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GENE EXPRESSION PROFILING AND PHYSIOLOGICAL ADAPTATIONS OF RED TILAPIA (*Oreochromis* sp.) UNDER DIFFERENT SALINITY LEVELS

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ABSTRACT

Aquaculture development depends on the availability of water. It is anticipate that climate change will alter the hydrological cycle, having significant consequences on the availability of water. One hundred twenty fish were reared in available sources of water with different levels of salinity 0.5,5,20 and 38ppt to investigate their effects on the following : growth performance, survival rate, haematological, biochemical, histological indicators and gene expression of stress-related genes (HSP27 and HSP70), ion-regulation genes (NKA α-1a and NKA α-1b) and immune-related genes (IgM) for red tilapia, *Oreochromis* sp. for 45 days. Analysis of variance showed no significant differences in weight gain of fish was observed among all groups with no mortality. No significant differences of haematological parameters among treatments, while biochemical parameters were affected significantly. Also, the mRNA expression levels of genes significantly affected ($p \leq 0.05$). NKA α-1a was reduced and α-1b upregulated with salinity exposure. A higher expression of liver-IgM at 38ppt than gills. Regarding the histological examination of gills and liver; two types of hepatic neoplasms and severe damage, vascular congestion, telangiectasis and round cells infiltration in gills in both high and low salinity was observed. Results deduced that, red tilapia has a wide range of adaptability to salinity, and it can be encouraged to rear in underground and sea water as aquaculture.

INTRODUCTION

Climate change and human effects have recently caused extensive high-temperature waves and high salinity, which have had a negative impact on the ecosystem and all human activities (aquaculture and agriculture) **(Gallardo-Hidalgo *et al*., 2021).** Fish growth performance, reproductive behaviour and immune responses can be adversely affected due to changes in water temperature and salinity **(Velmurugan *et al*., 2019)**. Salinity is a physical factor that exerts significant influence on various sides of physiology, including growth, osmoregulation, reproduction and more. All levels of biological organization, from the molecular to the organismal level, are affected، which are widespread and have an impact on a variety of physiological and biochemical functions. Based on the salinity tolerance range, fish can be divided into two categories: euclidean and stenosalin. Tilapia is one of the important a global aquaculture in world-wide, which accounted for 8% of the world's total fish production in 2016 **(FAO, 2018)**. As well, tilapia is highly valuable economically and ranks fourth among all fisheries commodities. Most countries pay special attention to tilapia cultivating Due to the high demand, market value and fish's tolerance to both of biotic and abiotic stressors **(Dawood *et al*., 2021; Mugwanya *et al*., 2021)**. The hybrid red tilapia is

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considered as being the vast significant for industrial fish farming. One of the main factors contributing to its the ease with which it adapts to culture conditions and confinement, as well as its saline tolerance and attractive colour, which adds to the retail value **(Brol *et al*., 2017; He *et al*., 2017; Nakphet *et al*., 2017**). Despite some previous studies conducted on the tilapia at different salinities (**Ninh *et al*., 2015; Gan *et al*., 2016**). Until now, there have only been a few studies that have explored the molecular-level genetic factors involved in fish adaptation to different salinity levels. This study aimed to estimate the effects of different salinity levels on red tilapia growth performance, survival rate, haematological, biochemical, histological indicators and the expression of genes implicated in osmoregulation.

MATERIALS AND METHODS

Experimental Design

This work was carried out at the Fish Research Centre (FRC), Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt. One hundred and twenty fish in twelve aquaria (60 × 40 × 50 cm; with water capacity of 80 L for each aquarium) were stocked at four treatments with different levels of water salinity. Ten fingerlings with initial weight (20 ± 2.0 g) and body length (8 ± 0.5 cm) were placed in each aquarium. Four treatments with three replicates for each treatment were performed. T1 (0.5 ppt obtained from Dechlorated tap water), T2 (5 ppt obtained from artesian well water), T3 (20 ppt obtained from artesian well mixed with sea water) and T4 (38 ppt obtained from sea water). The experimental period was conducted for 45 days.

Fish mortality was checked every day. Each aquarium's water was replaced daily with about one-third of its pre-adjusted water salinity, and completely each week. A 12 hr L: 12 hr D cycle was used to maintain the photoperiod. Fish pellets with 32% crude protein, ether extract 5.4%, crude fibre 4.8%, energy 3870 Kcal/kg from Skretting Egypt for Animal nutrition Company, Sharkia Governorate, Egypt at a ratio of 3% of their total biomass for fingerlings three-time per day at 08:00 am, 12:00 pm and 15:00 pm. The sea water was diluted by adding underground water to reduce the salinity rate to 20 ppt. The salinity levels in each treatment were adjusted by the refractometer. Fish wastes were removed from each aquarium every day. Fish were weighed and the diets were adjusted weekly during the experimental period, in accordance with the new body weight.

Fish samples were taken twice during the experimental period. The first sample was taken after 20 days. Gills and livers were taken from fish from each aquarium for assessment their physiological status and gene expression. The second sample was taken after the trial end. All fish were caught from each experimental aquarium, dissected, weighted, and collected blood samples to determine their hematological, physiological parameters, growth performance, feed utilization, histological investigation, and gene expression.

Growth Performance

These equations were used to measure growth performance: Weight gain (WG) = final weight (g)–initial weight (g); Relative weight gain % (RWG) = (WG/Wi) x 100; Average daily weight gain (ADWG) g/day = WG/t; Condition factor (K) = (W/L^3) x 100, where, W is weight of fish in g and L is total length of fish in cm; specific growth rate (SGR) = (LnWf – LnWi)/ t X 100, Where, Ln is the natural log; Wi is initial body weight and Wf is the final body weight in grams and "t" is the experimental period in days; Survival Rate (SR%)= Nf - Ni, Where, Nf = Number of fish at the end and Ni= number of fish at the beginning of the experiment.

Water Quality Measurement

The following parameters were measured weekly during the experiment: temperature, salinity, pH, dissolved oxygen (Do)and total ammonia-nitrogen (TAN). Temperature and pH were measured using an electric digital pH meter model (EZDO 7200). Do was measured by Hanna instrument HI8543 Oxygmeter. TAN was measured by ammonia nesslerization according to the method of **Eaton *et al.* (1992)**. Salinity was determined using hand refractometer S-28E (Salinity) Atago Salt 0-28 %.

Blood Sampling

After experimental duration, Fish were immediately anesthetized by clove oil at 0.05 ml per 500 ml of water according to the method of **Fernandes *et al.* (2016)**. Blood was extracted with a syringe from fish heart. The extracted blood was divided into two groups placed in two clean tubes. The first tube contained EDTA (Ethylene Diamine Tetra Acetic Acid) used as an anticoagulant for haematological indices. The second tube was used for biochemical parameters, the blood leave to clot then centrifuged at 3000 rpm for 15 min at room temperature. the serum was extracted from this tube and kept in Eppendorf tube at -20ºC until use. Most of the selected biochemical parameters were performed after collecting the blood samples directly.

Hematological Parameters

The method of **Dacie and Lewis (1991)** was used to calculate the red blood cells (erythrocytes) and white blood cells (leukocytes) (RBCs-WBCs) count. Platelets count (thrombocytes) was determined according to the method of **Brecher *et al.* (1953)**. Erythrocyte indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to **Seiverd (1964)**. Hemoglobin concentration (g dl^{-1}) was determined according to **Drabkin and Austin (1932)**. Hematocrit value was measured according to **Sorrell-Raschi and Tomasic (1998)**.

Biochemical Parameters

The method of **Doumas *et al.* (1975)** was used to determine total protein and albumin. While, globulin, was calculated according to **Kapale *et al.* (2008)**. Serum creatinine was measured according to **Henry (1974)** and according to method of **Reitman and Frankel (1957)** aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined.

Gene Expression

The liver and gill tissue samples were collected, saved in RNAlater, and stored at − 20°C then RNA extracted using QIAzol® reagent according to the instructions of manufacturer. The total RNA was isolated from each replicate of samples according to **Chomczynski and Sacchi, (1987)**. The quality of each RNA was determined by 1% agarose gel electrophoresis and a nanophotometer at 260/230 nm (Bio Drop, UK), and purity was evaluated at 260/280 ratio. Finally, its integrity and concentration were detected. Isolated RNA was converted to a cDNA using COSMO cDNA Synthesis Kit. Based on the manufacturer's instructions. PCR was used to check the cDNA using (β-actin) as a housekeeping gene.

Quantitative Real-time PCR

The qRT-PCR reaction was used to examine and analysis the gene expression of stress-related genes (HSP27 and HSP70)، ion-regulation genes (Na+/K+-ATPase (NKA) α-1a and α-1b) and immune-related genes (IgM) in both of liver and gills of red tilapia (Table 1).

Histological Examination by Light Microscopy

Samples from gills and livers were collected from five fish per each treatment. For about 24 hours, samples were fixed in Bouin's solution then preserved in 70% ethyl alcohol, after that cleared in xylene and finally embedded in paraffin wax as usual. On chemically clean glass slides, sections of 4-6 thickness were mounted. After the sections were prepared, Harri's Hematoxylin and Eosin (Hx and E) was used to stain them according to **Pearse (1972)**.

Table 1. The primers used to amplify the studied genes according to (NCBI)

Gene name	Primer sequence (5′ -3′)	Accession No.	Reference
HSP27	**F-** CTGAGGAGCTGGTGGTGAAG **R-**GATCAAAGGAGCCTCCACGGA	NM_001279530 .1	**(Hassan *et al*., 2017)**
HSP70	**F-** CTCCACCCGAATCCCCAAAA **R-** TCGATACCCAGGGACAGAGG	XM_019357557 .1	**(Hassan *et al*., 2017)**
NKA α-1a	**F-**AACTGATTTGGTCCCTGCAA **R-** ATGCATTTCTGGGCTGTCTC	GR645170 and GR644771	**(Qiang *et al*., 2012)**
NKA α-1b	**F-**GGAGCGTGTGCTTCATCACT **R-** ATCCATGCTTTGTGGGGTTA	TMU82549	**(Tipsmark *et al*., 2011)**
IgM	**F-**AGGAGACAGGACTGGAATGCACAA **R-** GGAGGCAGTATAGGTATCATCCTC	KJ676389.1	**(Phuyindee *et al*., 2011)**
β-actin (endogenous control)	**F-**GTACCACCATGTACCCTGGC **R-** TGAAGTTGTTGGGCGTTTGG	FN673689.1	**(He *et al*., 2017)**

Statistical Analysis

The statistical analysis system program, version 6.03 **(Statistical Analysis System, 1996)**, was used to analyze all the data. The individual effects of the factors were analyzed using a one-way ANOVA, and the results are shown as means with pooled standard error (SE). Duncan's new multiple range test was used to compare means **(Zar, 1996)**. The $2^{-\Delta\Delta CT}$ method was used to determine the relative expression of the target gene (**Livak and Schmittgen, 2001**).

Ethical Approval

The authors adhered to all applicable national, institutional, and/or international standards for the handling and use of fish. Arish University's ethical committee, research code :AGRI-04.

RESULTS

Growth Performance

Table 2 was presents growth performance and survival rate of red tilapia exposed to various levels of salinity. The initial body weight (IBW) was insignificant differences among the treatments. Results indicated a homogeneous distribution of the experimental treatment at the beginning of the study.

No significant differences were noted among treatments at most growth performance parameters. We recorded the highest value for each of FBW, WG, ADWG, SGR and RWG by treatment with salinity 38 ppt, then 0.5ppt. While the lowest one obtained through treatment with salinity (5and 20 ppt). Additionally, there were insignificant differences in survival rate (SR%) among various treatments.

Hematological and Biochemical parameters

No significant differences ($P>0.05$) were opined among various groups in RBCs, WBCs, Hb, Hct, PLT, MCV and MCH. While MCHC was significantly affected ($p \leq 0.05$), it was increased with salinity decrease. The highest values of MCHC (33%) was obtained at low salinity 0.5 ppt, while the lowest value was recorded at each of 38 ppt, 5 and 20 ppt.

Table 2. Effect of salinity on growth performance and survival rate of red tilapia

Parameter	Treatment			
	0.5 ppt	5ppt	20 ppt	38 ppt
IBW[1] (g fish $^{-1}$)	19.86±0.06	19.93±0.13	20 ±0.11	20.20±0
FBW[2] (g fish $^{-1}$)	24.78±0.79	23.29±0.68	23.51±1.01	25.48±1.41
WG[3] (g fish $^{-1}$)	4.92±0.74	3.36±0.55	3.51±1.02	5.28±1.41
ADWG[4] (g/day)	0.10±0.01	0.07±0.01	0.07±0.02	0.11±0.03
RGR[5] (%)	24.75±0.01^{a}	16.83±2.64^{b}	17.59±5.11^{b}	26.14±6.99^{a}
SGR[6] (%d^{-1})	0.48±0.06	0.34±0.04	0.35±0.09	0.50±0.12
SR (%)	96.66	100	100	100
IBL[8] (cm fish $^{-1}$)	9.68± 0.04	9.56±0.16	9.53±0.15	9.70±0.15
FBL[9] (cm fish $^{-1}$)	10.89±0.12	10.68±0.18	10.64±0.18	10.90±0.25
LG[10] (cm fish $^{-1}$)	1.21±0.10	1.12±0.10	1.11±0.06	1.20±0.10
K[11]	1.91±0.01	1.91±0.06	1.94±0.02	1.96±0.03

Values mean ± SE and different letters at same row indicate significant difference ($p \leq 0.05$).* 1.initial body weight,2. final body weight,3. weight gain,4. average daily weight gain,5. relative growth rate, 6. specific growth rate,7. survival rate,8. initial body length,9. final body length,10. length gain ,11. condition Factor.

Table 3. Hematological variables in red tilapia under different salinity levels

Parameter	Treatment			
	0.5 ppt	5ppt	20 ppt	38 ppt
RBCs ×10^{6}	1.85±0.14	1.47±0.24	1.51±0.18	1.92±0.02
WBCs×10^{3}	41.90±2.89	40.70±1.76	36.40±3.75	46.73±5.73
Hb (g dl^{-1})	5.56±0.43	4.43±0.73	4.53±0.56	5.76±0.08
Hct (%)	16.70±1.30	13.30±2.20	13.60±1.68	17.30±0.26
Platelets ×10^{3}	420.33±16.41	570.33±128.02	417.66±13.29	638.33±181.13
MCV (fl)	89.33±0.33	90±1.73	89±1.15	89±0.57
MCH (pg)	28.10±0.66	29.70±0.94	28.10±0.11	29.50±0.83
MCHC (%)	33^{a} ±0.00	32^{ab} ±0.57	32^{ab} ±0.57	31.33^{b} ±0.33

Values mean ± SE and different letters at same row indicate significant difference ($p \leq 0.05$).

Biochemical Parameters

Results in Table 4 presents the effect of different salinity levels on biochemical parameters. Serum albumin, globulin, A/G ratio and total protein were significantly differed within treatments. The highest most values of these parameters were recorded in 38 ppt group, except globulin the highest value was observed where in 0.5 ppt and 5 ppt. While no significant differences (P>0.05) were observed among different groups in creatinine. Creatinine was increased tendency when exposed to 20 ppt and decreased at 0.5 ppt. A gradual increase in AST and ALT activities were found under different levels of salinity, without significant differences among treatments (shown in Fig. 1).

Gene Expression

Expression analysis of stress-related genes (HSP27 and HSP70)

Analysis of relative gene expression levels of HSP27 and HSP 70 in liver and gills are shown in Figs. 2 and 3, respectively. In the liver, after 20 days of salinity-exposing, no significant difference (p>0.05) down regulation of HSP27 gene expression was observed in all treatments. Only a slight increase 1.49-fold was observed at 20 ppt group. Regarding other groups, HSP27 gene expression was down regulated at 0.5 ppt group. With increasing the exposure time of salinity to 45 days, gene expression levels of HSP27 were significantly ($p \leq 0.05$) up regulation. It was upregulated 2.14-folds at 20 ppt group. While a slight decrease was observed at 5 ppt group (Fig. 2). Fish liver HSP70 mRNA expression level showed significant ($p \leq 0.05$) down regulation 1.01- and 1.16-fold in both 5 ppt and 20 ppt groups, respectively. While it was upregulated significantly at 0.5 ppt and 38 ppt salinity concentration to 1.92- and 1.40- fold, respectively, after 20 days of salinity exposure. With increasing the time of salinity exposure to 45 days, HSP70 expression was still up regulated in red tilapia liver in response to stress caused by elevated salinity at 0.5, 5 and 38 ppt. However, it was downregulated 0.39-fold in 20 ppt group (Fig. 2).

In the fish gill, after 20 days of salinity-exposing, the gene expression of HSP27 expression was significantly ($p \leq 0.05$) upregulated in 0.5, 5 and 38 ppt groups. However, it was down regulated at 20 ppt group to 0.51-fold. With increasing the time of salinity exposure to 45 days, HSP27 expression was up regulated in red tilapia gills in response to stress caused by elevated salinity in 0.5 ppt and 38 ppt groups to 4.46 and 4.56-fold, respectively. While it was down regulated significantly at 5 ppt and 20 ppt salinity concentration to1.0 and 1.64-fold, respectively (Fig. 3).

Fish gills HSP70 mRNA expression level showed significant ($p \leq 0.05$) up regulation in 0.5 ppt group to 5.35-folds. While it was down regulation with increasing the water salinity. However, after 45 days, 38 ppt salinity group showed induced significant ($p \leq 0.05$) up regulation of HSP70 expression in the gills to 3.95-folds, as shown in (Figure. 3). While it was down regulation with decreasing salinity to 0.72-fold at 0.5 ppt group. It is markedly up regulated when the experimental fish were exposed to high salinity for long time.

Expression analysis of ion-regulation genes (NKA α-1a and α-1b)

Different expression patterns of NKA α1-a and α1-b in the liver and gills were detected in Figs. 4 and 5. In the liver, NKA α1-a significantly ($p \leq 0.05$) increased to 1.0-fold at 0.5 ppt group compared to other groups at a period of 20 days. It was significantly down regulated at 0.5, 20 and 38 ppt groups to 0.67-, 0.57- and 0.41-folds, respectively. After 45 days, liver NKA α1-a mRNA expression level revealed significant ($p \leq 0.05$) up regulation in 0.5 ppt group to 3.29-folds (Fig. 4). While lowest value of

Table 4. Biochemical variables in red tilapia under different levels of salinity

Parameter	Treatment			
	0.5 ppt	5ppt	20 ppt	38 ppt
Albumin (g dl^{-1})	2.55±0.47^{b}	2.37±0.32^{b}	3.66±0.73^{ab}	5.10±0.50^{a}
Globulin (g dl^{-1})	4.80±0.36^{a}	4.56±0.20^{a}	3.40±0.50^{b}	3.06±0.18^{b}
A/G ratio	0.54±0.13^{b}	0.52±0.09^{b}	1.17±0.33^{ab}	1.70±0.23^{a}
T. protein (g dl^{-1})	7.35±0.29^{ab}	6.94±0.13^{b}	7.06±0.24^{b}	8.16±0.46^{a}
Creatinine (mg dl^{-1})	0.66±0.28	1±0.32	1.51±0.04	1.37±0.34

Values are mean ± SE and different letters at same row indicate significant difference ($p \leq 0.05$).

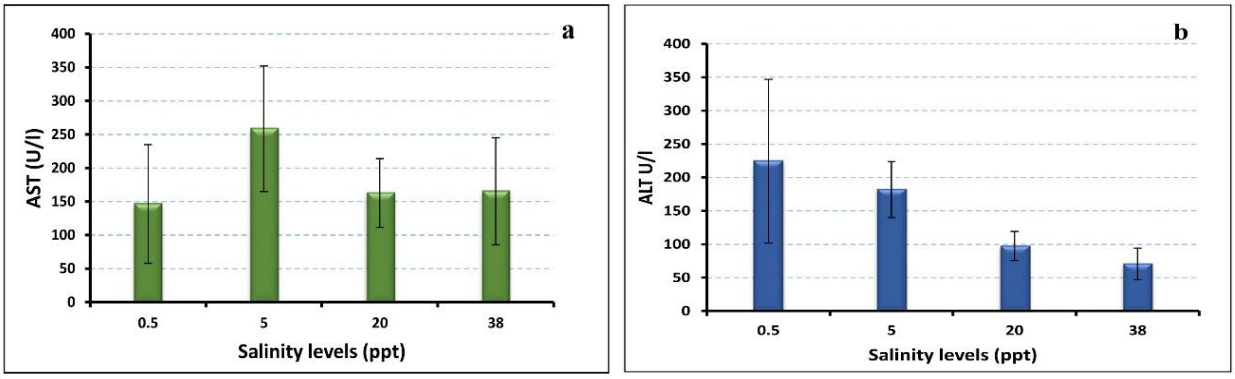

Fig. 1. Effect of different levels of salinity on aspartate transaminase (AST) (a), alanine transaminase (ALT) (b) in red tilapia

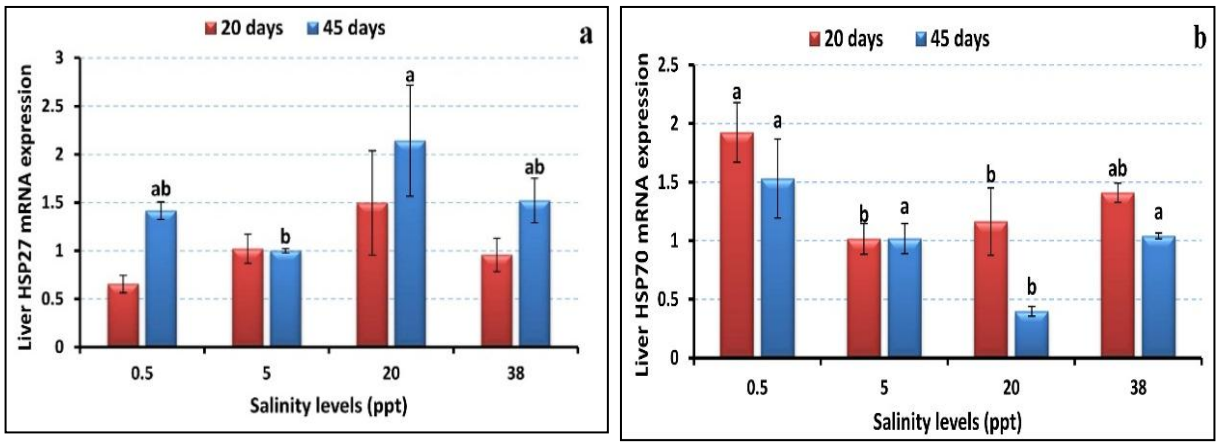

Fig. 2. Relative gene expression levels of HSP27 (a) HSP70 (b) in liver tissue of red tilapia exposed to different levels of salinity for 20 days and 45 days. The results were normalized using the β-actin gene as a reference gene. The results are displayed as the mean ± SE (n=3), with different letters denoting significant differences ($p \leq 0.05$)

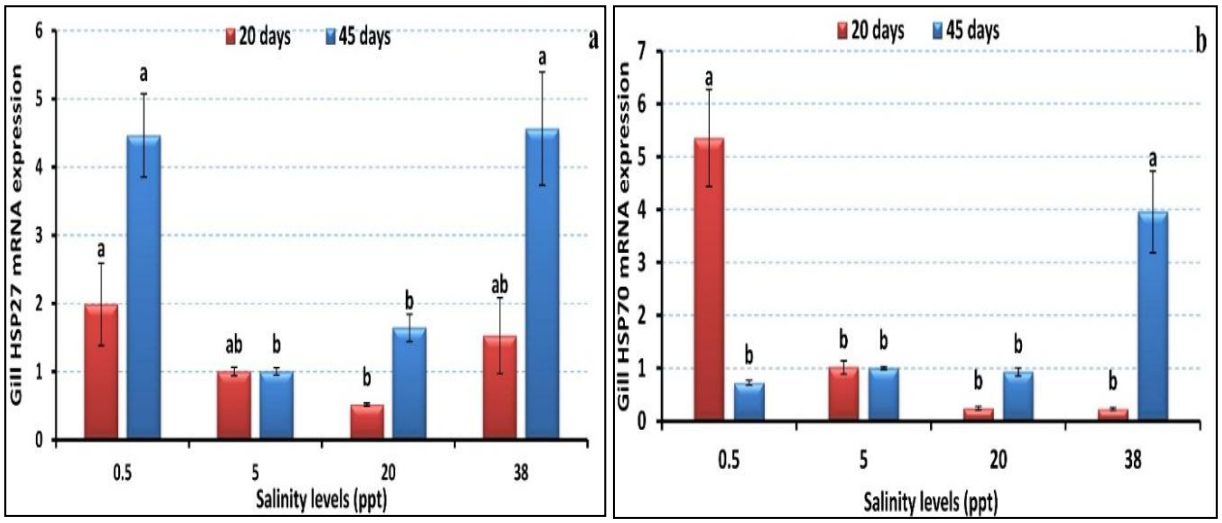

Fig. 3. Relative gene expression levels of HSP27 (a) HSP70 (b) in gill tissues of red tilapia exposed to different levels of salinity for 20 days and 45 days. The results were normalized using the β-actin gene as a reference gene. The results are displayed as the mean ± SE (n = 3), with different letters denoting significant differences ($p \leq 0.05$)

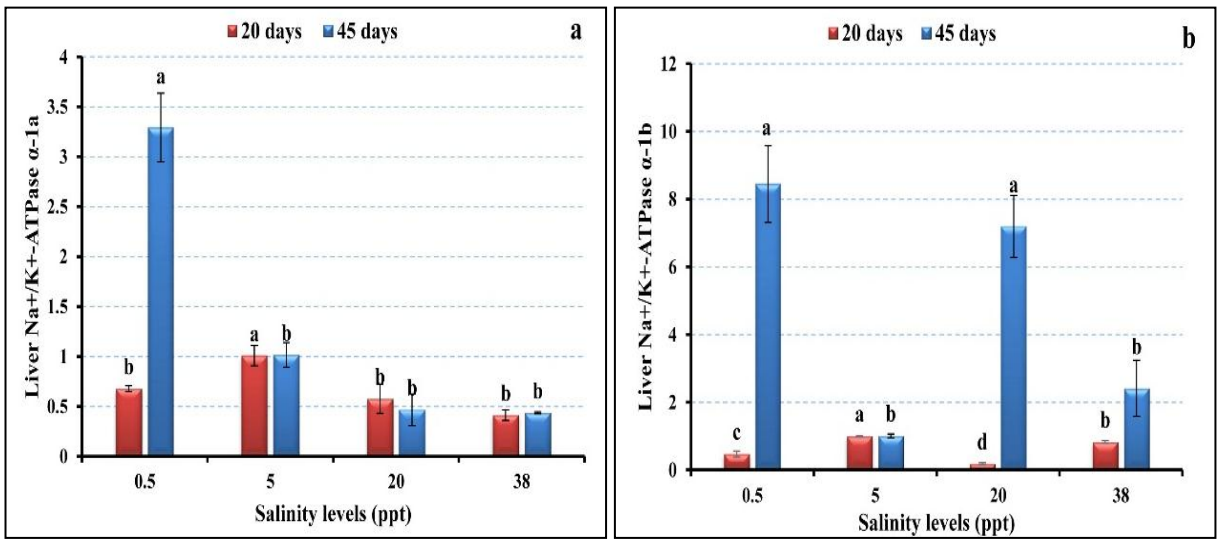

Fig. 4. Relative gene expression levels of NKA α1-a (a) NKA (b) in liver tissues of red tilapia exposed to different levels of salinity for 20 days and 45 days. The results were normalized using the β-actin gene as a reference gene. The results are displayed as the mean ± SE (n = 3), with different letters denoting significant differences ($p \leq 0.05$)

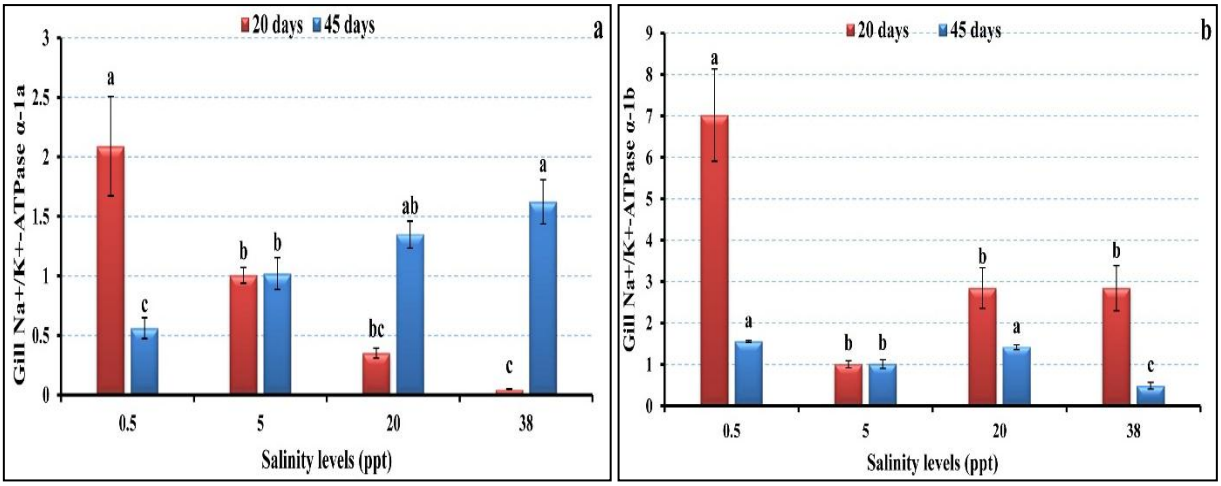

Fig. 5. Relative gene expression levels of NKA (a) NKA (b) in gill tissues of red tilapia exposed to different levels of salinity for 20 days and 45 days. The results were normalized using the β-actin gene as a reference gene. The results are displayed as the mean ± SE (n = 3), with different letters denoting significant differences ($p \leq 0.05$)

gene expression was obtained 0.43-fold at 38 ppt group. NKA α1-b mRNA expression level observed significant ($p \leq 0.05$) down regulation in 20 ppt group followed by 0.5, 38 ppt and 5 ppt groups which were 0.18, 0.47, 0.82 and 1.0-folds, respectively. However, gene expression levels of NKA α-1b showed significantly ($p \leq 0.05$) up regulation at 0.5 ppt group to 8.45-folds followed by 20 ppt group to 7.18-folds, after 45 days (Figure. 4). The fish gill NKA α1-a mRNA expression level showed a significant ($p \leq 0.05$) down regulation with increasing salinity for short-term (after 20 days). While it was up regulation significantly ($p \leq 0.05$) with increasing the time of salinity exposure to 45 days. NKA α1-a significantly ($p \leq 0.05$) increased 2.08-folds in 0.5 ppt group compared to 5 ppt group and other groups after 20 days. It was significantly down regulated to 0.56-fold at 0.5 ppt group after 45 days. While the lowest value of gene expression was obtained at 38 ppt group after 20 days then it was significantly ($p \leq 0.05$) up regulated 1.62-fold after 45 days. In the fish gill, NKA α1-b mRNA expression level showed a significant ($p \leq 0.05$) up regulation with decreasing salinity for short-term after 20 days. While it was down regulation significantly ($p \leq 0.05$) with increasing salinity for long-term after 45 days. NKA α1-b significantly ($p \leq 0.05$) increased 7.01-folds in 0.5 ppt group compared to 5 ppt and other groups after 20 days and it was significantly down regulated to 1.55-fold after 45 days. The lowest value of gene expression was obtained at 5 ppt group after 20 days. After 45 days, the lowest value of gene expression obtained 0.48-fold in 38 ppt group (Fig. 5).

Expression analysis of immune-related genes (IgM)

After 20 days, in the fish liver, IgM gene expression showed significant ($p \leq 0.05$) up regulation at 38 ppt group to 36.34-fold. Meanwhile, it is significantly down regulated to 0.54-fold at 0.5 ppt group compared to the other groups as shown in (Fig. 6a). However, decrease of IgM expression with non-significant ($p > 0.05$) differences at 20 ppt group to 0.74-fold after 45 days followed by 0.5 ppt group to 0.82-fold, then 1.02-fold at 5 ppt group and 1.55-fold at 38 ppt group.

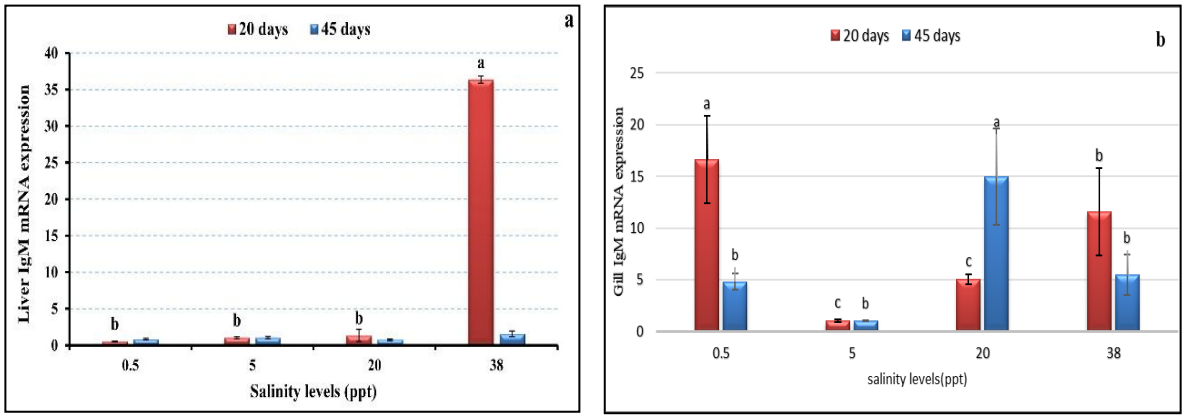

Fig. 6. Relative gene expression levels of IgM in liver (a) and gill (b) tissues of red tilapia exposed to different levels of salinity for 20 days and 45 days. The results were normalized using the β-actin gene as a reference gene. The results are displayed as the mean ± SE (n = 3), with different letters denoting significant differences ($p \leq 0.05$)

After 20 days, in the fish gills, IgM gene expression showed significant difference ($p \leq 0.05$) up regulation at 38 ppt group to 11.56-folds, followed by 0.5 ppt group to 16.59 -folds then 20 ppt group to 5.02-folds and 5 ppt group to 1.01 -fold as shown in (Fig. 6b). After 45 days, slight up regulation was observed at 20 ppt group to 14.96; followed by 38 ppt, 0.5 ppt and 5ppt groups as shown in (Fig. 6b).

Histological Investigation of Gills and Liver

The Gills

Examined sections from gills of red tilapia exposed to different levels of water salinity for 45 days showed marked vascular congestion, telangiectasis and round cells infiltration in all groups. However characteristic multi focal squamous and or chloride metaplastic changes were recorded in groups 0.5 and 38ppt. Gill filaments and rakers epithelial metaplasia was also seen in groups 20 and 38ppt. Moreover, generalised inter and intra flammmatoy edema and epithelial denudation with occasional focal necrotic change could also be detected (Fig.7 a, b).

The Liver

Sections for liver of fish exposed to water salinity for 45 days showed mild hepato-portal vascular dilation, peri-portal edema, disorganized partially in-active pancreatic acini, focal and diffuse macro-steatosis, and peri-portal fatty cystic changes. Hepatic sinusoids were consequently compressed. Focal melano-macrophage aggregations were noted in group (38ppt). Two types of hepatic neoplasms were recorded which are, interstitial cell neoplasm in which ‘spindle cell proliferation’ consists of an unspecified population of spindle-shaped cells which replaces portions of the hepatic parenchyma. Although spindloid biliary epithelial cells (biliary epithelioma) have been found to comprise portions of some neoplasms by keratin immunocytochemistry, a substantial population remains uncharacterized and may include fibroblasts, endothelial cells and/or perisinusoidal cells. The second recorded type was hepatocellular adenomas. No other types of hepatic neoplasms could be recorded in the examined cases and further study protocols of a longer time schedule could provide more data in the future (Fig. 8 a, b).

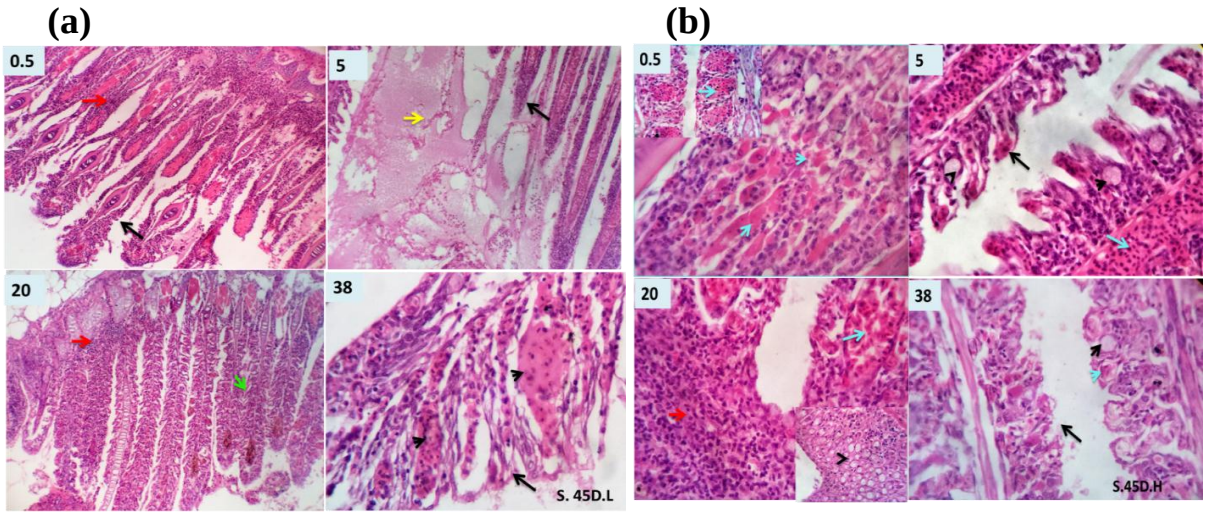

Fig. 7. Photomicrograph from gills of red tilapia exposed to different levels of water salinity for 45 days (a, b: low and high power magnification), showing marked vascular congestion, telangiectasis (light blue arrows, upper left corner window) and round cells infiltration in all groups (red arrow), however characteristic multi focal squamous and or chloride metaplastic changes are seen in groups (0.5 and 38ppt) (black arrow heads, lower right corner window, light blue arrow heads respectively). Gill filaments and rakers epithelial metaplasia was also seen in groups (20 and 38 ppt) (black arrow heads). Moreover, generalized inter and intra inflammatory edema and epithelial denudation with occasional focal necrotic change are seen (yellow arrow). H&E100, 200, 400 X

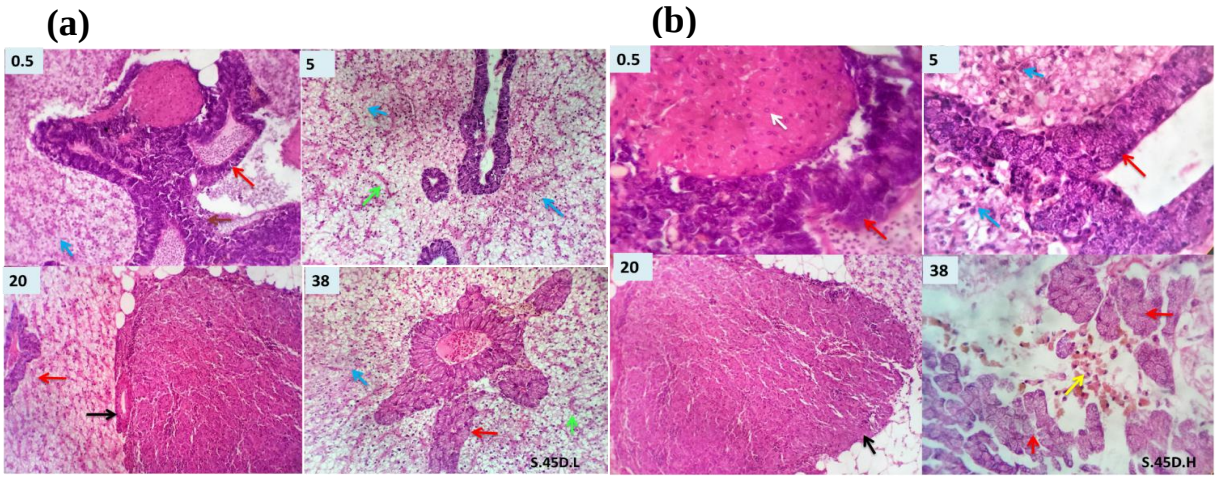

Fig. 8. Photomicrograph from liver of fish exposed to different levels of water salinity for 45 days (a, b: low and high -power magnification), showing mild hepato-portal vascular dilation, peri-portal edema, disorganized partially in-active pancreatic acini (red arrows), focal and diffuse macro-steatosis and peri-portal fatty cystic changes (blue arrows and star respectively). Hepatic sinusoids appear compressed (green arrows). Focal melano-macrophage aggregation is seen in group (38ppt) (yellow arrow). Two types of hepatic neoplasms are observable which are (biliary epithelioma) (white arrow) and hepatocellular adenomas (black arrows) .H&E100, 200, 400 X.

DISCUSSION

In the current study, no significant differences were noted between treatments on growth performance which was consistent with the results reported by **Young *et al.* (2022)** who presented that *Oreochromis* sp. fry production and grow-out farming were achievable under high salinity circumstances; it might be due to characteristics of the hybrid and numerous species of tilapia are euryhaline; tolerate variety range of salinity, mostly because of the descended from marine teleost's **(Suresh and Lin, 1992).** Also, it means that regulate the gradient concentration of salinity required a small amount of energy, saving energy that can instead be used to promote growth. These results are in agreement with **Martínez-Contreras (2003)** who stated that *Oreochromis* sp. hybrids compared to other species had greater growth and survival in higher salinities. Additionally, previous research revealed that decreasing growth-fish in extremely high or low salinity levels may has potential to suppress the appetite which is linked to a decrease in food consumption with a high energy demand for osmoregulation **(Mahmudul *et al.*, 2014).** Similarly, **Hassanen *et al.* (2014)** said that the best growth performance in 37 ppt with 24°C temperature.

In this study, the high value of survival fingerlings obtained in high and low level of salinity, and it's in agreement with **Fitwi, (2003)** who said that the magnitude of salinity adaptation can be achieved through rearing of healthy, well grown fish with lower mortalities at lower salt concentration. On the other hand, another species of tilapia (*O.mossambicus*) in a direct transferal to salinity levels from 0 to 35 ppt was zero mortality at all salinity concentrations up to 25 ppt and 100% at 35 ppt. similarly , **Sallam *et al.* (2017)** found that the best ultimate body weight, weight increase, average daily gain, specific growth rate and survival rate of red tilapia at 36 and 24 gL^{-1}, respectively, of water salinity and are in consistent with those reported by **Mirabent *et al.* (2020)** who showed that red tilapia fry can be adapted to seawater with a high survival rate, Contrary to what **Helmy and El Deeb (2018)** reported that by increasing the salinity levels, the mortality ratio of *O.niloticus* increased. And, with **Takishita *et al.* (2015)** who conducted a 45-day experiment on *O. niloticus* exposed to various salinity to investigate effect of water salinity on daily weight gain (0, 7, 14 and 21gL^{-1}). The best growth results were obtained on 0 and 7 gL^{-1} while increasing salinity levels caused decreased weight.

Hematological and biochemistry parameters are necessary to determine the health status of fish, as they provide important information for diagnosing fish diseases and used in determining the effect of endogenous and exogenous factors in fish. **(Alsaid *et al.*, 2014; Ahmed *et al.*, 2020)**. As a result of the above, Hematological, and biochemical responses were investigated in this study. The analysis of the results variance revealed that there were no significantly different between groups for RBCs, WBCs, Hb and Hct, these results corroborate **Sharaf *et al.* (2013)** who showed non-significant differences between Hb values in red tilapia in brackish and saline water.

In the current study, Hb increased with salinity increasing with non-significant differences, meanwhile, decreased on *O. niloticus* with significantly lower value at 35 ppt **(Bosisio *et al.*, 2017; Ali *et al.*, 2022)**. Our findings are supported by research on other tilapia species, including Tilapia *guineensis* **(Akinrotimi *et al.*, 2012)** and Mozambique tilapia **(Rauf and Arain, 2014)**. These findings might be explained by osmoregulatory dysfunction brought on by high salinity **(Fazio *et al.*, 2013; Soltanian *et al.*, 2016).** These

findings contrast with **Rani *et al*. (2016)**, which showed that *Labeo rohita* exposed to higher salinity levels (6 and 8 ppt) had lower haemoglobin levels, which caused the animals to reducing feeding because they were under stress.

Erythrocyte indicators: MCV, MCH and MCHC are crucial for determining the cause of anaemia in the majority of fish. **Coles, (1986)** found decrease in them with salinity increased. Due to a decrease in erythrocyte haemoglobin content, a decrease in (MCHC) and (MCH) could lead to hypochromic anaemia, which would result in a reduction in the colour of fish blood **(Zuckerman, 2007).** While **Elarabany *et al.* (2017)** reported that different salinity concentration induced changes in some hematological parameters on tilapia; RBCs were lower in the experimental groups (4, 8 and 12 gL^{-1}, respectively) than control group while, The Hct, Hb, PLTs was higher. Further, no obvious changes by salinity occurred in WBCs, MCV, MCH and MCHC. Regarding our experimental groups showed a significant effect of different salinity levels on some estimated biochemical parameters. Total protein, albumin and globulin are the principal serum proteins that are essential for the immunological response **(Wiegertjes *et al*., 1996).** All the biochemical indices except for creatinine were affected. In cases of severe kidney dysfunction, the kidneys' glomerular filtration process filtered serum creatinine, causing blood levels to rise. **Kulkarni and Pruthviraj, (2016)** found slight increased when exposed high salinity which mean that there is no dysfunction in kidneys. Albumin, A/G ratio and total protein increasing with salinity increasing. These finding in the same trend with **Abdel-Rahim *et al.* (2020)** who stated that in comparison to the groups reared at 8 and 16ppt salinity, the serum total protein, albumin, and globulin levels in *Argyrosomus regius* were significantly higher at 24 and 32 ppt salinity. And come into collision with **Ahirwal *et al.* (2021)** who noted that when exposed fish to 3 and 6 ppt salinities, fish's plasma protein levels were significantly lower than those of control fish (0 ppt salinity). Also, Liver function of red tilapia had been affected, there is a positive relationship between salinity and liver function. Similarly, **Al-Khshali and Al Hilali (2019)** reported that Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) increased with salinity increased to (5, 10 and 15 g/l respectively) in common carp. Also, when compared to their presence in fresh water, the ALT and AST enzyme concentrations in *Oncorhynchus keta* at the saline water were noticeably different, according to **Liu *et al.* (2010). Hegazi (2011)** mentioned an increase in the ALT and AST enzymes in the tilapia fish *O. niloticus*, as well as references to ongoing saline stress and fish breeding in high densities. With continuing exposure period to stress caused an increase in breaking down proteins in the cells, which in turn caused a decrease in the fish growth rate.

In the liver, the mRNA expression level of hsp70 was measured under different salinities. The results illustrated a significant up regulated in (Hsp70) mRNA expression at 0.5ppt, with 1.92- fold change after 20 days. Meanwhile, in gill the mRNA expression level of Hsp70 up regulation by fresh water (0.5ppt) to 5.35-folds, while it was down regulation with increasing salinity. The role of Hsp70 in fish acclimatization to salinity variations has been well documented in lot of experiments **Smith *et al*., 1999; Larsen *et al*., 2008)**. Conflicted with **Zhu *et al*. (2018)** who noted that the differences in the responses to saltwater could be attributed to differences in saltwater tolerance among the four tilapias (*Oreochromis mossambicus, O. urolepis hornorum, their hybrids O. mossambicus* ♀×*O. hornorum*♂ *and O. hornorum*♀×*O. mossambicus*♂).

In our study, we proved that with salinity exposure, the mRNA expression levels of NKA α-1a was reduced and α-1b increased, Similar to, **Wong *et al.* (2016)** observed that after transferring tilapia, medaka and salmon from freshwater to marine water, expression of NKA α1-a decreased and expression of NKA α1-b increased. Also, **El-Leithy *et al*. (2019)** reported a higher expression of NKA α-1b of the gill at 16 ppt and slightly increase at 20 ppt. Furthermore, **Tipsmark *et al*. (2011)** said that when freshwater-acclimated fish were transferred to seawater, NKA α-1a expression significantly decreased within 24 hr and α-1b expression increased, reaching its peak 7 days later. Additionally, α-1a expression increased within 2 days, whereas a-1b expression declined after 14 days, as an indicator of changes in expression in relation to salinity acclimatization in Mozambique tilapia.

The main role of endocrine-based switching between NKA isoform are certificated through the noted shutdown of NKA α1-a mRNA expression after hypophysectomy; meanwhile, α1-b expression was not affected **(Tipsmark *et al*., 2011).** Some authors suggest that the salinity adaptation induces a reciprocal switch between α1 (a and b) isoforms **(Bystriansky *et al*., 2006; Madsen *et al*., 2009; McCormick *et al*., 2009; Tipsmark *et al*., 2011; El-Leithy *et al*., 2019). Richards *et al*. (2003)** presented molecular genetic evidence supporting the expression of various isoforms of the α -subunit in rainbow trout gill tissue. The NKA α-1a isoform's mRNA level increases after transfer from seawater to freshwater, while the NKA α-1b isoform's mRNA level rises after the transfer from freshwater to seawater. Similar effects of salinity on these isoforms have been seen in Atlantic salmon (*Salmo salar* Linnaeus); additionally, During the parr-smolt metamorphosis, as juvenile salinity tolerance increases, the mRNA levels of gill NKA-1a and NKA-1b change in freshwater, NKA α -1a decreases while NKA α-1b increases **(Nilsen *et al*., 2007)**. **Blondeau-Bidet *et al.* (2016)** who found that expression of NKA α-1a in the gills and posterior intestine was significantly increased or decreased in low salinity. On the other hand, Branchial NKA α -1b, was significantly reduced in low salinity. Short-term adaptation appears to rapidly raise NKA α -1a transcript levels in the kidney, in contrast to gill tissues, where altered gene expression levels are only observed after long-term acclimatization and our finding in agreement with it.

It has been reported that at 16 and 20 ppt salt, the expression of the gills IgM gene showed a significant down regulation to the same level (0.01-fold) salt, and its expression in the kidney increased to 17.03 and 5.5-fold, respectively **(El-Leithy *et al*., 2019)** and our result have the same trend. Also, **Khansari *et al*. (2018)** noted down regulation of immune-related genes in sea bream and rainbow trout gills after exposure to various stressors. Likewise, **Huang *et al*. (2015)** found that the interaction between temperature and salinity increased the expression of IgM gene in kidney tissues more than in liver.

Kidney, gastrointestinal tract, and gill are thought to be the key organs in tilapia that regulate osmoregulation **(Evans *et al*., 2005; Sakamoto and McCormick, 2006)**. There are mitochondrial cells (MRs) epithelium of the gills, responsible for the capture of freshwater ions (FWs) and these creation of saltwater ions (SWs) **(Hirose *et al*., 2003)**. To keep equilibrium, the kidney's epithelial cells scan with stand various salinities and osmotic stresses **(Arun Kumar *et al*., 2020).** The examination of gill and liver histology provides valuable insights into the structural modifications that take place when organisms adapt to different salinity levels. In both laboratory and field research, histopathological alterations have been frequently employed

as biomarkers in the evaluation of the health of fish exposed to pollutants. The gills are the first osmoregulatory line and ensure that the isotonic balance is maintained **(Wilson and Laurent, 2002)**. Although the liver is not primarily an osmoregulatory organ, it is the primary site of glycogen/glucose turnover and, because of osmotic adaptation, its metabolism is enhanced to produce lot amount of biological fuel for osmoregulatory functions, particularly in major osmoregulatory organs such as kidney and gills **(Vijayan *et al*., 1996).** Regarding the histomorphological structure of the liver, two types of hepatic neoplasms at 38 ppt, no other types of hepatic neoplasms could be recorded in the examined cases and further study protocols of a longer time schedule could provide more data in the future.

In gills, severe damage, vascular congestion, telangiectasis and round cells infiltration in gills in both high and low salinity. Although there were no noticeable changes at 5 ppt, serious lesions, such as necrosis, lamellar fusion, and hyperplasia were revealed at 20 and 38 ppt in this study. In contrast to, **Takishita *et al*. (2015)** who reported that epithelial salinity increased by 10, 20, and 25 ppt while disregarding the occurrence of aneurysms and telangiectasia. And in agreement with **Ali *et al*. (2022)** who observed that fish exposed to salinities higher than 0 ppt have epithelial hyperplasia, lamellar fusion, epithelial lifting, and necrosis of the epithelium. And with **Mohamed *et al.* (2021)** who said that the histopathological transverse of *O. niloticus* exposed to Euryhaline revealed damage in liver, kidney and gills tissues which confirmed the harmful effects of increased salinity. Our findings are supported by many research on tilapia species such as **(Hassan *et al*., 2013; Nofal *et al*., 2019; Ali *et al*., 2022).**

Conclusion

Red tilapia's haematological, biochemical, gene expression and histological characteristics were all impacted by the high salinity, making them more resistance to environmental changes. We can develop effective strategies for managing fish in the face of climate change by enabling species such as red tilapia to thrive in diverse salinity conditions.

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الملخص العربي

تحديد ملامح التعبير الجيني والتكيفات الفسيولوجية لسمك البلطي الأحمر (*Oreochromis* sp.) تحت مستويات ملوحة مختلفة

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تعتمد تنمية الاستزراع المائي على توافر المياه. ومن المتوقع أن يؤدي تغير المناخ إلى تغيير الدورة الهيدرولوجية، مما سيكون له آثار كبيرة على مدى توافر المياه. تم تربية مائة وعشرين سمكة في مصادر المياه المتاحة بمستويات مختلفة من الملوحة ٠,٥ ، ٥ ، ٢٠ و٣٨ جزء فى الالف لدراسة تأثيرها على القدرة على التكيف وأداء النمو ومعدل بقاء الأسماك ومؤشرات الدم/المصل والكيمياء الحيوية والمؤشرات النسيجية والتعبير الجيني للأسماك. الجينات المرتبطة بالإجهاد HSP27وHSP70، وجينات تنظيم الأيونات NKA α-1a وNKA α-1b ، والجينات المرتبطة بالمناعة IgM للبلطي الأحمر، *Oreochromis sp.* لمدة ٤٥ يومًا. أظهر تحليل التباين عدم وجود فروق معنوية في معدل الزيادة فى الوزن للأسماك بين المجاميع الدراسة مع عدم وجود وفيات (SR100%) وعدم وجود فروق معنوية في المعايير الدموية، في حين تأثرت المعايير البيوكيميائية بشكل كبير. كما أن مستويات التعبير mRNA للجينات تأثرت بشكل كبير .($P \geq 0.05$) انخفض NKA α-1a وزاد NKA α-1b مع التعرض للملوحة بينما ارتفع التعبير للجينات المرتبطة بالمناعة فى الكبد عن الخياشيم عند ٣٨ جزءفى الالف. فيما يتعلق بالتركيب النسيجي للخياشيم والكبد؛ فقد وجدت اختلافات بينهم ظهراحتقان للأوعية الدموية، وتوسع الشعيرات، وارتشاح الخلايا المستديرة في الخياشيم في كل من الملوحة العالية والمنخفضة. ولذلك، فإن البلطي الأحمر يتمتع بقدرة واسعة على التكيف مع الملوحة، كما يتم تشجيع استخدام المياه الجوفية في تربية الأحياء المائية.

الكلمات الاسترشادية: مقاومة الملوحة،المؤشرات الفسيولوجية،التنظيم الأيوني، المناعة، التعبير الجين.

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