

## Comparative impact assessment of varying salinity concentration on growth, survival and blood chemistry of tilapia fingerlings

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### Abstract

We designed this investigation to compare the impact of varying salinity levels on growth, survival, and blood chemistry of Nile Tilapia (*Oreochromis niloticus*) fingerlings. Furthermore, we try to provide a comparative account of our and previous findings conducted in other parts of the world on different fish species. One hundred and eighty tilapia fingerlings with an average initial weight of  $11.6 \pm 3.42$ g were procured and acclimatized in laboratory conditions and transferred to twelve different aquaria sub-divided into four different salinity treatments viz. T<sub>0</sub> (0 ppt), T<sub>1</sub> (6 ppt), T<sub>2</sub> (10 ppt) and T<sub>3</sub> (14 ppt). Results revealed that certain water quality parameters (conductivity, Na, Cl, Bicarbonates, and total dissolved solids) significantly changed ( $P < 0.05$ ) with the increasing salinity. The maximum weight and length gain and FCR were recorded as  $15.11 \pm 2.80$ g,  $5.06 \pm 0.43$ cm, and  $2.61 \pm 0.92$  in T<sub>3</sub>, respectively. However, the survival rate was recorded as 100% in all treatments, indicating higher salinity tolerance in tilapia fingerlings. Blood chemical analyses revealed significant differences ( $P < 0.05$ ) among white blood cells (WBCs), red blood cells (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelets in all treatments, with the highest records in T<sub>3</sub>. In conclusion, our outcomes suggested that *Oreochromis niloticus* fingerlings could survive at higher salinity levels (14ppt) with better growth performance and improved blood chemistry factors. This investigation supports a potentially succeeding aquaculture of tilapia in moderately saline water bodies.

**Keywords:** Salinity, Growth, Survival, Blood chemistry, Fingerlings, FCR

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## Introduction

Conforming water quality conditions are of paramount relevance in modern aquaculture systems because it has a strong impact upon health, survival, and net yield of cultured aquatic species (Iqbal *et al.*, 2017; Haider *et al.*, 2018; Iqbal *et al.*, 2020a; Kim *et al.*, 2021; Atique and An, 2020). For the sustainable growth and survival, it is highly recommended to maintain vital physicochemical parameters of water quality at a sustainable level that would, otherwise, batter the survival and growth of aquatic fish species (Mehboob *et al.*, 2017; Atique and An, 2018; Atique *et al.*, 2020a; Iqbal *et al.*, 2020b). Among these parameters are the water temperature, dissolved oxygen (DO), pH, total dissolved solids (TDS), total suspended solids (TSS), alkalinity, hardness, and turbidity (Batool *et al.*, 2018; Khan *et al.*, 2018; Atique *et al.*, 2019). Besides these factors, stocking density and salinity level are two significant factors that can adversely affect the fish culture system. At times, these critical parameters act as limiting factors by creating stressful conditions for the aquatic organisms. These factors strongly affect the growth rate, survival rate, and feedings nature of cultivated fish species. Furthermore, salinity can modify teleost's hematology and enhance plasma corticosteroid (Yada and Nakanishi, 2002; Iqbal *et al.*, 2017).

Water scarcity is another issue that is exceedingly reported worldwide and forms the foothold to look into alternative water resource utilization for aquaculture (El-Sayed 2006; Rahman *et*

*al.*, 2020). Presently, Pakistan is also confronting critical freshwater crises despite having vast available water resources as temporary rainwater storage, freshwater reservoirs, canals, streams, and rivers (FAO, 2008; Atique *et al.*, 2020b). In Pakistan, the groundwater is mostly saline, while in some localities, it is turning saline at a faster pace rendering it unsuitable for agriculture and freshwater aquaculture species. Underground water and surface water resources of most regions turn unsupportive for aquaculture and agriculture due to high salinity. Such water bodies should be used to rear aquatic species that can tolerate high salinity conditions and show better growth (Farooq *et al.*, 2007; Jewel *et al.*, 2020).

Therefore, it is inevitable to find potential alternative approaches to utilize the impending higher salinity levels in our domestic water resources. This could be only possible by finding survivability, growth performance, and culture success of such species that can tolerate high salinity levels (Mateen, 2007; Gondal *et al.*, 2020).

*Oreochromis niloticus*, is a member of the family Cichlidae and at the top of the list among high market value fish species globally. It has recently been recognized as the second popular commercial edible fish species in Pakistan (Iqbal *et al.*, 2012; Iqbal *et al.*, 2020b). Keeping the above aspects in view, this study was planned to estimate the growth performance and survivability of *O. niloticus* fingerlings at different salinity levels under controlled conditions. We also

investigated the comparatively changing blood chemistry under varying salinity levels and how the chemical water quality indicated changes linked with different salinity levels. In the end, we provide a comparative account of various species in other parts of the world.

### **Material and methods**

#### *Selection and stocking of fingerlings.*

The present study was conducted in the Research Laboratory, Department of Zoology, The University of Lahore, Pakistan. Research trials were conducted for 90 days. One hundred and eighty fingerlings of *O. niloticus* having average initial weight ( $11.6 \pm 3.42$ g) were procured from a government fish hatchery in Bhalwal, Punjab, Pakistan. Fingerlings were live transported to the laboratory and acclimatized for seven days before initiation of the research trial. During the acclimatization period, diseased and sick fingerlings were separated manually from healthy ones.

#### *Experimental design*

After that, fingerlings were randomly divided into four treatment groups based upon four different salinity concentrations (0 ppt, 6 ppt, 10 ppt, and 14 ppt), and these groups were termed as T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub> respectively. All the experimental treatments were conducted in triplicates for 90 days. Three salinity levels were prepared by dissolving sea salt in the freshwater following (Rodriguez *et al.*, 2015). Salinity concentrations were maintained in each aquarium at a defined level

throughout the trial period. Rectangular glass aquaria having the dimensions (3.5" × 2.5" × 3.5" ) and water carrying capacity of 64 liters were filled with fresh water up to one-third capacity. In each aquarium, the oxygen level was maintained by aerators, model (Davio pumps 4200). Before the trial initiation, each aquarium's fish was weighed by electrical weight balance (KV2001-15), and the length was measured (Rahman, 2015).

#### *Experimental feed*

Commercial feeds "Oryza Organics" specially formulated for Tilapia fish culture, was dispensed at regular intervals as per defined rations calculated every fortnight. Proximate analysis of the feed was performed to estimate the moisture, protein, fat, and ash contents by following the standard methods of AOAC (2000) performed in triplicates. The feed was milled in powder form and dispensed to the fish twice a day at the rate of 4% of body weight.

#### *Water quality parameters*

Water quality parameters, i.e. temperature, pH, DO, salinity, and total dissolved solids, were maintained and recorded daily. A digital thermometer recorded the temperature, pH was recorded by pH tester (pocket pH tester, Romania), DO and electric conductivity (EC) were measured by an Oxygen meter (YSI model 355). TDS were calculated by using standard methodology (APHA, 1992). One-third water of each aquarium was changed

daily, while complete water replacement was carried out weekly.

#### *Growth parameters*

Growth parameters were recorded fortnightly, and new feeding rations

Weight gain=final weight - initial weight.

Length gain = final length - initial Length

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Weight of dry feed (g)}}{\text{Total weight gain (g)}}$$

The fish survival was calculated at the end of the culture period with the following relationship.

$$\text{Survival rate} = \frac{\text{final no. of fingerlings}}{\text{initial no. of fingerlings}} \times 100$$

#### *Hematological Analysis*

At the end of the feeding trial, the blood samples were collected from stocked tilapia fingerlings of each aquarium from three randomly selected fish individuals. Blood was collected with a 5cc syringe from the caudal region (Sardella *et al.*, 2004). Before blood sampling, fishes were starved for one day before sampling, and a blood sample was taken within less than 3 minutes to minimize the handling stress in fish. Vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA) were used to preserve extracted blood samples. An automated blood analyzer (Abacus 380) was used to determine the concentration of red blood cells (RBC), white blood cells (WBC), hemoglobin, hematocrit, platelets, mean corpuscular volume

were adjusted according to new weight gained. The following formulae were used to record the following growth-related parameters.

(MCV), mean corpuscular hemoglobin (MCH), lymphocytes and neutrophil.

#### *Statistical analysis*

Obtained data was arranged with mean± standard deviation and subjected to SAS version 9.1 for statistical analysis. One-way analysis of variance (ANOVA) was applied to check variance of means, and Duncan's multiple range test was applied to check significant differences between mean values ( $p<0.05$ ).

## **Results**

#### *Proximate analysis of feed*

The fish feed's proximate composition, termed here as *Oryza* organic feed as per the market name, used as an experimental diet in the present trial is given in Table 1. According to proximate analysis, moisture, protein, lipid, ash and fiber contents were as 9.76±0.20%, 39.96±1.45%, 7.33±0.90%, 7.36±1.10% and 5.4±0.81%, respectively. Protein concentration found in our experimental

diet was found within an optimum concentration of  $39.96 \pm 1.45\%$ .

**Table 1: Proximate analysis of Oryza organic feed used in the experiment.**

Composition	Moisture%	Protein %	Fats %	Ash%	Fiber %
1	10	40	8	7.3	4.7
2	9.7	38.5	6.3	6.3	5.2
3	9.6	41.4	7.7	8.5	6.3
Mean $\pm$ SD	$9.76 \pm 0.20$	$39.96 \pm 1.45$	$7.33 \pm 0.90$	$7.36 \pm 1.10$	$5.4 \pm 0.81$

#### Water quality parameters

Water quality parameters were recorded daily, and values are given in Table 2. Temperature, pH, DO, EC, bicarbonates, sodium, chlorine, sodium absorption and TDS were calculated, respectively. The temperature was recorded as  $T_3 (28.25 \pm 0.75^\circ\text{C})$  followed by  $T_2 (27.65 \pm 0.35^\circ\text{C})$ ,  $T_1 (27.40 \pm 0.10^\circ\text{C})$  and  $T_0 (27.00 \pm 1.00^\circ\text{C})$  whereas pH was recorded as  $T_3 (7.90 \pm 0.20)$ ,  $T_2 (7.70 \pm 0.30)$ ,  $T_1 (7.70 \pm 0.10)$  and  $T_0 (7.60 \pm 0.00)$ . DO level was recorded as

$T_0 (5.33 \pm 1.23)$  followed by  $T_1 (5.19 \pm 0.54)$ ,  $T_2 (5.45 \pm 1.22)$  and  $T_3 (4.69 \pm 0.88)$ . Recorded values of temperature, pH and oxygen remained constant and within optimum ranges during the trial. Statistical analysis showed that temperature, pH and Oxygen level are not significantly affected by salinity, and non-significant differences for these parameters were recorded among these treatments ( $p > 0.05$ ).

**Table 2: Water quality parameters in different treatments salinity levels recorded during the experimental period.**

Parameters	$T_0$	$T_1$	$T_2$	$T_3$	P-value
Temperature $^\circ\text{C}$	$27.00 \pm 1.00^a$	$27.40 \pm 0.10^a$	$27.65 \pm 0.35^a$	$28.25 \pm 0.75^a$	0.2016
pH	$7.60 \pm 0.00^a$	$7.70 \pm 0.10^a$	$7.70 \pm 0.30^a$	$7.90 \pm 0.20^a$	0.3234
Oxygen (mg/L)	$5.33 \pm 1.23^a$	$5.19 \pm 0.54^a$	$5.45 \pm 1.22^a$	$4.69 \pm 0.88^a$	0.3312
EC ( $\mu\text{S}/\text{cm}$ )	$1305.0 \pm 375.0^d$	$9635.0 \pm 15.0^c$	$15955.0 \pm 145.0^b$	$21415.0 \pm 1305.0^a$	<.0001
Bicarbonates (mg/L)	$2.80 \pm 0.40^b$	$3.30 \pm 0.30^b$	$4.00 \pm 0.20^a$	$4.10 \pm 0.10^a$	<.0001
Na (mg/kg)	$9.55 \pm 3.25^d$	$92.45 \pm 0.55^c$	$155.40 \pm 1.40^b$	$209.80 \pm 13.10^a$	<.0001
Cl (mg/kg)	$8.40 \pm 3.00^d$	$71.45 \pm 20.65^c$	$135.55 \pm 14.65^b$	$187.60 \pm 2.90^a$	<.0001
Sodium absorption	$7.05 \pm 1.95^d$	$66.65 \pm 3.75^c$	$107.90 \pm 0.20^b$	$142.25 \pm 9.35^a$	<.0001
TDS (mg/L)	$0.91 \pm 0.26^d$	$6.74 \pm 0.01^c$	$11.16 \pm 0.10^b$	$14.98 \pm 0.91^a$	<.0001

Values given in table are Means with SD. The numbers among same row having different alphabet are significantly different ( $p < 0.05$ ).

EC and bicarbonates were also recorded as  $T_3 (21415.0 \pm 1305.0)$ ,  $T_2 (15955.0 \pm 145.0)$ ,  $T_1 (9635.0 \pm 15.0)$ , and  $T_0 (1305.0 \pm 375.00)$ , whereas the highest bicarbonates concentration was ( $4.10 \pm 0.10$ ) in  $T_3$  and the lowest ( $2.80 \pm 0.40$ ) was recorded in  $T_0$ . The statistical analysis showed that EC significantly differed in all treatments

( $p < 0.05$ ). The sodium ions concentration was recorded in  $T_3 (209.80 \pm 13.10)$ ,  $T_2 (155.40 \pm 1.40)$ ,  $T_1 (92.45 \pm 0.55)$ , and  $T_0 (9.55 \pm 3.25)$ . However, the values of chlorine ions were recorded as  $T_3 (187.60 \pm 2.90)$ ,  $T_2 (135.55 \pm 14.65)$ ,  $T_1 (71.45 \pm 20.65)$ , and  $T_0 (8.40 \pm 3.00)$ . The statistical analysis showed that sodium and chloride ions

increased as the salinity level increased, and a significant difference was seen among all the treatments.

#### *Growth parameters*

Growth parameters, i.e. weight gain, length gain, FCR and survival rate, were recorded fortnightly. The recorded values of growth parameters are given in Table 3. Weight gain was observed as T<sub>3</sub>(15.11±2.80g), T<sub>2</sub>(14.66±1.90g), T<sub>1</sub>(13.00±2.44g) and T<sub>0</sub>(8.88±1.56g). Length gain was recorded as T<sub>3</sub> (5.06±0.77cm) followed by T<sub>2</sub> (5.03±1.05cm), T<sub>1</sub> (4.84±0.68 cm), and

T<sub>0</sub> (4.60±0.93 cm). Statistical analysis showed a significant difference in weight gain and length gain among different treatment groups ( $p<0.05$ ). FCR values were recorded as (4.13±0.67), (3.05±0.87), (3.63±0.53) and (2.61 ± 0.92) for T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub>, respectively. T<sub>3</sub> showed better FCR as compared to T<sub>0</sub>. A significant difference was found among all treatment groups ( $p<0.05$ ). No mortality was observed in our study among all treatment groups during the trial period.

**Table 3: Average fortnightly weight gain, length gain, FCR and survival of different salinity groups of Tilapia (*Oreochromis niloticus*)**

Parameters Value	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	P-
Weight gain (g)	8.88±1.56 <sup>c</sup>	13.00±2.44 <sup>b</sup>	14.66±1.90 <sup>a</sup>	15.11±2.80 <sup>a</sup>	<.0001
Length gain (cm)	4.60± 0.74 <sup>b</sup>	4.84±0.43 <sup>ab</sup>	5.03±0.38 <sup>a</sup>	5.06±0.43 <sup>a</sup>	0.0373
FCR	4.13±0.67 <sup>a</sup>	3.05±0.87 <sup>c</sup>	3.63±0.53 <sup>b</sup>	2.61±0.92 <sup>d</sup>	<.0001
Survival	100%	100%	100%	100%	<.0001

Values given in the table are Means with SD. The numbers among the same row having different alphabet are significantly different ( $p<0.05$ ).

#### *Hematological analysis*

Selected blood parameters were checked at the end of the trial. Recorded values of these parameters are presented in Table 4. According to recorded data, RBCs were recorded as 0.85±0.03, 0.27±0.04, 0.63±0.03 and 0.97 ±0.02 among T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. In T<sub>1</sub>, the RBCs count was the lowest while increasing the salinity number of RBCs increased and the

highest number of RBCs was observed in T<sub>3</sub>. Statistical analysis showed that the number of RBCs in all treatments showed a significant difference ( $p<0.001$ ). Similarly, the WBCs were recorded as 12.33 ±0.57, 11.43 ±0.05, 11.00±0.65 and 13.80± 0.45 in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>and T<sub>3</sub>, respectively. Statistical analysis showed a significant difference among treatment groups ( $p<0.001$ ).

**Table 4: Blood parameters of *Tilapia Oreochromis niloticus* in different salinity groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>0</sub>**

Parameters	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	P-value
RBCs count	0.85±0.03 <sup>b</sup>	0.27±0.04 <sup>d</sup>	0.63±0.03 <sup>c</sup>	0.97±0.02 <sup>a</sup>	<.0001
WBCs count	12.33±0.57 <sup>b</sup>	11.43±0.05 <sup>bc</sup>	11.00±0.65 <sup>c</sup>	13.80±0.45 <sup>a</sup>	0.0006
Hematocrit (%)	8.43±0.61 <sup>b</sup>	8.43±0.30 <sup>b</sup>	9.33±0.37 <sup>b</sup>	10.80±1.00 <sup>a</sup>	0.0055
Platelets	213.33±52.54 <sup>a</sup>	160.33±11.54 <sup>b</sup>	98.00±0.00 <sup>c</sup>	63.00±0.00 <sup>c</sup>	0.0006
Hemoglobin (gm/dl)	2.96±0.15 <sup>b</sup>	3.06±0.20 <sup>b</sup>	3.40±0.10 <sup>a</sup>	3.70±0.17 <sup>a</sup>	0.0022
MCV (fl)	99.0±0.00 <sup>c</sup>	95.60±5.29 <sup>c</sup>	106.43±3.05 <sup>b</sup>	114.30±3.60 <sup>a</sup>	0.0009
MCH (pg)	36.96±0.80 <sup>a</sup>	34.50±0.52 <sup>b</sup>	29.33±0.51 <sup>c</sup>	25.10±0.52 <sup>d</sup>	<.0001
Lymphocytes (%)	17.10±0.00 <sup>a</sup>	15.50±3.60 <sup>ab</sup>	14.93±3.05 <sup>ab</sup>	11.00±1.00 <sup>b</sup>	0.0321
Neutrophil (%)	1.70±0.20 <sup>a</sup>	1.43±0.25 <sup>ab</sup>	1.40±0.10 <sup>ab</sup>	1.13±0.15 <sup>b</sup>	0.0353
MCHC (g/dl)	37.46±0.66 <sup>a</sup>	34.06±1.71 <sup>b</sup>	28.00±1.70 <sup>a</sup>	33.53±5.05 <sup>a</sup>	0.0210

Values given in table are Means with SD. The numbers among same row having different alphabet are significantly different ( $p < 0.05$ ).

The platelets count was recorded as 213.33±52.54, 160.33±11.54, 98.00±0.00 and 63.00±0.00 in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. As the level of salinity increased, the number of platelets decreased. The statistical analysis showed a significant difference among treatment groups for platelets ( $p < 0.05$ ). The highest concentration of hemoglobin was recorded as 3.70±0.17 in T<sub>3</sub>. The statistical analysis showed the highest hemoglobin concentration as compared to other treatment groups. A significant difference was recorded between T<sub>0</sub>, T<sub>2</sub> and T<sub>3</sub>, while none significant difference was recorded in

T<sub>2</sub> and T<sub>3</sub>, respectively. The highest number of lymphocytes and neutrophils were recorded in T<sub>0</sub>(17.10±0.00), T<sub>3</sub>(1.70±0.20), whereas the lowest number of lymphocytes and neutrophils were recorded in T<sub>3</sub>(11.00±1.00), (1.13±0.15), respectively. However, the statistical analysis showed a non-significant difference between all the treatments for these parameters. Table 5 shows a comparison of different parameters that have already been explored in different localities and international studies using the tilapia fingerlings.

**Table 5: Comparison of present result with leading studies published in international journals previously.**

Sr. #	Parameters	<i>O. niloticus</i>	<i>O. niloticus</i>	<i>Oreochromis</i> spp.	<i>O. niloticus</i>
1	References	Present study	(Iqbal <i>et al.</i> , 2012)	(Rahmah <i>et al.</i> , 2020)	(Souza <i>et al.</i> , 2019)
2	Aquaria size	3.5' × 2.5' × 3.5'	2.896ft×0.762ft×0.914ft	---	---
3	Feed type	Moisture (9.76±0.20%), Protein (39.96±1.45%), Lipid (7.33±0.90%), Ash (7.36±1.10%) and fiber contents (5.4±0.81%)	Protein (24.27), Fat (4.15), Moisture (7.76), Fiber (11.97), Ash (13.84) and Phosphorus (0.25)	---	36% crude protein and others
4	Maximum weight gain (g)	T <sub>0</sub> (8.88±1.56), T <sub>1</sub> (13.00±2.44), T <sub>2</sub> (14.66±1.9), and T <sub>3</sub> (15.11±2.80)	T <sub>0</sub> (28.00±0.69), T <sub>1</sub> (36.25±0.96), T <sub>2</sub> (36.40±1.42), T <sub>3</sub> (34.20±0.93) and T <sub>4</sub> (36.65±1.30)	T <sub>0</sub> (147±1.0), T <sub>1</sub> (156±6.0), T <sub>2</sub> (154±7.9), T <sub>3</sub> (169±10.) and T <sub>4</sub> (299±30.3)	T <sub>0</sub> (280.4±7.6), T <sub>1</sub> (263.8±3.8), T <sub>2</sub> (256.1±10.3), T <sub>3</sub> (256.5±3.8) and T <sub>4</sub> (66.5±6.9)
5	Maximum length gain (cm)	T <sub>0</sub> (4.60±0.74), T <sub>1</sub> (4.84±0.43), T <sub>2</sub> (5.03±0.38), and T <sub>3</sub> (5.06±0.43)	T <sub>0</sub> (2.3±0.07), T <sub>1</sub> (3.15±0.10), T <sub>2</sub> (3.55±0.12), T <sub>3</sub> (3.15±0.09) and T <sub>4</sub> (3.7±0.11)	---	---

Sr. #	Parameters	<i>O. niloticus</i>	<i>O. niloticus</i>	<i>Oreochromis spp.</i>	<i>O. niloticus</i>
6	Water temperature	T <sub>0</sub> (27.00±1.00), T <sub>1</sub> (27.40±0.1), T <sub>2</sub> (27.65±0.35), and T <sub>3</sub> (28.25±0.75)	25-27	---	T <sub>0</sub> (27.65 ± 1.44), T <sub>1</sub> (27.77 ± 1.40), T <sub>2</sub> (27.57 ± 1.34), T <sub>3</sub> (27.65 ± 1.35) and T <sub>4</sub> (27.61±1.53)
7	Salinity	T <sub>0</sub> (0ppt), T <sub>1</sub> (6ppt), T <sub>2</sub> (10ppt) and T <sub>3</sub> (14ppt)	T <sub>0</sub> (800 ppm), T <sub>1</sub> (1600 ppm), T <sub>2</sub> (2400), T <sub>3</sub> (3200) and T <sub>4</sub> (4000 ppm)	T <sub>0</sub> (0 g L <sup>-1</sup> ), T <sub>1</sub> (4 g L <sup>-1</sup> ), T <sub>2</sub> (8 g L <sup>-1</sup> ), T <sub>3</sub> (12 g L <sup>-1</sup> ), and T <sub>4</sub> (16 g L <sup>-1</sup> ) Average 7	T <sub>0</sub> (0 g L <sup>-1</sup> ), T <sub>1</sub> (4 g L <sup>-1</sup> ), T <sub>2</sub> (8 g L <sup>-1</sup> ), T <sub>3</sub> (12 g L <sup>-1</sup> ), and T <sub>4</sub> (16 g L <sup>-1</sup> )
8	pH	T <sub>0</sub> (7.60±0.00), T <sub>1</sub> (7.70±0.10), T <sub>2</sub> (7.70±0.30), and T <sub>3</sub> (7.90±0.20)	---	---	T <sub>0</sub> (7.60 ± 0.23), T <sub>1</sub> (7.54 ± 0.23), T <sub>2</sub> (7.50 ± 0.23), and T <sub>3</sub> (7.45 ± 0.20) and T <sub>4</sub> (7.40 ± 0.22)
9	Oxygen (mg/l)	T <sub>0</sub> (5.33±1.23), T <sub>1</sub> (5.19±0.54), T <sub>2</sub> (5.45±1.22), and T <sub>3</sub> (4.69±0.88)	---	---	T <sub>0</sub> (2.24 - 7.62), T <sub>1</sub> (2.46 - 7.68), T <sub>2</sub> (2.17 - 7.73), T <sub>3</sub> (2.20 - 7.55) and T <sub>4</sub> (2.32 - 7.83)
10	Ec μS/cm	T <sub>0</sub> (1305.0±37.00), T <sub>1</sub> (9635.0±15.00), T <sub>2</sub> (15955.0±14.0), and T <sub>3</sub> (21415.0±13.0)	---	---	---
11	Bicarbonates (mg/l)	T <sub>0</sub> (2.80±0.40), T <sub>1</sub> (3.30±0.30), T <sub>2</sub> (4.00±0.20), and T <sub>3</sub> (4.10±0.10)	---	---	---
12	Na (mg/kg)	T <sub>0</sub> (9.55±3.25), T <sub>1</sub> (92.45±0.55), T <sub>2</sub> (155.40±1.4), and T <sub>3</sub> (209.80±13.10)	---	---	---
13	Cl (mg/kg)	T <sub>0</sub> (8.40±3.00), T <sub>1</sub> (71.45±20.6), T <sub>2</sub> (135.55±14.65), and T <sub>3</sub> (187.60±2.9)	---	---	---
14	Sodium absorption	T <sub>0</sub> (7.05 ± 1.95), T <sub>1</sub> (66.65±3.75), T <sub>2</sub> (107.90±0.20), and T <sub>3</sub> (142.25±9.35)	---	---	---
15	TDS (mg/L)	T <sub>0</sub> (0.91±0.26), T <sub>1</sub> (6.74±0.01), T <sub>2</sub> (11.16±0.1), and T <sub>3</sub> (14.98±0.91)	---	---	---
16	Initial fish weight	11.6±3.42g	T <sub>0</sub> (23.1 g±3.51), T <sub>1</sub> (22.5 g ±3.15), T <sub>2</sub> (22.9 g±3.29), T <sub>3</sub> (23.9 g±3.9) and T <sub>4</sub> (24.6 g±4.71)	---	---
17	Sample size (n)	180	225	---	---
18	Trail days	90	---	30	90

Values given in table are mean with standard deviation

## Discussion

The protein affects fish production, and a 40% protein level is considered best for Tilapia growth (Khattab *et al.*, 2000). The protein requirement depends on energy, dietary level, culturing system, size, and species. However, 27% to 37% protein levels are considered best for Tilapia (Khattab *et al.*, 2000). *O. niloticus* fingerlings showed optimum growth when protein

concentration is 40.00 ±1.34%, and it has a significant effect on its growth performance (Abdel-Tawwab *et al.*, 2010). The protein requirement for tilapia differs according to the season, age and sex. As the maturity level of *O. niloticus* increases, the requirement for protein decreased, and advanced juveniles because fingerlings of tilapia cannot use excessive protein efficiently (El-Sayed and Teshima, 1991). In the



present study, the proximate composition of dispensed feed showed that recorded nutrients were in line as other authors described (Khattab *et al.*, 2000; Abdel-Tawwab *et al.*, 2010). So, keeping in view previous researchers' work, *Oryza* organic feed was selected, containing optimum protein concentrations  $39.96 \pm 1.45$ .

The water quality studies provide vital information for identifying water type, quality, and feasible environment for aquaculture practices (Wendelaar Bonga, 1997; Hara *et al.*, 2020; Haque *et al.*, 2020; Khanom *et al.*, 2020; Saeed *et al.*, 2020). Fish showed the best growth and survival rate within pH 6-9 and at a temperature of  $26^{\circ}\text{C}$  –  $32^{\circ}\text{C}$ . The values of temperature and pH remained within the optimum ranges in the present study as described by previous researchers (Watanabe *et al.*, 1997; El-Sherif and El-Faky, 2009). At optimum temperature ( $27^{\circ}\text{C}$ ), salinity does not affect the growth rate of fish, however, as the temperature falls below  $25^{\circ}\text{C}$ , marked changes can be seen in the growth and survival of fish and a suitable range of temperature and pH recorded for fish farming remain between  $24^{\circ}\text{C}$  –  $30^{\circ}\text{C}$  and 6.7 – 9.5, respectively (Wendelaar Bonga, 1997).

The optimum DO level for better growth performance of fish is  $>5\text{ppm/l}$ , whereas an optimum range of temperature and pH is  $24^{\circ}\text{C}$  –  $30^{\circ}\text{C}$  and 6.7 – 9.5 (Bhatnagar *et al.*, 2004; Santhosh and Sing, 2007). A slight change in optimum pH and temperature i.e. above and below this range, creates a stressful *O. niloticus*. Below  $5\text{ppm}$ ,

fish growth rate is affected, and in severe conditions, it may cause fish mortality (Kim *et al.*, 2019; Bae *et al.*, 2020; Moon *et al.*, 2020). Our results showed that an increase in salinity does not significantly affect water quality parameters, i.e. temperature, DO and pH and these values were found within optimum range among all treatment groups. Previous researchers also supported our findings who reported that salinity has no profound effect on water quality parameters from 4 ppt up to 34 ppt (Watanabe, 1995; Hamed *et al.*, 2016). Salinity does not influence the DO, temperature and pH levels and optimum range of these parameters for the best growth of tilapia, i.e. temperature  $28.19 \pm 1.25^{\circ}\text{C}$ , DO  $4.75 \pm 0.63\text{ mg/l}$  and pH  $7.06 \pm 0.71$  for fish growth (De Azevedo *et al.*, 2015). So above recorded values were within the optimum range as described by previous researchers.

Growth parameters, i.e. weight gain, length gain, FCR and survival rate, are significantly affected by a change in salinity level. As we achieved the best value of FCR in  $T_3$  these findings are in line with previous results reported by Boeuf and Payen (2001) and Sparks *et al.* (2003) that as the salinity level increased, average weight gain and average length gain also get better. Similar findings have also been published previously by other authors (Kangombe and Brown, 2008; Basuki and Rejeki, 2015).

In this present study, as the salinity level increases, RBCs level also increased, and the maximum RBC count was recorded in  $T_3$ . The WBC

count is the best indicator for the expression of the physiological state of fishes. The leukocytes increased in *O. niloticus*, the level of salinity decreased, and similar findings were also recorded during *Oncorhynchus mykiss* culture in a saline medium (Elarabany *et al.*, 2017). The WBCs, Lymphocytes, Monocytes and Neutrophils increased as the salinity level decreased and as the level of salinity increased, the number of RBCs, Hemoglobin and HCT also increased significantly (Amiri *et al.*, 2009; Sahafi *et al.*, 2013). A possible reason for this fluctuation is hyperosmotic conditions; the fish reflexively lose water contents, increasing blood cell concentration. However, an increment in blood cell concentration may result from RBCs formation from the spleen, and in a hypersaline environment, the concentration of RBCs and hemoglobin increases due to splenic activities in response to a stressor (Martinez-Alvarez *et al.*, 2002; Soltanian *et al.*, 2016). The platelets packed cell volume (PCV), Lymphocytes and Hemoglobin were reduced, WBCs and Neutrophils increased as the salinity level declined. The reason for this increment in WBCs, Monocytes, and Neutrophil is that in a saline environment, fish try to maintain their ionic balance with cortisol and prolactin hormonal interaction blood parameters may fluctuate (Anyanwu *et al.*, 2007). This type of elevation could be the potential reason for ions stability in freshwater ecosystems (Eckert *et al.*, 2001).

#### *Present and previously reported impacts of varying salinity level*

We have also compared the present study outcomes with leading studies previously published in international journals (Table 5). In the current project, fingerlings were fed upon *Oryza* organic feed, Moisture ( $9.76 \pm 0.20\%$ ), Protein ( $39.96 \pm 1.45\%$ ), Lipid ( $7.33 \pm 0.90\%$ ), Ash ( $7.36 \pm 1.10\%$ ), and fiber contents ( $5.4 \pm 0.81\%$ ). After 90 days, fingerlings gained maximum weight  $T_0$  ( $8.88 \pm 1.56$ ),  $T_1$  ( $13.00 \pm 2.44$ ),  $T_2$  ( $4.66 \pm 1.9$ ) and  $T_3$  ( $15.11 \pm 2.80$ ) and maximum length  $T_0$  ( $4.60 \pm 0.74$ ),  $T_1$  ( $4.84 \pm 0.43$ ),  $T_2$  ( $5.03 \pm 0.38$ ), and  $T_3$  ( $5.06 \pm 0.43$ ). In contrast, Iqbal *et al.* (2012) and Souza *et al.* (2019) used specially formulated feed having protein (24.27), fat (4.15), moisture (7.76), fiber (11.97), ash (13.84), phosphorus (0.25). Their fish attained maximum weight  $T_0$  ( $28.00 \pm 0.69$ ),  $T_1$  ( $36.25 \pm 0.96$ ),  $T_2$  ( $36.40 \pm 1.42$ ),  $T_3$  ( $34.20 \pm 0.93$ ), and  $T_4$  ( $36.65 \pm 1.30$ ). Souza *et al.* (2019) fish attain maximum weight  $T_0$  ( $280.4 \pm 7.6$ ),  $T_1$  ( $263.8 \pm 3.8$ ),  $T_2$  ( $256.1 \pm 10.3$ ),  $T_3$  ( $25 \pm 3.8$ ), and  $T_4$  ( $66.5 \pm 6.9$ ), respectively. To check the effect of water quality parameters on fingerling growth performance, we observed temperature, salinity, pH, DO, EC, bicarbonates, sodium, chlorine, sodium absorption, and TDS. Temperature as observed as  $T_0$  ( $27.00 \pm 1.00$ ) followed by  $T_1$  ( $27.40 \pm 0.10$ ),  $T_2$  ( $27.65 \pm 0.35$ ), and  $T_3$  ( $28.25 \pm 0.75$ ), whereas pH was recorded as  $T_0$  ( $7.60 \pm 0.00$ ),  $T_1$  ( $7.70 \pm 0.10$ ),  $T_2$  ( $7.70 \pm 0.30$ ), and  $T_3$  ( $7.90 \pm 0.20$ ). However, the level of salinity was maintained as  $T_0$  (0ppt),  $T_1$

(6ppt), T<sub>2</sub> (10ppt) and T<sub>3</sub> (14ppt). Previous authors, Iqbal *et al.* (2012) reported the water temperature 25-27°C and Souza *et al.* (2019) observed water temperature T<sub>0</sub> (27.65 ± 1.44), followed by T<sub>1</sub> (27.77 ± 1.40), T<sub>2</sub> (27.57 ± 1.34), T<sub>3</sub> (27.65 ± 1.35) and T<sub>4</sub> (27.61 ± 1.53). On the other hand, Rahmah *et al.* (2020) recorded pH as 7 while Souza *et al.* (2019) reported pH T<sub>0</sub> (7.60 ± 0.23) followed by T<sub>1</sub> (7.54 ± 0.23), T<sub>2</sub> (7.50 ± 0.23), T<sub>3</sub> (7.45 ± 0.20) and T<sub>4</sub> (7.40 ± 0.22). However, the level of salinity was calculated by Iqbal *et al.* (2012) T<sub>0</sub> (800 ppm), T<sub>1</sub> (1600 ppm), T<sub>2</sub> (2400), T<sub>3</sub> (3200), and T<sub>4</sub> (4000 ppm) whereas, Rahmah *et al.* (2020) calculated water salinity T<sub>0</sub> (0 g L<sup>-1</sup>), T<sub>1</sub> (4 g L<sup>-1</sup>), T<sub>2</sub> (8 g L<sup>-1</sup>), T<sub>3</sub> (12 g L<sup>-1</sup>), and T<sub>4</sub> (16 g L<sup>-1</sup>) and Souza *et al.* (2019) T<sub>0</sub> (0 g L<sup>-1</sup>), T<sub>1</sub> (4 g L<sup>-1</sup>), T<sub>2</sub> (8 g L<sup>-1</sup>), T<sub>3</sub> (12 g L<sup>-1</sup>), and T<sub>4</sub> (16 g L<sup>-1</sup>). As compared to previous authors in our study, temperature and pH were controlled, and it did not affect the weight gain, whereas, with the increasing level of salinity, the ratio of weight gain increased. In our study, maximum weight was gained in T<sub>3</sub> (15.11 ± 2.80), whereas in Iqbal *et al.* (2012) study, maximum weight was gained in T<sub>4</sub> (36.65 ± 1.30), in Rahmah *et al.* (2020) study, maximum weight was gained in T<sub>4</sub> (299 ± 30.3) and in Souza *et al.* (2019) study maximum attained weight was recorded in T<sub>0</sub> (280.4 ± 7.6). In our study, DO level was calculated as T<sub>0</sub> (5.33 ± 1.23) followed by T<sub>1</sub> (5.19 ± 0.54), T<sub>2</sub> (5.45 ± 1.22), and T<sub>3</sub> (4.69 ± 0.88) whereas Souza *et al.* (2019) observed DO in T<sub>0</sub> (2.24 - 7.62), T<sub>1</sub> (2.46 - 7.6),

T<sub>2</sub> (2.17 - 7.73), T<sub>3</sub> (2.20 - 7.5) and T<sub>4</sub> (2.32 - 7.83), respectively.

The present research work results indicated that Nile tilapia (*O. niloticus*) could survive in salinity level as higher as 14 ppt and it showed better growth performance compared to lower salinity concentrations. A significant difference was observed in growth parameters, but salinity levels do not impact the critically important water quality parameters.

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