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Effects of Salinity on Some Haematological and Biochemical Parameters in Nile Tilapia, *Oreochromus niloticus*

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Abstract: Any change in haematological and biochemical parameters could be a predictor of unfavorable environment or effect of different stress factors. The present study was designed to assess different salinity concentration induced changes in some haematological and biochemical parameters in 64 *O. niloticus* fishes captured from Manzala Lake (Egypt), they were acclimatized and fed with commercial fish diet for one week before starting the experiment for another 2 weeks. Fishes were divided into 4 equal groups assigned as; control, 4g NaCl\L, 8 g NaCl\L and 12 g NaCl\L. RBCs, HCT, Hb concentration, platelets count, superoxide dismutase activity, catalase activity, potassium level and serum total protein were estimated. The HCT, Hb concentration, platelets count and potassium levels were significantly higher in (4gNaCl\L, 8gNaCl\L and12gNaCl\L, respectively). The Superoxide dismutase activity (SOD), total protein, RBCs and catalase activit were significantly lower in (4gNaCl\L, 8g NaCl\L and 12gNaCl\L, respectively) compared to the control group. Other parameters such as WBCs, haematimetric indices (MCV, MCH, MCHC), malonedialdhyde (MDA) level, carbonyl protein (CP), glutathione reductase (GR) activity, as well as serum sodium, glucose, cortisol and IgM, did not show any significant differences in the estimated salinity concentrations.

Keywords: Tilapia, Salinity, Haematologic Parameters, Oxidative Stress Markers, Electrolytes

1. Introduction

Fish aquaculture is essential to enhance food security, in addition to give another source of income, especially in Egypt that has high population density [1, 2].

It is outstanding that oceanic life forms, including fishes, are influenced by both inside (e.g. hormones and compounds) and outside components (e.g. their condition). Stress is any change in the physical or systemic elements that affect body health leading to disease or many causes death [3]. Moreover, it may cause disturbing homeostasis, stress reaction including many physiological changes including blood composition and immune mechanisms [4].

Fish response to any stressor could be achieved by many physiological changes to maintain homeostasis, osmolality and hematology [5, 6]. Salinity is a main abiotic factor in aquaculture and its optimum degree are specific and may affect growth, and survival success [7]. There are variations in salinity tolerance among different species [8]. The physiological responses to high or low salinity levels in the aquatic environment have been studied in different fresh and marine species [9-11].

Nile Tilapia, *Oreochromis niloticus* is one member of Cichlidae family. It is the most common cultured freshwater species worldwide [12], although it could adapt to different levels of water salinities, it has the least salinity tolerance compared to other tilapia species [13]. Salinity usually affects

the growth rate of euryhaline fishes because a part of the energy available for growth is consumed for osmoregulation [14, 15]. The perfect salinity level is the one that insures higher growth rate and lowers energetic cost of osmoregulation.

Stress due to salinity changes has been reported to alter the standard haematological characteristic of teleosts, elevating plasma corticosteroids [16, 17] reducing the levels of some blood parameters and also increasing the values of some blood components. These changes can affect oxygen transport in the blood and across the gills.

Both haematological and biochemical parameters have been frequently used as an indicator of the general condition in different aquatic species [18-21]. Many studies were conducted to estimate the effects of the change in salinity levels on fish physiology [22, 23].

Due to the economic importance of *O. niloticus* in aquaculture in Egypt, the present study aimed to study the effects of different salinities on some haematological and biochemical measurements in *O. niloticus*.

2. Materials and Methods

2.1. Fishes and Experimental Design

Sixty-four *O. niloticus* (mean body weight= 112±14.5 g) were obtained from Manzala Lake, Damietta, Egypt. Fishes, apparently healthy, were transported to the lab and acclimatized in dechlorinated water for one week and fed on a basal fish diet. After acclimation, the fishes were divided randomly into equal four groups with 8 fishes in all aquaria(40×35×70 cm) with capacity of 60L water were used in the experiment, each group with different salinity level (Control, 4 g/l, 8 g/l and 12 g/l)for another 14 days.

The experimental salinity levels were obtained by adding 2 g/l sodium chloride (NaCl) gradually per day until 4 g/l, 8 g/l and 12 g/l were reached. Water salinity was measured daily to avoid any change. The experiment was done in natural photoperiod and the fishes were fed twice per day by 3% of the total stock biomass.

2.2. Blood Sampling

Fishes were fasted for 24 hours before sampling, blood was obtained from the caudal vein using 3 ml syringe within less than 3 minutes to minimize handling stress. The collected blood was divided into two tubes, one containing heparin as anticoagulant agent for haematological assessment and the other was anticoagulant free for biochemical estimation.

Haematological measurements were assayed within one hour of sampling using blood cell automated counter, red blood cells count (RBCs), haematocrit (HCT)%, haemoglobin content (Hb), platelets count, and white blood cells count(WBCs)were evaluated. Different blood indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated.

Anticoagulant free samples were used for serum preparation by centrifugation for 20 minutes at 1207 g within one hour of sampling, the sera samples were used for determination of malondialdehyde (MDA), protein carbonyl (PC), catalase (CAT), glutathione reductase (GR), sodium (Na^+), potassium (K^+), glucose (GLU), total protein (TP), cortisol and immunoglobulin M (IgM) while superoxide dismutase (SOD) was determined in RBCs lysate later on the same day with commercial kits. Absorbance values of samples and standards were measured using an UV spectrophotometer.

2.3. Statistical Analysis

Data were expressed as mean ± SEM of different treated groups compared to control ones. Normal distribution of all parameters was tested. The results were analyzed using one way analysis of variance (ANOVA) followed by Tukey (HSD) test to compare groups with each other and Dennett two-sided test for comparisons with the control group. $P<0.05$ was considered significant. All statistical analyses were performed using XLSTAT program.

3. Results

Results tabulated in table 1, 2 showed a significant change in RBCs, haematocrit, haemoglobin, platelets count and potassium level. Haemoglobin content, RBCs and haematocrit were significantly lower 8 g/l, 12g/l compared to control group. On the contrary, platelets count was significantly higher in the 8 g/l, 12g/l groups with respect to the control group and that with salinity 4g/l (Table 1).

Table 1. *Means ± SEM of haematological parameters of Nile tilapia O. niloticus treated with different concentrations of salinities for 14 days.*

Parameter	*Salinity concentration*				*P*
	Control	(4 g/L)	(8 g/L)	(12g/L)	
Erythrocytes (10^6/µl)	1.89± 0.13^{a}	2.02± 0.08^{ab}	1.88± 0.08^{ab}	1.83± 0.14^{b}	0.046
HCT (%)	22.22± 0.78^{ab}	24.28± 0.85^{a}	19.56± 0.91^{b}	19.53±0.88^{b}	<0.01
Hb (g/dl)	6.31± 0.38^{ab}	7.01 ±0.22^{a}	5.69± 0.27^{b}	5.78± 0.30^{b}	< 0.001
platelets (10^3/µl)	120±1.50^{a}	130.79± 11.59^{a}	210± 7.15^{b}	203.± 8.28^{b}	< 0.0001
Leucocytes (10^3/µl)	4.760±2.22	4.587± 1.86	4.116± 2.19	5.215±1.164	NS
MCV (fl)	117.5± 7.45	122.83 ±4.31	106.21± 3.23	113.77± 7.75	NS
MCH (Pg)	33.38± 0.75	32.99 ±1.73	30.56± 0.89	32.99± 1.73	NS
MCHC (g/dl)	28.4± 1.36	28.67 ± 0.48	28.81± 0.36	29.58 ± 0.81	NS

Values are means ±S.E.M. Values with different superscript letters within each row are significantly different (analysis of variance, $P<0.05$).

On the other hand, superoxide dismutase activity, serum total protein and catalase activity were significantly lower in (4g/l, 8g/l and 12g/l, respectively) compared to the control group. Other parameters such as MDA, PC and GR did not show any significant differences. The highest levels of GR were in the 4g/l group; while the highest levels of MDA and PC was found in that with salinity 12g/l.

No significant differences were found also between different salinity treated group in sodium, glucose, cortisol, and IgM. The highest levels of glucose and total protein were found in 4g/l group; levels of sodium and IgM were found in12g/l group. Potassium concentration in all treated groups was significantly higher than the control group (Table 2).

Table 2. *Means± SEM of biochemical parameters of Nile tilapia O. niloticus treated with different concentrations of salinities for 14 days.*

Parameter	*Salinity*				*P*
	Control	(4 g/L)	(8 g/L)	(12g/L)	
MDA (nmol/ml)	29.06±5.67	29.18± 3.43	33.72± 4.93	36.72± 5.81	NS
PC (nmol/mg)	301.73±20.27	271.40± 26.72	293.60±36.46	338.51±58.70	NS
SOD(U/ml)	362.74±3.10^{a}	341.37± 6.93^{b}	355.23± 5.29^{ab}	356.32± 3.76^{ab}	< 0.016
CAT (U/L)	619.02±56.43^{a}	541.6± 25.3^{ab,}	614.36± 42.48^{a}	446±40.1^{b}	< 0.001
GR (U/L)	38.18±4.43	46.07± 5.31	34.94± 5.21	32.80± 6.06	NS
Sodium (mEq/L)	150.40±1.34	148.21± 2.76	147.79± 1.46	160.71± 6.37	NS
Potassium (mEq/L)	6.12±0.75^{a}	12.38± 2.07^{b}	12.65± 1.67^{b}	13.30± 1.15^{b}	< 0.05
Glucose (mg/dl)	72.88±2.98	78.20± 4.41	67.8± 6.48	65.54± 3.03	NS
Serum total protein (g/dl)	6.85±0.14^{a}	6.57± 0.22^{a}	5.97± 0.20^{b}	6.40± 0.32^{a}	0.043
Cortisol (ng/ml)	194.15±41.21	163.70± 23.71	183.73± 22.48	159.26± 24.48	NS
IgM (mg/dl)	193.46±14.71	189.06± 10.61	206± 24.82	232.08± 31.00	NS

Values are means ± S.E.M. Values with different superscript letters within each row are significantly different (analysis of variance, P<0.05).

4. Discussion

Tilapia culture needs more precise information on stress effect and control to ensure fish good health conditions, particularly those introduced to a new environment. Haematological parameters can be good indicators of the physiological condition for fish farmers, which are crucial to prevent and control of stress-induced pathologies due to environmental changes [24].

The current study results showed a significant effect of different salinity levels on some estimated parameters. The results revealed a decrease in both haemoglobin content, RBCs and haematocrit in 8g/l and 12g/l treated groups, These findings are in agreement with other researcher who found a significant effect of salinity on RBCs, HCT and Hb in different species, this result may be associated with osmoregulatory dysfunction induced by high salinity levels [25-27]. Low haematocrit percentages in fishes under stress could be explained by reduced volume of RBCs due to osmotic changes caused by ion leakage from the plasma [28]; while no significant differences were found in MCV, MCH and MCHC values in treated groups, these results are in according with the findings of previous results [27, 29, 30].

White blood cells count in fishes is a good indicator of physiological stress [31]. The current study results showed an increase in the leucocytes count in the highest salinity treatment (12g/l), similar results were found in rainbow trout, *Oncorhynchus mykiss*, placed in high salinity levels showed increased leucocytes count compared to those placed in fresh water [32], and silver barb, *Barbonymus gonionotus*, when placed in brackish water [33]. This elevation may result from the interaction of prolactin and cortisol in a non-specific immune response [27].

Although WBCs are the main immune cells, in fishes, thrombocytes have the capability to play a role immune response by producing and releasing a wide array of bioactive proteins [34] and their ability of phagocytosis [35].

Platelets count in the 8g/l and 12g/l groups increased significantly compared to control and 4g/l group. Elevation in platelets count may indicate a non-specific immune response due to stress induced by high salinity [32, 33]. These results are in agreement with the significant increase in thrombocytes due to increased salinity levels in *Tilapia guineensis* [30].

Superoxide dismutase activity and catalase activity play an important role to protect cells against H_2O_2 production, [36]. In the current study, SOD and CAT activity were significantly lower in (4g/l and 12g/l, respectively) compared to the control group. Similar results were found in *Acipenser naccarii* where SOD and CAT activities were higher in (12g/l and8g/l, respectively) compared to the other groups [37]. Increased SOD activity appears to be an adaptive response to increased generation of the superoxide anion [38]. Where catalase activity decreased in *Apostichopus japonicas* when exposed to 20% salinity for 72 hours. Moreover, 10 ppm salinity induced elevation in catalase activity in *Dicentrarchus labrax* [11].

It was documented that stress usually causes imbalance in hydromineral [39]. Moreover, electrolytes are a good indicator of osmoregulation problems [40]. In the current study, the highest sodium level was found in the highest salinity treatments. This result is may be to effects increase salinity on renal tissue according findings [41]. The present study showed that serum K^+ concentrations had a positive relation to salinity level as it increased with increasing salinity, the same pattern was found in *O niloticus*, by Karsi and Yildiz (2005), where plasma K^+ increased significantly following direct transfer from fresh water to different

salinities. Moreover, did not find any significant change in both serum sodium and potassium levels associated with different salinity levels [26, 42].

Glucose concentration is maintained within very narrow limits, regulated by hormonal control, even in fasting state, because glucose is the main source of energy for the central nervous system. Glucose concentration in groups 8g/l and 12g/l decreased by increasing salinity; while increased in group 4g/l compared to the control group, this result may be due to the high rate of glycogenolysis to meet high energy requirements to overcome stress [43, 44]. Moreover, Fishes live in high salinities level could be consuming more amounts of glucose to cover the higher energy demands of different osmoregulatory organs as observed in other euryhaline fish species [45, 46].

Total protein is also thought to be related to a stronger innate immune response in fish [47]. In the present study, total protein was significantly lower in 8g/l group compared to the control group. This decrease in plasma proteins with an increment of salinities may be the high osmoregulatory vitality request. This result is in agreement with other studies where protein levels were significant decline with increased salinity [48, 49].

Cortisol is well known to suppress fish immune functions in relation to various stresses [50]. In the present study, the highest level of cortisol was found in the 8g/l group; while its lowest concentration was detected in the 12g/l group. The increase of plasma cortisol value is considered to be a primary indicator of stress response [51]. Similar results were found in tambaqui, (*Colossoma macropomun*), where higher salinity levels caused an increase in cortisol level [52], Moreover, in tongue sole (*Cynoglossus semilaevis*), cortisol levels were low in moderate salinity levels [53].

IgM is the main class of antibody molecules and has been previously detected in fish mucus [54]. In the present study, IgM was highest in the 12g/l group, this result might be explained by the effects increase salinity on IgM, the similar increment was found when tilapia (*O. mossambicus*) was transferred from fresh water to seawater [17].

5. Conclusion

In conclusion, the present study showed that change in salinity concentration leads to stress that of the metabolic, biochemical as well as hematologic parameters in order to adapt the environmental changes to overcome oxidative stress.

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