

Zagazig J. Agric. Res., Vol. 43 No. (3) 2016

http://www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master



EFFECT OF WATER SALINITY AND NaCI SUPPLEMENTAION ON GROWTH PERFORMANCE, FEED UTILIZATION, BLOOD CONSTITUENTS AND BODY COMPOSITION OF NILE TILAPIA, *Oreochromis niloticus*

Abd Elrahman M. Abd Elrahman^{1*}, A.M.A-S. Goda¹, G.A. Abd Rhman² and M.S. Ayyat

1. Fish Nut. Lab., Nat. Inst. Oceanography and Fisheries (NIOF), Cairo, Egypt 2. Anim. Prod. Dept., Fac. Agric., Zagazig Univ., Egypt

ABSTRACT

A 12-week growth study was carried out to investigate the effects of water salinity (10 and 15 ppt) and dietary sodium chloride, NaCl supplementation (0, 3 and 6% NaCl) levels on growth performance, survival rate and blood components of juvenile of Nile tilapia, Oreochromis niloticus. The results indicated that final live body weight, daily growth rate, relative growth and specific growth significantly (P<0.001) affected with water salinity, also feed conversion was significantly (P<0.001) improved. Final live body weight and growth rate increased in fish group reared at low water salinity level when compared with fish group reared at the high water salinity. Serum total protein, albumin, globulin, aspartate amino transferase (AST), alanine amino transaminase (ALT), glucose and total lipids concentration insignificantly affected with water salinity. Uric acid and serum creatinine concentration significantly (P<0.001) affected with water salinity. Red blood cells and white blood cells counts increased significantly (P<0.001) with increasing salinity level in pond water, while lymphocytes, monocytes and neutrophil cells insignificantly affected with water salinity. Live body weight, daily weight gain, relative growth rate and specific growth rate, significantly (P<0.001) affected with NaCl supplementation. Fish group fed diets supplemented with 3% NaCl recorded final body and daily gain higher by 11.18 and 25.58% when compared with those fed diet without supplementation, while fish fed diets supplemented with 6% NaCl recorded 4.95 and 18.6%, respectively. Fish group fed diets supplemented with 3% NaCl recorded the best survival rate. Daily feed intake affected significantly (P<0.001) with NaCl supplementation, while feed conversion ratio insignificantly affected with NaCl supplementation. Fish group fed diet supplemented with 3% NaCl recorded higher glucose concentration than the other experimental groups, while this group recorded lower plasma creatinine and uric acid. The obtained results indicated that salinity is a key factor in controlling growth of Nile tilapia.

Key words: Water salinity, NaCl, nile tilapia, growth rate, feed efficiency, blood components.

INTRODUCTION

The importance of aquaculture in meeting human protein requirements from fish is evident from the fact that aquaculture contributes over 50% to total world fish supply. With improvements in fish culture practices, aquaculture's contribution to total fish supply is expected to exceed 60% by 2020. Tilapia is mainly lacustrine fish and is well adapted to enclosed water from low salinity to high salinity (FAO, 2012).

The shortage of fresh water in many countries, together with the competition requirement for agriculture and other urban activities has increased the pressure to develop aquaculture in brackish and sea water. Control of salt and water balance within a narrow limit is critical to life in all multicellular organisms, including teleost fishes. Salt tolerance is a term

^{*}Corresponding author: Tel. : +201095982368 E-mail address: abdelrhman niof@hotmail.com

describing the overall fitness, or productivity, of the fish in a saline environment. It is a combination of different quantitative traits, such as metabolism, growth, osmoregulation, immunocompetence and fecundity (Cnaani and Hulata, 2011).

Nile Tilapia, *O. niloticus* belongs to family Cichlidae, which dominates freshwater fish culture is excellent breed for aquaculture in brackish water due to their ability to tolerate a wide range of water salinity. Inter-specific variation in salinity tolerance may be used to select salt-tolerant species and develop salttolerant hybrids. Growth of *O. niloticus* at high salinity is significantly lower than that in freshwater (Fineman, 1988), whereas survival is not affected by salinity. High salinity does seem to suppress, or at least delay, onset of reproduction in *O. niloticus*, thus presenting a practical method of population control.

Osmoregulation, somatic growth, and reproduction are among the most energetically costly metabolic activities engaged by teleost fishes. Boeuf and Payan (2001) discussed four possible pathways of interaction between osmoregulation and growth: (1) difference in standard metabolic rate, (2) increase in food intake, (3) increase in digestibility, and (4) hormonal stimulation. These four pathways can interact, and none can be considered as a unique route connecting osmoregulation and growth.

The salinity of the culture system is known to influence metabolism and homeostatic processes in fish, meaning the organism needs for nutrients and protein particularly, may differ in freshwater and saltwater systems (Altinok and Grizzle, 2001). Yao et al. (2008) investigated the best conditions for transfer of Nile tilapia (O. niloticus) from freshwater to salt water. Fingerlings (8 to 12 g) were transferred, either directly or gradually, from freshwater to water of variable salinities, and survival was monitored after 3 weeks. Larumbe-Moran et al. (2010) reported that culture of Nile tilapia, O. niloticus is feasible in saline environments at up to 25 ppt of salinity without affecting growth or survival range, and with no increases required in dietary protein if the standing biomass remains under critical maxima. Nonetheless, the tilapia do require higher protein intake at higher

salinities to produce growth rates equivalent to those observed in freshwater environments.

The present study was undertaken to determine the effects of water salinity and dietary supplementation of sodium chloride on growth performance, feed utilization, survival rate, and blood components of juvenile of Nile tilapia.

MATERIALS AND METHODS

Experimental Fish and Culture Technique

The present study was conducted out at Department of Animal Production, Faculty of Agriculture. Zagazig University. Zagazig. Egypt. The practical work and chemical analysis were carried out at Baltim Research Station, Kafr El-Sheikh Governorate, National Institute of Oceanography and Fisheries (NIOF), Egypt for 12 weeks (84 days, August to October 2014). Two hundred and seventy Nile tilapia, O. niloticus juveniles with an average initial body weight of 0.8 g were stock raised obtained from at Baltim Research Station Farm,. The juveniles were stocked into six cement ponds (each with 20 m³, $2 \times 5 \times 2$ m). Each cement pond was divided into three equal compartments (pens) by netting (each of 3 m^3) and each pen was stocked with 15 fish. Two different water salinity levels (10 and 15 ppt,) and three different dietary sodium chloride, NaCl supplementation levels (0, 3 and 6% diet) in a factorial manner (3×2) was conducted to represented six different experimental treatments, Three replicates pens were randomly assigned to each treatment. Prior to the start of experiment, the fish were acclimated to the experimental conditions for two weeks.

Experimental Diets

Three experimental diets were formulated to be isonitrogenous, 35% crude protein (CP) and isocaloric, 10.26 MJ/kg digestible energy (DE). All diets were identical except for the variation in NaCl levels. The basal experimental diet (control diet) had no NaCl added. Diets 2-3 each contained sodium chloride, NaCl at levels of 3 and 6%, respectively (Table 1).

Ingredient	Percent in basal experimental diet ^a
Fish meal	30
Soybean meal	19
Yellow corn	11
Wheat bran	33
Sunflower oil	5
Vitamin and Mineral premix ^b	2
Proximate composition (%)	
Dray matter	91.89
Crude protein	35.10
Crude fat	7.61
Total carbohydrate	47.36
Ash	9.93
Gross energy ^c (MJ/kg)	10.26

 Table 1. Formulation and proximate composition of the experimental basal diet (% as-fed basis)

^a The basal experimental diet (control diet) had no NaCl added. Diets 1-2 each contained NaCl at levels of 3 and 6%, respectively.

Vitamin and mineral mixture (supplements per kg of the mixed feed): Vit. A 72000IU, Vit. B₁ 6 mg, Vit. B₃ 12000 IU, Vit. B6 9 mg, B12 0.06 mg, Vit E 60 mg, Vit. 12 mg, Pantothonic acid 60 mg, Nicotinic acid 120 mg, Folic acid 6 mg, Biotin 0.3 mg and Choline chlorids 3 mg.

Each one Kg of mineral mixture contained: Zinc sulphat hepahydrate 3.0, Mg, sulphat 0.335, Coppous chloride 0.10, Calcium phosphate monobasic 135.8, Calcium Lactate 327.0, Ferric citrate 29.7, Potassium phosphate dibasic anhydrous 239.8, Sodium phosphate monobasic 87.2, Sodium chloride 43.6, Aluminium chloride anhydrous 0.15, Potassium iodide 0.15, Cobalt chloride 1.0, Sodium selenite 0.011 and L-cellulose 132.25 (as g/Kg mineral mix) Gatlin and Wilson (1984).

^c Calculated using gross caloric values of 23.62, 39.52, and 17.15 kJ/g for protein, fat, and carbohydrate, respectively, according to Brett (1973).

The diets were processed by blending the dry ingredients into a homogenous mixture. Pellets of 2 mm were made in Baltim laboratory pellet. The pelleting temperature did not exceed 40°C and all diets were air dried for 4 hr., (moisture content of about 10%). All diets was packed in cellophane bags and cooled at 4°C prior to use.

During the 12-weeks experimental period, all fish were fed their respective diets at a level of 5% of body weight for a week. The daily ration was divided into three equal amounts and offered three times a day (09 : 00 am, 12 : 00 pm and 15:00 pm). Random samples of at least 75% of fish from each replicate pen were weighed biweekly and the amount of daily allowance was adjusted accordingly.

The cement ponds were supplied with dechlorinated tap freshwater by stored the tap water in fiberglass tanks for 24 hr., under aeration for dechlorination. The turnover rate of water was 50% /pen/day with adjustment of water salinity for each pond and fish were held under natural light (12:12 hr., light : dark schedule).

Water temperature, dissolved oxygen, pH, salinity and ammonia were monitored during the trial, to maintain water quality at optimum range for Nile tilapia. Water temperature was recorded daily at 13.00 pm using a mercury thermometer suspended at 30-cm depth. Dissolved oxygen (DO) was measured at 05.00 hr., using YSI model 56 oxygen meter and pH at 09.00 hr., by

using a pH meter. Salinity by TDS. Ammonia and alkalinity were measured three times a week according to APHA (1998).

Growth Indices

Mean final body weight (FBW) of each experimental treatment was determined by dividing total fish weight in each pen by number of fish. Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), fat retention (FR) and energy retention (ER) were calculated using the following equations:

WG = Final body weight (g) - Initial body weight (g); SGR = (ln FBW - ln IBW)/t \times 100; where: FBW is final body weight (g); IBW is initial body weight (g); ln= natural logarithmic; t = time in days; FCR = Feed intake (g)/weight gain (g).

Blood Samples and Analysis

Blood samples were collected at the end of the experiment. Each of the experimental treatment was sampled once, with three fish/pen for hematological indices analyses. The fish were anesthetized with t-amyl alcohol and the blood samples were taken by puncturing the caudal vessels. Heparin was used as anticoagulants and the plasma separated by centrifugation at 3000 rpm for 20 min and stored at -20°C until further analysis. Total plasma protein (TPP) and albumin (TPA) were determined according to (Sundeman, 1964) However, the total plasma globulin (TPG) was calculated by subtracting the total plasma protein from total plasma albumin. Glucose and Urea-N, creatinine according to Henery (1974), plasma transaminase enzymes (aspartate amino transferase, AST and alanine amino transferase, ALT), creatinine were determined according to Reitman and Fankel, (1957).

The red blood cell counts (RBCs) were determined by using a Bürker counting chamber and Hayem solution. The findings and instructions published by Blaxhall and Daisley (1973) and Hrubec *et al.* (2000) were followed when the RBCs were determined. Hematocrit (Hct) were determined by using microhematocrit-heparinized capillary tubes and a microhematocrite centrifuge (10000 g for

5 min). The values of Hct were determined within 30 min alter bleeding. Hemoglobin concentrations (Hb) were determined by the cyanhemoglobin method, at 540 nm. RBCs and Hb values were determined within 6 hr., after blood sampling. The haematological parameters are expressed in international units (SI).

Analytical Methods

At the beginning of the trial, a random pooled sample of 10 fish was collected, for determination of initial whole-body proximate composition. At the termination of the feeding trial, five fish were randomly selected from each pen, homogenized in a blender, to determine the final whole-body proximate composition. The fish were pooled for each pen, oven-dried, ground, and stored at -20° C for subsequent analysis. The chemical composition of fish and diet samples were determined according to procedures of AOAC (1980). Dry matter was determined after drying the samples in an oven (105°C) for 24 hr. Ash by incineration at 550°C for 12 hr. Crude protein was determined by micro-Kieldhal method, N% \times 6.25 crude fat by soxhlet extraction with diethyl ether (40 - 60°C).

Statistical Analysis

Data were statistically analyzed by ANOVA using SAS (2002) according to the following model:

$$Y_{ijk} = \mu + S_i + A_j + SA_{ij} + E_{ijk}.$$

Where,:

 μ is the overall mean, S_i is the fixed effect of ith water salinity level, A_j is the fixed effect of jth sodium chloride supplementation, SA_{ij} is the interaction effect of ith water salinity and jth dietary supplementation with NaCl and E_{ijk} is the random error. Means were tested for significant differences using Duncan's Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth Performance

Final body weight, daily weight gain, relative growth rate and specific growth rate significantly (P<0.01 or 0.05) decreased with increasing water salinity (Table 2). Final live body weight, daily weight gain, relative growth

Item	Initial weight (g)	Final body weight at 12 weeks (g)	Daily weight gain at 0-12 weeks	Relative growth rate at 0-12 weeks	Specific growth rate at 0-12 weeks
Water salinity level	1				
10 ppt	$0.873 {\pm} 0.001$	9.592±0.179	0.104 ± 0.002	217.831±8.092	0.012 ± 0.001
15 ppt	$0.873 {\pm} 0.001$	8.709±0.381	0.093 ± 0.005	181.864±15.544	0.011 ± 0.001
Significance	NS	***	***	***	***
Feed additives					
control	0.873 ± 0.002	$8.947 \pm 0.142^{\circ}$	$0.086 \pm 0.004^{\circ}$	156.584±14.597 ^e	0.012 ± 0.001 ^c
3% NaCl	0.875 ± 0.002	$9.947{\pm}0.085^{a}$	0.108 ± 0.001^{a}	233.831±3.941 ^a	0.013 ± 0.001 ^a
6% NaCl	0.872 ± 0.002	9.390 ± 0.200^{b}	0.102 ± 0.002^{b}	209.127 ± 8.942^{b}	0.012 ± 0.001 ^b
Significance	NS	***	***	***	***
Interaction betwee	n water salini	ty and feed ad	ditives		
10 ppt					
control	0.873±0.003	8.947±0.141 b	$0.096 \pm 0.002^{\circ}$	188.640±6.070 ^b	0.012 ± 0.001 ^b
3% NaCl	0.877±0.003	10.05±0.102 ^a	0.109±0.001 ^a	238.249±4.797 ^a	0.013 ± 0.001 ^a
6% NaCl	0.870 ± 0.000	9.780±0.154 ^a	0.106±0.002 ^a	226.604±7.271 ^a	$0.013{\pm}0.001^{a}$
15 ppt					
control	0873±0.003	7.283±0.029 ^c	$0.076 \pm 0.001^{\circ}$	124.529±0.988 °	0.011 ± 0.001 ^c
3% NaCl	0.873±0.003	9.843±0.123 ^a	$0.107{\pm}0.002^{a}$	229.413±5.927 ^a	$0.013{\pm}0.001^{a}$
6% NaCl	0.873±0.003	9.000±0.156 ^b	0.097 ± 0.001^{b}	191.651±6.443 ^b	0.012 ± 0.001 ^b
Significance	NS	***	***	***	***

Table 2. Live body weight, growth performance of Nile tilapia juveniles at different experimental treatments

Means in the same column within each classification with different letters differ significantly (P<0.01).

rate and specific growth rate increased with 10.14, 11.83, 19.78 and 9.09%, respectively in fish group reared at 10 ppt water salinity when compared with those reared at 15 ppt salinity. Fish group reared at 10 ppt salinity recorded 217.83 g/100 of live body weight, while fish group reared at 15 ppt recorded only 181.86 g/100 g of live weight. Nile tilapia juvenile has been documented as surviving in salinities up to 25 ppt with acclimation. With stepwise acclimation of 5 ppt per 48 hr., till 20 ppt and 1 ppt thereafter, juvenile *O. niloticus* were found to tolerate salinities up to 28 ppt (Al Asgah, 1984). Boeuf and Payan (2001) reviewed the literature on salinity influence on growth in fish

and concluded that salinity is also a key factor in controlling growth. They observed that the changes in growth rate that depend on salinity result from an action on metabolic rate, food intake and food conversion. Better growth at intermediate salinities (8-20 ppt) is very often but not systematically, correlated to a lower standard metabolic rate.

Live body weight, daily weight gain, relative growth rate and specific growth rate significantly (P<0.001) affected with NaCl supplementation (Table 2). Fish group fed diet containing 3% NaCl recorded the higher live body weight, daily weight gain, relative growth rate and specific growth rate than the other

experimental groups. Fish group fed diets supplemented with 3% NaCl recorded final body and daily gain higher by 11.18 and 25.58% when compared with those fed diet without supplementation. while fish fed diets supplemented with 6% NaCl recorded 4.95 and 18.6%, respectively. Fish group fed diet supplemented with 3% NaCl recorded 233.83 g/100 of live body weight, while fish group fed diet without supplementation recorded only 156.58 g/100 g of live weight. The results of the present study coincide with some other studies, Smith et al. (1995) found that feeding rainbow trout (Oncorhynchus mykiss) with a 12% saltadded diet promoted better growth, survival and feed conversion ratio, meanwhile the inclusion of over 12% salt resulted in a deterioration in all growth performances. The addition of salt to the diet of freshwater carps (Cyprinus carpio) at a level of 1.5% resulted in significantly better growth (Nandeesha et al., 2000).

Interaction between water salinity and dietary NaCl supplementation was significantly (P<0.001) affected live body weight, daily weight gain, relative growth rate and specific growth rate (Table 2). Fish groups which fed on diets supplemented with 3% NaCl (within the low and high water salinity) recorded higher live body weight, daily weight gain, relative growth rate and specific growth rate than the other experimental groups. Feeding with diets supplemented with NaCl is another acclimation method that has been used with some success for adapting freshwater fish to salt water. Feeding NaCl-supplemented may have modest benefit to the osmoregulatory ability of Nile tilapia transferred to sea water (Lim et al., 2006).

Survival Rate (%)

Nile tilapia gradually transferred from freshwater to salinities up to 15 ppt showed good survival (Table 3). It has been recognized that some tilapia may have higher survival, with higher growth, in saline conditions than in freshwater. Prolonged acclimation over days or even weeks from freshwater to saltwater also increases survival values (Al Asgah, 1984).

Yao *et al.* (2008) reported that survival of Nile tilapia fingerlings transferred directly to saline water was increased (84.3% to 96.8%) up

to 17 ppt, but mortalities were significant decreased above that level of salinity (60-70%). Higher survival rate (78 to 81%) was achieved by gradual acclimation to salinity of 30 ppt over two days.

Survival rate not affected with dietary NaCl supplementations. Fish group fed diets supplemented with 3% NaCl recorded the best survival rate (%) (Table 3). Results of Lim *et al.* (2006) showed that juvenile Nile tilapia receiving dietary NaCl feeding regimes, even for a two-week period, exhibited consistently better survival values (%).

The higher water salinity (15 ppt), and dietary NaCl supplemented improved the Nile tilapia survival values. Fish groups fed diets with NaCl (3 or 6%) recorded the highest survival values (Table 3). Salt tolerance in tilapia can be improved by optimizing acclimation protocols, adding salt to the diet.

Feed Utilization

Daily feed intake insignificantly affected with water salinity, while feed conversion ratio affected significantly (P<0.001) with water salinity (Table 3). Feed conversion decline with 11% in fish group reared at 15 ppt when compared with those reared at 10 ppt water salinity. Florida red tilapia physiologically function more efficiently in brackish and saline waters, with lower feed conversion ratios and faster weight gain than those raised in freshwater (Head *et al.*, 1994).

Daily feed intake affected significantly (P<0.001) with NaCl supplementation, while feed conversion ratio insignificantly affected supplementation with NaCl (Table 3). Gangadhara et al. (2004) reported that Rohu, Labeo rohita fish recorded significantly better feed conversion ratio, protein efficiency ratio and net protein retention with increased dietary salt content compared to the other treatments. Smith et al. (1995) found that feeding rainbow trout (Oncorhynchus mykiss) with a 12% saltadded diet promoted better feed conversion ratio, but the inclusion of over 12% salt resulted in a deterioration in all growth performances

Interaction between water salinity and dietary NaCl supplementation was significantly (P < 0.001) affected daily feed intake and feed

Item	Daily feed intake 0-12 weeks (g)	Feed conversion (g feed/g gain)	Survival percent (%)
Water salinity level			
10 ppt	0.161±0.002	1.545 ± 0.018	97.04
15 ppt	0.163±0.009	1.736±0.026	99.26
Significance	NS	***	
Feed additives			
control	0.141 ± 0.006^{b}	1.636±0.024	100.00
3% NaCl	0.174±0.005 ^a	1.615±0.046	97.78
6% NaCl	0.169±0.004 ^a	1.671 ± 0.070	96.67
Significance	***	NS	
Interaction between wat	ter salinity and feed additives		
10 ppt			
control	0.153±0.002 °	1.599±0.010 ^c	100.00
3% NaCl	0.165±0.001 ^b	5±0.001 ^b 1.514±0.016 ^d	
6% NaCl	0.161 ± 0.005^{bc}	1.521 ± 0.036^{d}	93.33
15 ppt			
control	$0.128{\pm}0.003$ ^d	1.672 ± 0.037^{bc}	100.00
3% NaCl	$0.184{\pm}0.004^{a}$	1.715 ± 0.020^{b}	97.78
6% NaCl 0.177±0.003 ^a		1.822±0.022 ^a	100.00
Significance	***	***	

Table 3. Feed utilization of Nile tilapia juveniles at different experimental treatments

Means in the same column within each classification with different letters differ significantly (P<0.01)

conversion ratio (Table 3). Fish groups which fed on diets supplemented with 3% NaCl (within the low and high water salinity) recorded higher daily feed intake. Fish groups which fed on diets supplemented with 6% NaCl (within high water salinity) recorded higher feed conversion ratio.

Blood Components

Serum total protein and its fractions, glucose, AST, ALT and total lipids concentrations affected insignificantly with increasing water salinity levels, while uric acid and creatinine significantly (P<0.01 or 0.001) affected with water salinity (Tables 4 and 5). Concentration of serum creatinine and uric acid decreased by

20.34 and 7.21%, respectively, in fish group reared at higher water salinity (15 ppt) when compared with those reared at 10 ppt level. Red blood cells and white blood cells counts increased significantly (P<0.01 or 0.001) with increasing water salinity while level. lymphocytes, monocytes and neutrophil cells insignificantly affected with water salinity (Table 6). Red blood cells and white blood cells increased by 2.85 and 12.13%, respectively, in fish group reared at 15 ppt water salinity when compared with those reared at 10 ppt water salinity. This hemoglobin molecule had been reported to have a better affinity for oxygen at elevated osmotic and temperature levels than in the conventionally recognized hemoglobin. This

Item	m Total protein (g/100 ml)		Globulin (g/100 ml)	Glucose (mg/100 ml)	
Water salinity level					
10ppt	3.942±0.441	2.424±0.075	1.517±0.055	80.811±3.796	
15ppt	3.804±0.104	2.213±0.079	1.591±0.081	80.864±3.581	
Significance	NS	NS	NS	NS	
Feed additives					
control	3.855±0.139	2.336±0.129	1.519±0.070	87.463±2.712 ^a	
3% NaCl	3.758±0.125	2.256±0.051	1.502±0.097	84.775±4.495 ^a	
6% NaCl	4.006±0.126	2.363±0.122	1.643±0.084	70.274±1.897 ^b	
Significance	NS	NS	NS	***	
Interaction between water salinity and feed additives					
10ppt					
control	4.061±0.129	2.479±0.115	1.582±0.065	93.036±2.129 ^a	
3% NaCl	3.710±0.167	2.281±0.047	1.428±0.153	77.103±6.188 ^{bc}	
6% NaCl	4.056±0.239	2.513±0.194	1.543±0.046	72.293±3.488 ^{bc}	
15 ppt					
control	3.649±0.194	2.194±0.222	1.455±0.127	81.890±1.082 ^b	
3% NaCl	3.806±0.219	2.231±0.101	1.575±0.136	92.448±1.969 ^a	
6% NaCl	3.957±0.140	2.213±0.121	1.743±0.153	68.255±1.320 ^c	
Significance	NS	NS	NS	***	

Table 4. Plasma total protein and its fractions (g/dl) and glucose concentrations as affected by water salinity, NaCl and their interactions of Nile tilapia juveniles at different experimental treatments

946

Table 5. Plasma transaminase enzymes (AST and ALT; U/l), uric acid and creatinine (mg/dl) as kidney function diagnose and total lipids concentration as affected by water salinity, NaCl and their interactions of Ni.0le tilapia juveniles at different experimental treatments

Item	ALT (U/l)	AST (U/l)	Uric acid (mg/L)	Creatinine (mg/L)	Total lipids (mg/100 ml)
Water salinity lev	el				
10ppt	14.658±0.689	41.653±1.070	7.936±0.370	1.213±0.031	24.000±0.408
15ppt	15.888±1.174	41.821±0.852	7.402±0.140	1.008±0.059	23.667±0.527
Significance	NS	NS	**	***	NS
Feed additives					
control	16.253±1.862	40.253±0.614	$7.964{\pm}0.252^{a}$	0.994±0.52 °	23.500±0.428
3% NaCl	14.988±0.816	43.105±1.331	7.043±0.254 ^b	1.264±0.011 ^a	23.833±0.703
6% NaCl	14.578±0.489	41.853±1.236	8.000±0.416 ^a	1.075 ± 0.080^{b}	24.167±0.601
Significance	NS	NS	***	***	NS
Interaction betwe	en water salinity	and feed additiv	ves		
10ppt					
control	12.624±0.880 ^c	39.107±0.427	$8.471 {\pm} 0.090^{a}$	$1.102{\pm}0.041^{b}$	23.000±0.577
3% NaCl	16.603±0.437 ^b	45.011±0.909	6.532±0.163 ^c	1.285±0.011 ^a	24.000±0.557
6% NaCl	14.746±0.879 ^{bc}	40.842±1.877	8.804±0.326 ^a	1.251±0.009 ^a	25.000±0.577
15ppt					
control	19.882±1.842 ^a	41.399±0.623	$7.457 {\pm} 0.228^{b}$	0.886±0.009 ^c	24.000±0.577
3% NaCl	13.373±0.730 ^{bc}	41.200±2.098	7.553±0.191 ^b	1.243±0.003 ^a	23.667±1.453
6% NaCl	14.410±0.628 ^{bc}	42.864±1.761	7.196±0.336 ^{bc}	0.898 ± 0.027 ^c	23.333±0.882
Significance	***	NS	***	***	NS

Table 6.	Red blood cells, white blood cells and lymphocytes as affected by water salinity, NaCl
	and their interactions of Nile tilapia juveniles at different experimental treatments

Items	RBCs counts x 10 ⁶ (cells/µl)	WBCs counts x 10 ³ (cells/µl)	lymphocytes cells/l	Monocytes cells/l	Neutrophil cells/µl		
Water salinity level							
10ppt	4.3222±1.673	54.99±0.354	69.778±0.662	31.444±0.626	12.778±0.465		
15ppt	4.4489±1.634	62.58±0.686	68.667±0.745	33.333±0.553	13.111±0.351		
Significance	**	***	NS	NS	NS		
Effect of feed a	dditives						
Control	3.9500±0.428 ^c	57.49±1.718 ^b	67.833±0.749 ^b	32.500±0.764	12.000±0.258 ^b		
3% NaCl	4.2333±0.760 ^b	60.32±2.099 ^a	$68.833 {\pm} 0.703^{ab}$	32.667±0.843	13.333±0.558ª		
6% NaCl	5.0333±0.558 ^a	58.55±1.379 ^b	71.000±0.683 ^a	32.000±0.931	13.500±0.428 ^a		
Significance	***	***	*	NS	*		
Interaction bet	ween water salin	ity and feed add	itives				
10ppt							
control	3.9000±0.577	53.71±0.261 ^d	68.000±1.155	31.667±0.882	11.667±0.333		
3% NaCl	4.1000±0.577	55.66±0.384°	70.000±0.577	32.333±1.453	12.667±0.882		
6% NaCl	4.9667±0.882	55.60±0.256 °	71.333±0.882	30.333±0.882	14.000±0.577		
15ppt							
control	4.0000±0.577	61.26±0.669 ^b	67.667±1.202	33.333±1.202	12.333±0.333		
3% NaCl	4.3667±0.882	64.98±0.432 ^a	67.667±0.882	33.000±1.155	14.000±0.577		
6% NaCl	5.1000±0.577	61.51±0.837 ^b	70.667±1.202	33.667±0.882	13.000±0.577		
Significance	NS	*	NS	NS	NS		

948

secondary hemoglobin could transfer oxygen at elevated salt levels when conventional hemoglobin would be less effective (Perez and Maclean, 1976).

Plasma total protein and its fractions, AST, ALT and total lipids concentrations affected insignificantly, on the other hand, glucose, uric acid and creatinine significantly (P<0.001) affected with NaCl supplementation in fish diets (Tables 4 and 5). Fish group fed diet supplemented with 3% NaCl recorded higher glucose concentration than the other experimental groups, while this group recorded lower serum creatinine and uric acid.

Red blood cells, white blood cells counts, neutrophil lymphocytes and significantly (P<0.001 or 0.05) affected with NaCl supplementation, while monocytes cells insignificantly affected (Table 6). They are the main type of cell found in lymph, which prompted the name lymphocyte (Abbas and Lichtman, 2003). Neutrophil granulocytes are a kind of white blood cells, forming an essential part of the body's defense system. Neutrophils protect the host against pyogenic infections (Amulic et al., 2012). Their function is closely related with that of lymphocytes and macrophages, cells that are also involved in the response to infection. They are formed from stem cells in the bone marrow (Witko-Sarsat et al., 2000).

Plasma total protein and its fractions, AST, and total lipids concentrations insignificantly affected, on the other hand glucose, ALT, uric acid and creatinine significantly (P<0.001) affected with NaCl supplementation in fish diets (Tables 4 and 5). white blood cells counts significantly (P<0.05) affected with Interaction effect between water salinity and NaCl supplementation, while red blood cells, lymphocytes, monocytes cells and neutrophil insignificantly affected with (Table 6) salinity and NaCl.

Body Composition

Ether extract and crude protein affected significantly (P<0.001) with water salinity, while moisture and ash content insignificantly affected (Table 7). Body ether extract content in carcass increased with increasing water salinity, while body protein content decreased. Moisture, ether extract and crude protein content in fish body affected significantly (P<0.001) with NaCl supplementation while ash content insignificantly affected (Table 7). The contrast results were obtained by Keshavanath et al. (2012), who found that carcass composition was not affected as salt addition in fish diets.

Ether extract and crude protein affected significantly (P<0.001) with interaction effect between water salinity and NaCl supplementation, while moisture and ash content were insignificantly affected (Table 7).

Conclusion

Control of salt and water balance within a narrow limit is critical to life in all multicellular organisms, including teleost fishes. Salt tolerance is a term describing the overall fitness, or productivity, of the fish in a saline environment. The results of the present study revealed that juveniles of Nile tilapia has been documented as surviving in salinities up to 15 ppt with acclimation. The results demonstrating the beneficial effect that supplemental dietary salt can have beneficial effects on fish growth. Abd Elrahman, et al.

Items	Moisture (%)	Ether extract (%)	Crude protein (%)	Ash (%)
Water salinity level				
10 ppt	74.122±0.364	19.611±0.200	63.767±0.183	14.544±0.109
15 ppt	74.300 ± 0.403	21.078±0.305	62.422±0.351	14.411 ± 0.108
Significance	NS	***	***	NS
Feed additives				
control	72.867 ± 0.217^{b}	19.967 ± 0.126^{b}	$63.400{\pm}0.000^{b}$	14.367±0.115
3% NaCl	74.717±0.300 ^a	19.933 ± 0.512^{b}	63.633±0.401ª	14.550±0.081
6% NaCl	75.050±0.148 ^a	21.133±0.421 ^a	62.250±0.516°	14.517±0.189
Significance	***	***	***	NS
Interaction between w	vater salinity and f	feed additives		
10 ppt				
control	72.867±0.260	19.767±0.067 ^c	$63.400{\pm}0.000^{b}$	14.600±0.058
3% NaCl	74.767±0.578	$18.867{\pm}0.088^{d}$	64.500±0.000ª	14.667±0.120
6% NaCl	74.733±0.033	$20.200 \pm 0.000^{\circ}$	$63.400{\pm}0.000^{b}$	14.367±0.318
15 ppt				
control	72.867±0.410	$20.167 \pm 0.186^{\circ}$	$63.400{\pm}0.000^{b}$	14.133±0.088
3% NaCl	74.667±0.338	$21.000{\pm}0.404^{b}$	62.767±0.233°	14.433±0.067
6% NaCl	75.367±0.088	22.067±0.120 ^a	$61.100{\pm}0.100^{d}$	14.667±0.233
Significance	NS	***	***	NS

 Table 7. Body composition at different experimental periods as affected by water salinity, NaCl and their interactions of Nile tilapia juveniles at different experimental treatments

REFERENCES

- Abbas, A.K. and A.H. Lichtman (2003). Cellular and Molecular Immunology (5th Ed.). Saunders, Philadelphia
- Al Asgah, N.A. (1984). The effect of salinity on eggs and larvae of *Oreochromis niloticus* (Linnaeus). Arab Gulf J. Sci. Res., 2 : 673-681.
- Altinok, I. and J.M. Grizzle (2001). Effects of brackish water on growth, feed conversion and energy absorption efficiency by juvenile euryhaline and freshwater stenohaline fishes. J. Fish Biol., 59: 1142-1152.
- APHA (1998). American Public Health Association. Standard Methods for the Examination of Water and Wastewater. Am.
 Public Health Ass. Ed. Inc., Washington DC 20th Ed.
- Amulic, B., C. Cazalet, G.L. Hayes, K.D. Metzler and A. Zychlinsky (2012). Neutrophil function: from mechanisms to disease. Annual Rev. Immunol., 30: 459-89.
- AOAC (1980). Official Methods of Analysis, 13th Ed. Association of Official Analytical Chemists, Virginia.
- Blaxhall, P.C. and K.W. Daisley (1973). Routine hematological methods for use with fish blood. J. Fish Biol., 5: 771-781.
- Boeuf, G. and P. Payan (2001). How should salinity influence fish growth? Comp. Biochem. Physiol. C., 130:411–423.
- Brett, J.R. (1973). Energy expenditure of Sockeye salmon *Oncorhynchus nerka*, during sustained performance. J. Fish. Res. Board Can., 30: 1799-1809.
- Cnaani, A. and G. Hulata (2011). Improving salinity tolerance in tilapias: Past experience and future prospects. Isr. J. Aquacult. Bamidgeh, 63 (63) : 521-533.
- Duncan, D.B. (1955). Multiple Range and Multiple F-test. Biometrics, 11:1-42.
- El-Sayed, A.F.M. (1999). Alternative dietary protein sources for farmed tilapia, Oreochromis spp. Aquac., 179: 149–168.

- FAO (2012). The stat of the world fisheries and aquaculture: World review of fisheres and aquaculture, Rome.
- Fineman, A.S. (1988). Preliminary observations on the effect of salinity on the reproduction and growth of freshwater Nile tilapia, *Oreochromis niloticus* (L.), cultured in brackishwater ponds. Aquacult. Fish. Manage., 19:313-320.
- Gangadhara, B., M.C. Nandeesha, P. Keshavanath and T.J. Varghese (2004). Growth response and biochemical composition of rohu, Labeo rohita, fed saltincorporated diets. J. Appl. Aquacult., 16: 169-176.
- Gatlin, D.M. and R.P. Wilson (1984). Studies of the manganese requirement of fingerling channel catfish. Aquacult., 41: 85-92.
- Head, W.D., A. Zerbi and W.O. Watanabe (1994). Preliminary observations on the marketability of salt-water cultured Florida red tilapia in Puerto Rico. J. World Aquacult. Soc., 25: 432-441.
- Henery, R.J. (1974). Clinical Chemistry, Principles and Technique, 2nd Ed., Harper and Row.
- Hrubec, T.C., J.L. Cardinale and S.A. Smith (2000). Hematology and plasma chemistry Reference intervals for cultured tilapia (*Oreochromis* Hybrid). Vet. Clin. Pathol., 29 (1): 7-12.
- Keshavanath, P., Oishi, C.A., Leao da Fonseca, F.A., Affonso, E.G. and Filho, M.P. (2012). Growth Response of Tambaqui (*Colossoma macropomum*) Fingerlings to Salt (Sodium Chloride) Supplemented Diets. J. Fisheries and Aquatic Sci., 7: 439-446.
- Larumbe-Morán, E., M.P. Hernández-Vergara, M.A. Olvera-Novoa and C.I. Pérez Rostro (2010). Protein requirements of Nile tilapia (*Oreochromis niloticus*) fry cultured at different salinities. Aquacult. Res., 41: 1150 -1157.
- Lim, C.E., M. Aksoy, T.L. Welker and K. Veverica (2006). Effect of feeding duration of sodium chloride containing diets on growth performance and some osmoregulatory parameters of Nile tilapia

(*Oreochromis niloticus*) after transfer to water of different salinities. J. Appl. Aquacult., 18: 1-17.

- Nandeesha, M.C., B. Gangadhar, P. Keshavanath and T.J. Varghese (2000). Effect of dietary sodium chloride supplementation on growth, biochemical composition and digestive enzyme activity of young *Cyprinus carpio* (Linn) and *Cirrhinus mrigala* (Ham). J. Aquacult. in the Tropics, 15(2): 135-144.
- Perez, J.E. and N. Maclean (1976). The haemoglobins of the fish Sarotherodon mossambicus (Peters): functional significance and ontogenetic changes. J. Fisheries Biol., 9: 447-455.
- Reitman and S. Fankel (1957). A method for determination of plasma AST and ALT. Am. J. Clin. Path, 28:56.
- SAS (2002). SAS Institute Inc., Cary, NC, USA. NOTE: SAS Proprietary Software Version 9.00 (TS M0).

- Smith, N.F., F.B. Eddy and C. Talbot, 1995. Effect of dietary salt load on trans-epithelial Na+ exchange in freshwater rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol., 198 : 2359-2364.
- Sundeman, M.F.W. (1964). Studies of the serum proteins. Am. J. Clim. Path, 1-21.
- Witko-Sarsat, V., Rieu, P., Descamps-Latscha, B., Lesavre, P., Halbwachs-Mecarelli, L. (2000). Neutrophils: molecules, functions and pathophysiological aspects. Lab Invest., 80 (5): 617–53.
- Yao, K., M. Ouattara and A.F.A. Ahoussi (2008). Survie du Tilapia du Nil (*Oreochromis niloticus*) en eaux salées durant un transfert direct et progressif [Survival of the Nile Tilapia (*Oreochromis niloticus*) in salt water during a direct and progressive transfer]. Livest. Res. Rural Develop. 20: Article #72. (http://www.lrrd.org/lrrd20/5/yao20072.htm; in French with English abstract).

الهدف من هذه الدراسة هو تحديد تأثير الملوحة وإضافة كلوريد الصوديوم على أداء النمو، ومعدل البقاء ومكونات الدم لأسماك البلطي النيلي، وتشير النتائج إلى أن وزن الجسم النهائي، ومعدل الزيادة اليومية، ومعدل النمو النوعي تأثرت معنويا (P <0.001) مع ملوحة المياه، كما أن معدل التحويل الغذائي تحسن معنويا (P <0.001)، وزن الجسم النهائي ومعدل ألزيادة اليومي زَّاد في مجموعه السمك المرباة في مستوي منخفض من ملوحه المياه ١٠ جزء في الألف عند مقارنتها بالمجموعة ألمرباة في مستوى عالى من ملوحة المياه ١٥ جَزء في الألف، لم يتأثر كل من البروتين الكلي في الدم والألبيومين والجلوبيولين، (AST)، (ÂLT) والجلوكوز والدهون الكليَّة مع ملوحَة المياه، بينما تأثر حمض اليوريك وتركيز الكرياتينين معنويا (P <0.001) مع ملوحة المياه، انخفضت خلايا الدم الحمراء والبيضاء معنويا (P <0.001) مع زيادة مستوى الملوحة ، في حين لم يتأثَّر كلَّ من الليمفوسيت والمونوسيت والنيتروفيل مع ملوحة المياه، تأثر كل من وزنّ الجسم النهائي، معدل الزيادة اليومية، معدل النمو النوعي معنويا (P <0.001) مع إضافة كلوريد الصوديوم، أعطت مجموعه الأسماك التي غذيت على عليقه تحتوي علي ٣% كلوريدُ الصوديوم جسم نهائي وزيادة يومية مرتفعه بنسبة ١١,١٨ و ٢٥,٥٨% مقارنة بالمجموعة القياسية، في حين أن الأسماك التي غذيت على عليقه تحتوي على ٦% كلوريد الصوديوم سجلت ٤,٩٥ و ١٨,٦% على التوالي، أعطت مجموعة الأسماك التي غذيت على عليقه تحتوي على ٣% كلوريد الصوديوم أفضل معدل للبقاء على قيد الحيَّاة، تأثر الاستهلاك اليومي للغذاء معنويا (P <0.001) مع إضَّافة كلوريد الصوديوم ، في حين أن نسبة التحويل الغذائي لم يتأثر معنويا مع إضافة كلوريد الصوديوم، أعطت مجموعه الأسماك التي غذيت على عليقه تحتوي على ٣% كلوريد الصوديوم أعلى تركيز لجلوكوز الدم مقارنه بالمجموعات الأخرى، في حين سجلت هذه المجموعة أقلُ تركّيز من الكرياتينين وحمض اليوريك، ودلت النتائج المتحصل عليها أن الملوحة هي عامل مهم في نمو اسماك البلطي النيلي.

المحكمون :

١ - أبد. محمد أحمد عبدالله زكي

۲ - أ.د. صفاء محمود شرف

أستاذ رعاية الأسماك – كلية الزراعة – جامعة الإسكندرية.

شرف أستاذ فسيولوجي الأسماك – كلية الزراعة – جامعة قناة السويس.