplied with aerated freshwater. All the fish were acclimatized in the tanks at least for 21 days before the onset of the following experiments. Fish were immediately anesthetized with MS222 (Ethyl 3-aminobenzoate methane sulfonate salt; Sigma), and blood was collected within 5 min from the caudal vessels using a 3-Ml plastic syringe. Blood was allowed to sit at 4 °C and then centrifuged at 5000 ×g for 10 min; serum was collected for cortisol, glucose, IgM and lysozyme levels.

Effect of salinity:

To investigate the effect of salinity on serum, cortisol, glucose, IgM and lysozyme levels, ninety fish each were transferred to fifteen aquaria as five salinities levels treatments and three replicates. Following acclimatization of the fish in the aquaria with freshwater under basic experimental conditions for 21 days, salinity of water in the aquarium was adjusted to 0, 2, 4, 6 and 8 ppt by diluting seawater with freshwater. Increase of salinities was done within 24 hrs. Water temperature of each aquarium was maintained at 28 ^{o}C and half of the water was changed every 2 day, water salinity was readjusted after water change. Blood was collected from nine individuals at each 0, 2, 4 and 8 weeks after salinity treatments were started from each treatment.

Measurement of serum immunoglobulin M concentration:

Total IgM concentration in the serum of tilapia was measured by enzyme-linked immunosorbent assay (ELISA) according to the method of Takemura (1993). Purified tilapia IgM, rabbit anti-tilapia IgM antibody (a-IgM) and a-IgM labeled with horseradish peroxidase (a-IgM HRP) was prepared in advance (Takemura, 1993).

Serum lysozyme content measurement:

The serum lysozyme content was determined by a turbidimetric method (Ellis, 1990). Briefly, lysozyme of chicken egg white (Sigma) diluted with 0.04 M phosphate buffer (PB) for various concentrations served as the standard solution. The substrate used was *Micrococcus lysodeikticus* (0.25 mg/ml of 0.05 M PB; pH 7.4). Diluted lysozyme (15 µl standard solutions) or serum with 250 µl *M. lysodeikticus* was added to every well of a 96-well microtiter plate for 10 min at 37 °C. The absorbance at 450 nm was read at 1 min and then at 1-min intervals. The lysozyme content was defined as the amount of lysozyme that caused a decrease in absorbance of levels per min. The amount of lysozyme in the sample was calculated using the formula of the standard curve.

Serum glucose and cortisol:

Cortisol levels in serum were measured by immunological method (Sibar, Perugia, Italy) (Arakawa et al., 1979). Glucose in serum was carried out immediately according to the method of Trinder (1969).

Statistical analysis:

Serum cortisol, glucose, IgM and lysozyme concentrations were subjected to two-way analysis of variance (ANOVA) to test the effect of water salinity at different degree and time as the two factors simultaneously tested and were expressed as mean± S.D. Duncan's Multiple Range test was used as a post-hoc test to compare between

means at $P \leq 0.05$. The software SPSS, version 10 (SPSS, Richmond, VA, USA) was used as described by Dytham (1999).

RESULTS

The mean levels of body weight have no significant difference between the different levels of salinities up to 4 weeks of the onset of the experiment (Table 1), whereas, after 8 weeks, there is a very highly significant increase in fresh water 65.2±1.1 g and low salinity concentrations 2 ppt was highly significant increase 60.6±1.12g, whereas, the other treatments (4, 6 and 8 ppt) was significant decrease in comparing with fresh water rearming and represented as following 58.5±1.1g, 57.6±1.2g and 57.3±0.8g respectively. While, there is a significant difference increase in the same treatment with increasing time. The represented data showed that the body weight was very highly significant increase in fresh water and ranged from 46.3±1.4 to 65.2±1.1 g, followed by 2ppt was ranged from 43.5±1.2 to 60.6±1.12g, then 4ppt was ranged from 43.3±1.2 to 58.5±1.1g, 6ppt was ranged from 43.4±1.3 to 57.6±1.2g and the lowest final weight was recorded at 8ppt was ranged from 44.5±1.4 to 57.3±0.8g.

The mean levels of serum cortisol have no significant difference between the different levels of salinities and fresh water up to 4 weeks of the onset of the experiment (Table 2), whereas, after 8 weeks, there is significant difference decrease in fresh water 24.8±2.1 mg/ml and low salinity concentrations 2 ppt 30.5±1.4 mg/ml, whereas serum cortisol was highly significant increase in comparing to fresh water at 4 and 8 ppt 39.4±1.8 and 43.2±2.1 mg/ml respectively. whereas, the other side treatments (2, 4, and 6 ppt) and fresh water fish were non-significant difference in comparing with time experiment and represented as following 28.7±1.9 to 30.5±1.4; 30.8±1.3 to 33.8±1.6; 33.6±2.2 to 39.4±1.8 and 25.9±1.5 to 24.8±2.1 mg/ml respectively. But treatment 8

ppt was showed a significant difference in between at zero time and the experimental end as following 37.5±2.3 and 43.2±2.1 mg/ml.

The mean levels of serum glucose have a significant difference between the different levels of salinities and fresh water from the onset of the experiment (Table 3), where, after 2 weeks, there is significant difference decrease in fresh water 65.4±2.4 mg/100ml and low salinity concentrations 2 ppt 70.2±4.3 mg/100ml and 4 ppt 72.3±2.6mg/100ml, whereas serum glucose was highly significant increase in comparing to fresh water at 6 and 8 ppt 78.5±3.5 and 83.2±3.7 mg/100ml respectively. Also, after 4 weeks, there is a significant difference decrease in fresh water 68.2±4.3 mg/100ml and low salinity concentrations 2 ppt 72.4±6.2 mg/100ml and 4 ppt 75.4±5.2mg/100ml, whereas serum glucose was highly significant increase in comparing to fresh water at 6 and 8 ppt 80.2±4.1 and 84.5±5.1 mg/100ml respectively. Also, after 8 weeks, there is a significant difference decrease in fresh water 63.2±3.2 mg/100ml and low salinity concentrations 2 ppt 68.6±2.5 mg/100ml followed by 4 ppt 70.8±3.1 mg/100ml, whereas serum glucose was highly significant increase in comparing to fresh water at 6 and 8 ppt 84.6±3.6 and 80.7±2.8 mg/100ml respectively.

Whereas, in Table (3) also showed the mean values of glucose have no significant difference between the different times of the same salinity from onset up to the end the experiment for fresh water, 2, 4, 6 and 8 ppts treatments and ranged from 65.4±2.4 to 63.2±3.2; 70.2±4.3 to 68.6±2.5; 72.3±2.6 to 70.8±3.1; 78.5±3.5 to 84.6±3.6 and 83.2±3.7 to 80.7±2.8 mg/100ml respectively.

The mean levels of serum immunoglobulin M have a significant difference between the different levels of salinities and fresh water from the onset of the experiment (table 4), where, after 2 weeks, there is significant difference decrease in fresh water 0.202±0.017 mg/ml and low salinity concentrations 2 ppt 0.215±0.021 mg/ml followed by 4 ppt

0.319±0.034 mg/ml, whereas serum immunoglobulin M was highly significant increase in comparing to fresh water at 6 and 8 ppt 0.468±0.035 and 0.547±0.041 mg/ml respectively. Also, after 4 weeks, there is a significant difference decrease in fresh water 0.218±0.015 mg/ml and low salinity concentrations 2 ppt 0.220±0.017 mg/ml followed by 4 ppt 0.289±0.011 mg/ml, whereas serum immunoglobulin M was highly significant increase in comparing to fresh water at 6 and 8 ppt 0.302±0.023 and 0.416±0.029 mg/ml respectively. Also, after 8 weeks, there is a significant difference decrease in fresh water 0.231±0.018 mg/ml and low salinity concentrations 2 ppt 0.225±0.016 mg/ml followed by 4 ppt 0.241±0.017 mg/ml, whereas serum immunoglobulin M was highly significant increase in comparing to fresh water at 6 and 8 ppt 0.282±0.025 and 0.336±0.024 mg/ml respectively.

While, in Table (4) also showed the mean values of immunoglobulin M have no significant difference between the different times of the same salinity from onset up to the end the experiment for fresh water and 2 ppt treatments and ranged from 0.202±0.017 to 0.231±0.017 and 0.215±0.021 to 0.225±0.016 mg/ml respectively. But the mean values of immunoglobulin M have significant difference between the different times of the same salinity from onset up to the end the experiment for 6 and 8 ppt.

The mean levels of serum lysozyme (µg/mg protein) have a significant difference between the different levels of salinities and fresh water from the onset of the experiment (Table 5), where, after 2 weeks, there is significant difference decrease in fresh water 0.514±0.011 µg/mg protein and low salinity concentrations 2 ppt 0.502±0.014 µg/mg protein followed by 4 ppt 0.523±0.016 µg/mg protein, whereas serum lysozyme was highly significant increase in comparing to fresh water at 6 and 8 ppt 0.542±0.015 and 0.569±0.018 µg/mg protein respectively. Also, after 4 weeks, there is a significant difference decrease in fresh water

0.503±0.016 µg/mg protein and low salinity concentrations 2 ppt 0.496±0.018 µg/mg protein followed by 4 ppt 0.534±0.019 µg/mg protein, whereas serum lysozyme was highly significant increase in comparing to fresh water at 6 and 8 ppt 0.542±0.017 and 0.552±0.013 µg/mg protein respectively. Also, after 8 weeks, there is a significant difference decrease in fresh water 0.526±0.014 mg/ml and low salinity concentrations 2 ppt 0.523±0.016 µg/mg protein followed by 4 ppt 0.542±0.019 µg/mg protein, whereas serum lysozyme was highly significant increase in comparing to fresh water at 6 and 8 ppt 0.551±0.014 and 0.568±0.021 µg/mg protein respectively. While, in table (5) also showed the mean values of lysozyme have no significant difference between the different times of the same salinity from onset up to the end the experiment for fresh water and 2, 4, 6 and 8 ppt treatments.

Table (1): Values (mean ±SD) of body weight (g) at different water salinities (2, 4, 6 and 8 ppt) and fresh water of *Tilapia aurea* were recorded at different times.

Treatment	**Zero time (g)**	**After 2 weeks (g)**	**After 4 weeks (g)**	**After 8 weeks (g)**
0 ppt	48.3 ± 1.4^{ax}	52.2 ± 1.4^{bx}	56.4 ± 1.4^{cx}	65.2 ± 1.1^{dx}
2 ppt	43.5 ± 1.2^{ax}	51.2 ± 1.2^{bx}	55.5 ± 1.4^{cx}	60.6 ± 1.3^{dy}
4 ppt	43.3 ± 1.2^{ax}	49.9 ± 1.4^{bx}	54.6 ± 1.5^{bx}	58.5 ± 1.6^{cz}
6 ppt	43.4 ± 1.3^{ax}	49.5 ± 1.3^{bx}	53.7 ± 1.2^{bx}	57.6 ± 2.1^{cz}
8 ppt	44.5 ± 1.4^{ax}	50.3 ± 1.4^{bx}	54.6 ± 1.3^{bx}	57.3 ± 1.1^{cz}

The means of different letters (a-d) in the same row and different letters (w-z) in the same column show significant difference ($P< 0.05$).

Table (2): Changes in serum cortisol (mg/ml) of *Tilapia aurea* exposure to different salinities (2, 4, 6 and 8 ppt) and fresh water were recorded at different times.

Treatment	After 2 weeks	After 4 weeks	After 8 weeks
0 ppt	25.9 ± 1.5^{az}	26.5 ± 1.7^{az}	24.8 ± 2.1^{az}
2 ppt	28.7 ± 1.9^{az}	29.2 ± 2.2^{az}	30.5 ± 1.4^{ay}
4 ppt	30.8 ± 1.3^{ay}	32.1 ± 1.5^{ay}	33.8 ± 1.6^{ay}
6 ppt	33.6 ± 2.2^{ay}	36.2 ± 1.3^{ax}	39.4 ± 1.8^{ax}
8 ppt	37.5 ± 2.3^{ax}	40.8 ± 3.2^{ax}	43.2 ± 2.1^{bx}

The means of different letters (a-d) in the same row and different letters (x-z) in the same column show significant difference ($P< 0.05$).

Table (3): The concentrations (mean± SD) of serum glucose (mg/100ml) at different salinities (0, 2, 4, 6 and 8ppt) of *Tilapia aurea* at fixed water temperature 28^{o}C and pH value 8 were recoded.

Treatment	After 2 weeks	After 4 weeks	After 8 weeks
0ppt	65.4 ± 2.4^{ay}	68.2 ± 4.3^{ay}	63.2 ± 3.2^{az}
2ppt	70.2 ± 4.3^{ay}	72.4 ± 6.2^{ay}	68.6 ± 2.5^{az}
4ppt	72.3 ± 2.6^{axy}	75.4 ± 5.2^{ay}	70.8 ± 3.1^{ay}
6ppt	78.5 ± 3.5^{ax}	80.2 ± 4.1^{ax}	84.6 ± 3.6^{ax}
8ppt	83.2 ± 3.7^{ax}	84.5 ± 5.1^{ax}	80.7 ± 2.8^{ax}

The means of different letters (a-d) in the same row and different letters (x-z) in the same column show significant difference ($P< 0.05$).

Table (4): The concentrations (mean±SD) of serum immunoglobulin M (mg/ml) at different salinities (0, 2, 4, 6 and 8 ppt) of *Tilapia aurea* at fixed water temperature 28^{o}C and pH value were recoded.

Treatment	After 2 weeks	After 4 weeks	After 8 weeks
0 ppt	0.202 ± 0.017^{az}	0.218 ± 0.015^{az}	0.231 ± 0.018^{ay}
2 ppt	0.215 ± 0.021^{az}	0.220 ± 0.017^{az}	0.225 ± 0.016^{ayz}
4 ppt	0.319 ± 0.034^{cy}	0.289 ± 0.011^{by}	0.241 ± 0.017^{ax}
6 ppt	0.468 ± 0.035^{bx}	0.302 ± 0.023^{ax}	0.282 ± 0.025^{ax}
8 ppt	0.547 ± 0.041^{aw}	0.416 ± 0.029^{bw}	0.336 ± 0.024^{aw}

The means of different letters (a-c) in the same row and different letters (w-z) in the same column show significant difference (P< 0.05).

Table (5): The values (mean±SD) of serum lysozyme (µg/mg protein) at different salinity (0, 2, 4, 6 and 8 ppt) of *Tilapia aurea* at fixed water temperature 28^{o}C and pH values were recorded.

Treatment	After 2 weeks	After 4 weeks	After 8 weeks
0 ppt	0.514 ± 0.011^{ayx}	0.503 ± 0.016^{ayx}	0.526 ± 0.014^{ayx}
2 ppt	0.502 ± 0.014^{ay}	0.496 ± 0.018^{ay}	0.523 ± 0.016^{ayx}
4 ppt	0.523 ± 0.016^{ayx}	0.534 ± 0.019^{ax}	0.542 ± 0.019^{ax}
6 ppt	0.542 ± 0.015^{ax}	0.542 ± 0.017^{ax}	0.551 ± 0.014^{ax}
8 ppt	0.569 ± 0.018^{ax}	0.552 ± 0.013^{ax}	0.568 ± 0.021^{ax}

The means of different letters (a-c) in the same row and different letters (x-z) in the same column show significant difference (P< 0.05).

DISCUSSION

From the experimental data in this work noticed that body weight is a significant decrease in low salinity concentrations 2, and 4ppt and highly significant decrease in 6 and 8ppt in comparing with fresh water group, these results are in agreement with Sameh *et al*., (2007) they found that environmental salinity is among the important factors affecting fish growth. Providing the most suitable environment is particularly important to organizing the growth of juvenile fish in aquaculture. Riley *et al*., (2003) they suggested that environmental salinity is among the crucial factors that affect fish growth, particularly in euryhaline fishes such as the tilapia.

The mean levels serum cortisol have no significant difference between the different levels of salinities and fresh water up to 4 weeks of the onset of the experiment , whereas, after 8 weeks, there is significant difference increase in comparing with fresh water. Also, the values of glucose have no significant difference between the different times of the same salinity from onset up to the end the experiment. But within the same time for different salinities are significant in comparing to fresh water. These results may be in agreement with the following authors It is well established that cortisol, along with other osmoregulatory hormones, plays a key role in seawater adaptation, resulting in overall salt secretion to maintain body fluids and ion homeostasis (McCormick, 2001; Evans *et al*., 2005). Plasma cortisol levels increase after salinity exposure in tilapia, but most studies have examined gradual acclimation to full-strength seawater over a longer period of time (Vijayan *et al*., 1996). However, abrupt seawater exposure did not significantly alter plasma cortisol levels at 6 h, whereas plasma glucose levels and gill Na^+/K^+-ATPase activity were elevated suggesting metabolic and branchial adjustments to cope with the hyperosmotic shock. The higher plasma glucose response in seawater supports a key role for this fuel to

meet the increased branchial energy demands, including activation of gill Na+/K+-ATPase activity (Mommsen, 1984). Also, it has been shown that cortisol-mediated gluconeogenesis is involved in the elevated glucose response during seawater acclimation (Vijayan *et al*., 1996, 1997; Mommsen *et al*., 1999). The cortisol treatment prior to seawater exposure prevented mortalities in tilapia (Assem and Hanke, 1981). As expected, cortisol stimulation elevated glucose levels (Abo Hegab and Hanke, 1984; Vijayan *et al*., 1997), providing energy substrates to cope with the increased energy demand to activate gill ion transporters in seawater (Mommsen *et al*., 1999).

The specific defense system of teleost fishes is continuously affected by periodic or unexpected changes in different factors of the aquatic environment. Fishes under suitable environmental conditions have a functional immune system and, consequently, high disease resistance is expected. On the contrary, unsuitable environmental conditions may act as acute or chronic stressors against fish and cause the suppression of the specific defense system.

These results suggest that Tilapia aurea, *Oreochromis aureus* has an optimal salinity range for immune function. The effect of gradual increase in salinity from 2 up to 8 ppt on serum concentration of IgM after 2, 4 and 8 weeks exposure condition showed that did not alter serum IgM production. These results are in agreement with Yada *et al.,* (2002) they found that *O. mossambicus* which were transferred from freshwater to 21 or 35 ppt salinity did not alter serum IgM concentrations. A similar result was reported in rainbow trout, *Oncorhynchus mykiss*, it has been reported that transfer of the fish from freshwater to 12 ppt or full-strength 29 ppt salinity did not alter serum IgM concentrations (Yada *et al.,* 2001). In contrast with those results, Miriam *et al.,* (2004) showed that clear changes in IgM concentration with increasing salinity. Because *O. mossambicus* exhibits higher salinity tolerance than *O. niloticus*

(Popma and Masser, 1999), the difference in the results between the two tilapia species may be partially due to tolerance to high salinity. Additionally, it is possible that fish size is related to high salinity tolerance, because *O. mossambicus* weighing 50 to 100 g were used in the experiment by Yada *et al.* (2002). Another study showed that hypophysectomy of rainbow trout reared in freshwater caused a significant reduction in serum IgM levels (Yada and Azuma, 2002).

The mean levels of serum lysozyme (µg/mg protein) have a significant difference between the different levels of salinities and fresh water from the onset of the experiment (Table 5), where, after 2 weeks, there is significant difference decrease in fresh water these may be explained with the following, in the brown trout *Salmo trutta*., the phagocytic activity of leucocytes and plasma lysozyme activity were increased after transfer from fresh water FW to seawater SW, and there were positive correlations between plasma GH level and phagocytic or lysozyme activity Marc *et al*., 1995. Lysozyme is known to act as a non-specific immune function against parasitic, bacterial and viral infections Yano, 1996. In the brown trout, there is a positive correlation between plasma GH level and plasma lysozyme activity during acclimation from FW to SW Marc *et al*., 1995. In fish, lysozyme is distributed in the leucocyte-rich tissues such as the head kidney, skin, gills and eggs, and lysozyme activity has also been identified in neutrophils and macrophages Yano, 1996.

CONCLUSION

This study provides significant evidence that environmental salinity can impact the concentration of blood cortisol, glucose, IgM and lysozyme of Tilapia aurea. Second, it confirms that tilapia can be adapted for different levels of salinities up to 9 ppt with longer time 8 weeks.

REFERENCES

Abo Hegab, S. and W. Hanke. 1984. The significance of cortisol for osmoregulation in carp (*Cyprinus carpio*) and tilapia (*Sarotherodon mossambicus*). Gen. Comp. Endocrinol. 54: 409-417.

Almeida, J.A.; Y.S. Diniz; S.F. Marques; L.A. Faine; B.O. Ribas; R.C. Burneiko and E.L. Novelli. 2002. The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. Environ. Int., 27: 673-679.

Arakawa, H.; M. Maeda and A. Tsujl. 1979. Modified cortisol estimation by immunosystem. Anal. Biochem., 97: 248-251.

Assem, H. and W. Hanke 1981. Cortisol and osmotic adjustment of the euryhaline teleost, Sarotherodon mossambicus. Gen. Comp. Endocrinol., 43: 370-380.

Axelrod, H.R. 1993. The most complete colored lexicon of cichlids. Tropical Fish Hobbyist Publications, Inc., Neptune City, NJ.

Axelrod, H.R.; W.E. Burgess; N. Pronek; and J.G. Walls. 1985. Dr. Axelrod's atlas of freshwater aquarium fishes. Tropical Fish Hobbyist Publications, Inc., Neptune City, NJ.

Courtenay, W.; R., Jr and D. A. Hensley. 1979a. Survey of introduced non-native fishes. Phase I Report. Introduced exotic fishes in North America: status 1979. Report Submitted to National Fishery Research Laboratory, U.S. Fish and Wildlife Service, Gainesville, FL.

Crawshaw L.I. 1979. Responses to rapid temperature change in vertebrate ectotherms. American Zoologist, 19: 225-237.

Cuesta A; N.R. Laiz-Carrio´; M.P. Del Rı´o; J. Meseguer; J.M. Mancera; and MA. Esteban 2005. Salinity influences the humoral immune parameters of gilthead seabream (*Sparus aurata L.*). Fish & Shellfish Immunology, 18: 255-261.

D'Amato, M.E.; M.M. Esterhuyse; B.C.W. Van Der Waal, D. Brink and F.A.M. Volckaert. 2007. Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. Conservation Genetics, 8: 475-488.

Dange, A.D. 1986. Branchial Na^+/K^+ATPase activity in freshwater or saltwater acclimated tilapia, Oreochromis (Sarotherodon) mossambicus: effects of cortisol and thyroxine. Gen. Comp. Endocrinol., 62: 341-343.

Dytham, C. 1999. Choosing and using statistics: a biologist's guide. Blackwell Science Ltd., London, UK.

Ellis, A.E. 1990. Lysozyme assay. In: Stolen, J.S., Fletcher, T.C., Anderson, D.P., van Muiswinkel, W.D. (Eds.), Techniques in Fish Immunology. SOS Publications, Fair Haven, NJ, 101-104.

Emerson, K.; R.C. Russo; R.F. Lund; and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: Effects of pH and temperature. J. Fish Res. Board Canad., 32: 2379-2383.

Evans, D.H.; P.M. Piermarini and K.P. Choe. 2005. The multifunctional fish gill:dominant site of gas exchange, osmoregulation, acid-base regulation and excretion of nitrogenous waste. Physiol. Rev. 85: 97-177.

Fiess, J.C.; A. Kunkel-Patterson; L. Mathias; L.G. Riley; P.H. Yancey; T. Hirano and E.G. Grau. 2007. Effects of environmental salinity and temperature on osmoregulatory ability, organic osmolytes, and plasma hormone profiles in the Mozambique tilapia

(Oreochromis mossambicus). Comp. Biochem. Physiol. A., 146: 252-264.

Geir, O.M. and I.W. Heidrun, 2000. Serum protein and IgM profiles in connection with the smolting and vaccination of out-of-season Atlantic salmon (*Salmo salar L*.). Aquaculture ,188: 189-201.

Ingram, G.A. 1980. Substances involved in the natural resistance of fish to infection-A review. Journal of Fish Biology, 16: 23-60.

Jollès, P. and J. Jollès. 1984. What's new in lysozyme research? Always a model ystem, today as yesterday. Molecular Cell Biochemistry, 63: 165-189.

Lie O; O. Evensen; A. Sorensen and E. Froysadal. 1989. Study on lysozyme activity in some fish species. Diseases of Aquatic Organisms, 6: 1-5.

Marc AM, C. Quentel; A. Severe; PY. Le Bail and G. Boeuf. 1995. Changes in some endocrinological and non-specific immunological parameters during seawater exposure in the brown trout. Journal of Fish Biology; 46:1065-81.

Marc, A.M.; C. Quentel; A. Severe; P.Y.Le Bail; G. Boeuf. 1995. Changes in some endocrinological and non-specific immunological parameters during seawater exposure in the brown trout. J. Fish Biol., 46: 1065-1081.

McCormick, S.D. 1995. Hormonal control of gill Na^+/K^+ATPase and chloride cell function. In: Wood, C.M., Shuttleworth, T.J. (Eds.), Fish physiology, vol. XIV, Ionoregulation: Cellular and Molecular Approaches. Academic Press, pp. 285-315.

McCormick, S.D. 2001. Endocrine control of osmoregulation in teleost fish. Am. Zool., 41: 781–794.

McGeachin, C.B.; I. Robert; Bori L.O. Wicklund and R.W. James. 1987. Growth of *Tilapia aurea* in Seawater Cages. Journal of the World Aquaculture Society, 18 (1): 31-34.

McGowan, E.G. 1988. An illustrated guide to larval fishes from three North Carolina piedmont impoundments. Report by Carolina Power and Light Company, Shearon Harris Energy and Environmental Center, New Hill, NC. 113 pp.

Miriam D., T. Akihiro; T. Makoto and N. Shigeo. 2004. Impact of different environmental factors on the circulating immunoglobulin levels in the Nile tilapia, *Oreochromis niloticus*. Aquaculture, 241: 491-500.

Mommsen, T.P. 1984. Metabolism of the fish gill. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology. Academic, New York, pp. 203-238.

Mommsen, T.P.; M.M. Vijayan and T.W. Moon. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fisheries, 9: 211-268.

Nagae M.; K. Ogawa; A. Kawahara; M. Yamaguchi; T. Nishimura and F. Ito. 2001. Effect of acidification stress on endocrine and immune functions in carp, *Cyprinus carpio*. Water, Air, and Soil Pollution, 130: 893-898.

Naylor, R.L.; R.J. Goldberg; J.H. Primavera; N. Kautsky; M.C.M. Beveridge; J. Clay; D. Folke; J. Lubchenco; H. Mooney and M. Troell. 2000. Effect of aquaculture on world fish supplies. Nature, 405: 1017-1024.

Page, L. M., and B. M. Burr. 1991. A field guide to freshwater fishes of North America north of Mexico. The Peterson Field Guide Series, volume 42. Houghton Mifflin Company, Boston, MA.

Popma, T. and M. Masser. 1999. Tilapia: Life History and Biology. Southern Regional Aquaculture Center Pub. SRAC., 283, 4.

Riley L. G.; T. Hirano and E. U. Grau 2003. Effect of transfer from seawater to fresh water on the growth hormone/insulin-like growth factor-I axis and prolactin in the tilapia, (*Oreochromus mossambicus*) Comp. Biochem. Physiol., B: 136: 647-655.

Sameh M.; K. Uchida; T. Hirano; E. Gordon; A. Abdel Fattah and M. Nozaki. 2007. Effect of environmental salinity on somatic growth and growth hormone/like insulin-growth factor-laxis in juvenile tilapia *Oreochromus mossambicus*. Fisheries Science, 73:1025-1034.

Sankaran, K. and S. Gurnani. 1972. On the variation in the catalytic activity of lysozyme in fishes. Indian Journal of Biochemistry and Biophysics, 9: 162-165.

Skelton, P.H. 1993. A complete guide to the freshwater fishes of southern Africa. Southern Book Publishers, Halfway House, South Africa.

Takemura, A. 1993. Changes in an immunoglobulin M (IgM)-like protein during larval stages in tilapia *Oreochromis mossambicus*. Aquaculture, 115: 233-241.

Trewavas, E. 1983a. Tilapine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. Cornell University Press, Ithaca, NY.

Trinder, P. 1969. Serum glucose determination. Ann. Clin. Biochem., 6: 24. Cited from Boehringer Mannheim Gmth Diagnostica kit. 540 Tripathi, G. and P. Verma 2004. Sex-specific changes in the annual reproductive cycle of freshwater catfish. Comp. Biochem. Physiol., B137: 101-106.

Vijayan, M.M.; C. Pereira; E.G. Grau and G.K. Iwama. 1997. Metabolic responses associated with confinement stress in tilapia: the role of cortisol. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol., 116: 89-95.

Vijayan, M.M.; J.D. Morgan; T. Sakamoto; E.G. Grau and G.K. Iwama. 1996. Fooddeprivation affects seawater acclimation in tilapia: hormonal and metabolic changes. J. Exp. Biol., 199: 2467-2475.

Weng C.F.; C. Chia-Chang; H.Y. Gong; M.H.C. Chen; C.J.F. Lin and W.T. Huang. 2002. Acute changes in gill Naþ-Kþ ATPase and creatine kinase in response to salinity changes in the euryhaline teleost, tilapia (*Oreochromis mossambicus*). Physiological and Biochemical Zoology, 75: 29-37.

Yada, T. and T. Azuma. 2002. Hypophysectomy depresses immune functions in rainbow trout. Comp. Biochem. Physiol., 131C: 93-100.

Yada, T.; K. Uchida; S. Kajimura; T. Azuma; T. Hirano and E.G. Grau 2002. Immunomodulatory effects of prolactin and growth hormone in the tilapia, Oreochromis mossambicus. J. Endocrinol. 173: 483-493.

Yada, T.; T. Azuma and Y. Takagi. 2001. Stimulation of non-specific immune functions in seawater-acclimated rainbow trout, *Oncorhynchus mykiss* with reference to the role of growth hormone. Comp. Biochem. Physiol. 129B, 695-701

Yano, T. 1996. The nonspecific immune system: humoral defense. In: Iwama, G., Nakanishi, T. (Eds.), the Fish Immune System: Organism, Pathogen, and Environment. Academic Press, New York, pp. 105-157.

Yousif, A. N.; L. J. Albright and T. P. T. Evelyn. 1991. Occurrence of lysozyme in the eggs of coho salmon *Oncorhynchus kisutch.* Diseases of Aquatic Organisms 10: 45-49.

تأثير الملوحة على الكورتيزول والجلوكوز والامينوجلوبيولوين والليزوزوم فى دم سمكة البلطى الاوريا

محمد وفيق ، محمد عبدالسلام ، امانى عبدالعزيز غريب

قسم بحوث التفريخ والفسيولوجى، المعمل المركزى لبحوث الثروة السمكية، مركز البحوث الزراعية، القاهرة، مصر.

الملخص العربى

في هذه الدراسة تم دراسة تأثير الملوحة كعامل بيئي في دم سمكة البلطى الاوريا لقياس تركيز الكورتيزول والجلوكوز والامينوجلوبيولوين و نشاط الليزوزوم فى مصل سمكة البلطى الاوريا. وفى كل المعاملات تمت اقلمة كافة الاسماك فى ظروف المعمل العادية لمدة ٢١ يوم طبقا لظروف التجربة. تمت رعاية الاسماك في معدلات مختلفة من الملوحة وكانت مستويات الامينوجلوبيولوين و نشاط الليزوزوم تزداد مع زيادة الملوحة. وكانت مستويات الامينوجلوبيولوين و نشاط الليزوزوم تزداد زيادة معنوية بزيادة الملوحه من ٢ الى ٨ جزء فى الالف وتتراوح من ٠.٠٢١±٠.٢١٥ الى ٠.٠٤١±٠.٥٤٧ مجم/مل و ٠.٠١٤±٠.٥٠٢ الى ٠.٠١٨±٠.٥٦٩ ميكروجرام /ملجم بروتين عند الاسبوع الثانى من التعرض على التوالى. واوضحت النتائج ان الجهاز المناعى للسمكة يتغير بتغير الملوحة. بينما لا يتغير الكورتيزول والجلوكوز مع تغير الوقت لنفس الملوحه من بداية التجربة الى نهايتها ولكن يوجد زيادة معنويه مع زيادة مستويات الملوحة.