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Investigation of the influence of different salinities on the growth, survival and physiological responses of GIFT Tilapia (*Oreochromis niloticus*) in Inland saline water

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Abstract
Oreochromis niloticus, a member of the Cichlidae family, is a globally cultivated finfish species and is commonly raised in environments with varying levels of salinity. The state of Gujarat in India has a vast inland saline water area, offering potential for aquaculture development. This study aimed to evaluate the impact of varying salinities on the growth, survival, and physiological responses of Tilapia in this inland saline environment. The experimental study was conducted at Aqua Fish Farm in Ranagadh, Surendranagar, Gujarat, using a Completely Randomized Design with four treatment groups with different salinity [(Control, T_0, 0 ppt), (T_1, 5 ppt), (T_2, 10 ppt) and (T_3, 15 ppt)] and a duration of experiment was 90 days. Healthy advanced fry of the *Oreochromis* sp. were stocked at a density of 30 individuals per experimental unit. The results showed that the optimal growth performance was observed in the T_1 treatment group (5 ppt), while the lowest growth performance was observed in the T_3 treatment group with 15 ppt salinity and lowest Feed Conversion Ratio (FCR) was recorded in the T_1 treatment group as indicated by various growth parameters including Specific Growth Rate (SGR), (Protein Efficiency Ratio) PER, Feed Efficiency Ratio (FER), and Average Weight Gain (AWG). The study also assessed the physiological responses of Tilapia to varying salinities, and the results showed a positive correlation between increasing salinity levels and the activities of Alanine Transaminase (ALT) and Aspartate Transaminase (AST). The activities of ALT and AST were highest in the T_3 treatment group and lowest in the control group, which suggest that salinity is a significant environmental factor affecting liver health and function in the fish. The study concludes that *Oreochromis niloticus* can be cultured in the inland saline region of Surendranagar at salinities up to 5 ppt without significantly compromising growth performance.

Keywords: Nile tilapia, salinity, growth performance, physiological responses, saline area

1. Introduction
The family Cichlidae comprises a diverse group of freshwater fish, including commercially significant species known as Tilapia, that are utilized worldwide in aquaculture systems, primarily due to their omnivorous feeding habits. The *Oreochromis* genus, consisting of important Tilapia species such as Nile Tilapia (*Oreochromis niloticus*), Mozambique Tilapia (*O. mossambicus*), Blue Tilapia (*O. aureus*), and Wami Tilapia (*O. urolepis hornorum*), is widely used for aquaculture (Fitzsimmons *et al.*, 1997; Fitzsimmons, 2000; Dawood *et al.*, 2021) [13, 14, 6]. Nile Tilapia, with its high growth rate and tolerance to stress, is a promising candidate for intensive and super-intensive aquaculture, and is primarily farmed in freshwater environments. The adaptability and hardiness of Tilapia are major reasons for their successful culture. They can tolerate a wide range of environmental conditions and possess omnivorous feeding habits, allowing them to subsist on various diets. Nile Tilapia is renowned for its rapid growth and ability to thrive in high-density farming environments. Overall, the *Oreochromis* genus and Tilapia are recognized as significant resources for efficient and sustainable aquaculture practices, making them vital components of global food production.
Tilapia fish is hypothesized to have evolved from marine ancestors (Kirk, 1972) [21] and are primarily distributed in freshwater habitats. Nevertheless, they display broad salinity tolerance and have the capacity to thrive and reproduce in brackish water, with some tilapia species even able to persist in high-salinity environments (Suresh and Lin, 1992) [35]. Nonetheless, the culture of tilapia in brackish and seawater remains relatively underexplored compared to freshwater environments. Nile tilapia (*O. niloticus*) is a popular freshwater fish species for aquaculture because of its adaptability and fast growth.

However, due to the scarcity of freshwater in various regions, alternative water resources such as inland saline water hold potential for tilapia culture (Enciso-López and García-Trejo, 2019) [12].

Salt-affected soils represent a substantial ecological entity, accounting for 6% of the world's land area and 2.6% of the geographic region in India (Mandal and Sharma, 2006) [26]. In India, approximately 8.62 million hectares of land are affected by salt, with an additional 1.93 million hectares impacted by saline water (Lakra, 2014) [24]. These areas are primarily distributed in the arid and semi-arid regions of various states (Dhawan *et al.*, 2009, 2010) [10, 11]. In Gujarat, inland saline areas span over 12.18 lakh hectares (Mandal *et al.*, 2010) [27]. Specifically, in the Surendranagar district of Gujarat, 26 villages are affected by salinity and 4608.80 sq.km of inland saline area (Haskoning, 1998) [40], with a salinity range of 4-150 ppt, rendering it unsuitable for agriculture and aquaculture. There is limited information available on GIFT tilapia culture in inland saline water, and no studies have been conducted on GIFT tilapia culture in the inland saline water of Surendranagar district, Gujarat, which presents a potential area for inland saline aquaculture.

2. Materials and Methods

2.1 Fish and the methodology of experimentation

Healthy and active *Oreochromis niloticus* fry were obtained from the Aqua Fish Farm in Ranagadh, Surendranagar, and subjected to acclimation for a period of 10 days to varying salinities corresponding to the experimental treatments. Only individuals weighing between 1.5-1.6 gm were selected for stocking in the experimental tanks, with a density of 30 individuals per tank. Rectangular plastic tanks with a capacity of 150 litres were filled with inland saline and freshwater from the study site to obtain salinity levels of four treatments with triplicates: T_0 (0±0.5), T_1 (5±0.5), T_2 (10±0.5), and T_3 (15±0.5). Each tank was continuously aerated using 2 HP blowers and 4 diffuser stones, except during feeding and sampling. The required salinity was maintained throughout the experimental period, and various water quality parameters, including dissolved oxygen, salinity, pH, and temperature, were measured daily using specific instruments. Total hardness, total alkalinity, total ammonia nitrogen (TAN), nitrite, and nitrate were assessed every 15 days using a multi-parameter photometer, COD, and a digital pH meter (230V).

2.2 Experimental diet

During the experimental period, the *Oreochromis niloticus* individuals were fed a commercially available feed (GROWFIN) at a rate of 5% of their body weight. The GROWFIN feed had a biochemical composition containing 32% crude protein, 5.0% crude fat, 5.5% fiber, and 11.5% moisture. The feeding schedule involved two daily feedings, which was consistent with the standard feeding frequency observed in commercial fish farming.

2.3 Physico-chemical parameters of water

On a daily basis, a diverse array of water quality parameters encompassing dissolved oxygen, salinity, pH, and temperature were assessed. Additionally, total hardness, total alkalinity, and the concentrations of total ammonia nitrogen (TAN), nitrite, and nitrate were evaluated every 15 days in accordance with established procedures.

2.4 Growth parameters

Fortnightly random sampling was performed from each tank to estimate growth parameters. Animal weight was measured using an electronic weighing machine with minimal stress. The collected data was used to estimate the following growth parameters, which were evaluated using the following mathematical equations:

Total weight gain (TWG)

Total weight gain = Final body weight – Initial body weight

Average weight gain (AWG)

$$\text{AWG} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \text{x} 100$$

Specific growth rate (SGR)

$$\text{SGR} = \frac{\text{Log}_e(\text{Final Weight}) - \text{Log}_e(\text{Initial Weight})}{\text{Number of Days}} \text{x} 100$$

Survival (%)

$$\text{Survival (\%)} = \frac{\text{No. of fish survived after rearing}}{\text{No. of fish stocked}} \text{x} 100$$

Food conversion ratio (FCR)

$$\text{FCR} = \frac{\text{Amount of feed given (g)}}{\text{Body weight gain(Wet weight)(g)}}$$

Feed efficiency ratio (FER)

$$\text{FER} = \frac{\text{Body weight gain (Wet weight)(g)}}{\text{Feed is given (Dry weight)(g)}}$$

Protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Body weight gain (g)}}{\text{Protein fed (g)}}$$

2.5 Physiological parameters

Various physiological parameters were analysed, specifically Aspartate aminotransferase activity (AST) and Alanine transaminase activity (ALT). The analysis was performed at the PGIFER laboratory, Kamdhenu University, Himmatnagar. The determination of AST and ALT was carried out using alpha-keto-glutarate and DL-aspartic acid as substrate, following Wooten's method (1994). To prepare test samples, homogenate solution was added to the substrate and incubated at 37 °C for 1 hour. After incubation, 2, 4-DNPH was added to both samples, and they were kept at room temperature for 20 minutes. Finally, 0.4 N NaOH was added to both samples, and the absorbance was measured at 540 nm after 10 minutes.

2.6 Statistical analysis

The statistical analysis of growth and physiological parameters was carried out using SPSS Version 16.0 software. One-way analysis of variance (ANOVA) was used to evaluate the data. Post hoc comparisons of the means (p<0.05) among different groups were made using Duncan's multiple range tests. All data presented in the text, figures, and tables are expressed as

mean±standard error, and statistical significance was determined at $p<0.05$.

3. Result and Discussion

3.1 Effect of salinity on physico-chemical parameters of water

The results of water quality parameters are given in Table 1. The present study observed no significant temperature differences between the treatment groups, which could be due to seasonal variation. Nile tilapia is a hardy species that grows best between 27-32 °C (Pandit and Nakamura, 2010) [30]. However, recent research suggests that GIFT tilapia can thrive in temperatures ranging from 18-27 °C under varying salinity conditions. The pH value of aquatic environments is crucial for aquatic organism's growth and physiology. The study found pH values ranging from 8.13-8.25, with no significant differences between the control and treatment groups. Total hardness values varied significantly across all treatments, ranging from 1261.5±43.38 to 7530.9±31.06 mg/L. Total alkalinity values ranging from 195.39±0.64 to 227.28±0.56 mg/l in T_0 and T_3, respectively. Alkalinity, salinity, and hardness were positively correlated. Total alkalinity values were significantly different ($p<0.05$) between the treatments. Nitrite mean values ranged from 0.21±0.00 to 0.41±0.01 mg/L in T_0 and T_3, respectively, while the highest mean nitrate values (0.41±0.01 mg/L) were observed in treatment T_3, and the lowest (0.21±0.00) in T_0 at the end of the experiment. Nile tilapia requires a minimum dissolved oxygen (DO) level of 5 mg/L for optimal growth (Mengistu *et al.*, 2020) [28]. Mean DO values differed significantly ($p<0.05$) across all treatments throughout the experimental period.

Table 1: Observation of various water quality parameters during experimental period

Treatments	DO (ppm)	Temp. (°C)	pH	NO_2 (mg/L)	NO_3 (mg/L)	Alkalinity (mg/L)	Ammonia (mg/L)	Hardness (mg/L)
Control (T_0) (0 ppt)	8.28±0.00^b	24.5±0.00	8.13±0.03^a	0.21±0.00^c	2.67±0.06^b	195.39±0.64^b	0.23±0.00^b	1261.5±43.38^b
T_1 (5 ppt)	8.12±0.00^b	24.5±0.00	8.16±0.04^a	0.24±0.00^d	2.50±0.04^a	205.78±0.58^a	0.25±0.00^a	2285.2±22.25^a
T_2 (10 ppt)	7.93±0.01^b	24.5±0.00	8.23±0.04^a	0.34±0.00^b	2.06±0.07^c	216.33±0.48^c	0.35±0.00^c	4602.7±21.77^c
T_3 (15 ppt)	7.70±0.00^d	24.5±0.00	8.25±0.04^a	041±0.01^a	1.59±0.06^d	227.28±0.56^d	0.40±0.00^d	7530.9±31.06^d

3.2 Effect of salinity on growth performance

Salinity exerts a significant influence on the growth and development of fish, with variations based on the species involved. Some fish exhibit greater tolerance for high salinity levels than others (Bœuf and Payan, 2001) [2]. Some researchers propose that the changes in growth rate could be attributed to an effect on standard metabolic rate or food intake (NASA, 2011) [39]. It is also important to bear in mind that salinity levels can have an impact not just on fish growth but also on their survival and reproductive success. The relationship between salinity and fish growth is intricate and varies depending on the fish species and the salinity levels in their habitat. *Oreochromis niloticus* is a globally significant fish species that is extensively farmed for human protein supply and economic benefits. Salinity is a crucial determinant in the distribution of aquatic species and it plays a fundamental role in the growth and development of *O. niloticus.* Growth parameters of present study is mentioned in Table 2, Fig. 2. Which shown average weight gain (AWG) of *O. niloticus* in different treatments, where AWG ranged from 402.2±3.23 to 679.7±8.06 in T_3 and T_1, respectively. AWG, specific growth rate (SGR), and total weight gain values varied considerably among all treatments compared to the control. T_1 exhibited the highest mean values of AWG, SGR, and total weight gain, suggesting superior growth performance and feed utilization capacity of *O. niloticus*, regardless of water salinity. The study postulates that the improved AWG in low salinity levels (T_0 and T_1) could be attributed to reduced energy expenditure needed for ionic regulation. Fish living in isotonic environments, where there are minimal ionic gradients between the blood and water, need to expend energy to maintain ionic balance. In low salinity levels, this energy can be redirected towards growth, resulting in better AWG. This finding aligns with previous research by Likongwe *et al.* (1996) [25] and Iqbal *et al.* (2012), [19] who also reported that low salinity levels have a positive impact on fish growth. Moreover, the study suggests that *O. niloticus* can be cultured in environments with moderate levels of water salinity, as demonstrated by de Azevedo *et al.* (2015) [7]. Payne (1983) [31] recommended that the optimum salinity range for *O. niloticus* is between 5 and 10 ppt, and the current study found no adverse effects on growth, survival, and hematological parameters up to 7 ppt.

The study demonstrated that the T_0 and T_1 treatments resulted in 100% survival, whereas T_3 had a survival rate of 96.66% (Fig. 3). This finding is partially in agreement with Watanabe (1985) [37] study that reported high survival rates of Nile tilapia fry up to 10 ppt. The survival rate in T_3 was significantly different ($p<0.05$) from that in the other treatments and the control. The decline in survival at higher salinities may be attributed to osmoregulatory challenges, as gills play a crucial role in fish health by regulating osmotic pressure, removing nitrogenous waste, and facilitating gas exchange. Saline water can affect the chloride cells and Na+-K+-ATPase activity in the gill epithelium (Kızak, V., Ozden, O. & Guner, Y. 2013) [22], which can damage the filaments and branchial lamellae, leading to functional interference and compromising the survival of these animals (Reis *et al.*, 2009) [33]. The study's findings are consistent with de Azevedo *et al.* (2015) [7] results that Nile tilapia can survive at rates of 80-95% in water salinities of 0-21 ppt.

The food conversion ratio (FCR) in fish can be significantly influenced by salinity. The study calculated the feed efficiency ratio (FER) as the reciprocal of FCR, and observed the lowest FCR in treatment T_1 (1.76±0.00) and the highest in T_3 (2.36±0.00) (Table 2, Fig. 4). T_1 also had the highest mean FER value (0.014±0.00), while T_3 had the lowest (0.018±0.00) (Table 2, Fig. 6). This higher FCR in T_3 was attributed to the excess salt ions in high-salinity water, which can affect fish feed intake and conversion ratio. The study's findings are consistent with previous research reporting higher FCR at higher salinities. The mean values of FCR and FER showed a significant difference ($p<0.05$) among all treatments compared to the control. Similar results were reported by Likongwe *et al.* (1996) [25] and De Silva *et al.* (1985) [8] for different fish species at varying salinities. The study's observations suggest that salinity affects fish feed intake, digestion, and metabolism, leading to an impact on the conversion efficiency of feed into body mass. Therefore, salinity should be considered when formulating fish feed and determining optimal conditions for fish growth and production. The study also found significant

differences (P<0.05) in the mean values of protein efficiency ratio (PER) among treatments and the control, with T_1 having the highest mean PER value (0.058±0.000) and T_3 having the lowest (0.045±0.000) (Table 2, Fig. 7). PER is a commonly used method for assessing the quality of protein, especially in animal feed.

Table 2: Growth performance and survival of *O. niloticus*

	Total weight gain (g)	Average weight gain (%)	Survival (%)	FCR	SGR	PER	FER
Control(T_0) (0 ppt)	9.55±0.024^b	583.7±4.6^b	100±0.0^a	1.85±0.00^c	0.927±0.003^b	0.056±0.000^b	0.017±0.00^b
T_1 (5 ppt)	11.19±0.015^a	679.7±8.0^a	100±0.0^a	1.76±0.00^d	0.991±0.005^a	0.058±0.0001^a	0.018±0.00^a
T_2 (10 ppt)	8.28±0.049^c	506.3±5.0^c	98.88±1.1^a	1.93±0.03^b	0.869±0.004^c	0.054±0.0003^c	0.017±0.00^c
T_3 (15 ppt)	6.62±0.013^d	402.2±3.2^d	96.66±0.0^b	2.36±0.00^a	0.778±0.003^d	0.045±0.0001^d	0.014±0.00^d

*Values are presented as mean±SE in the same column within each classification bearing different letters are significantly (p<0.05) different at different treatments during the sampling

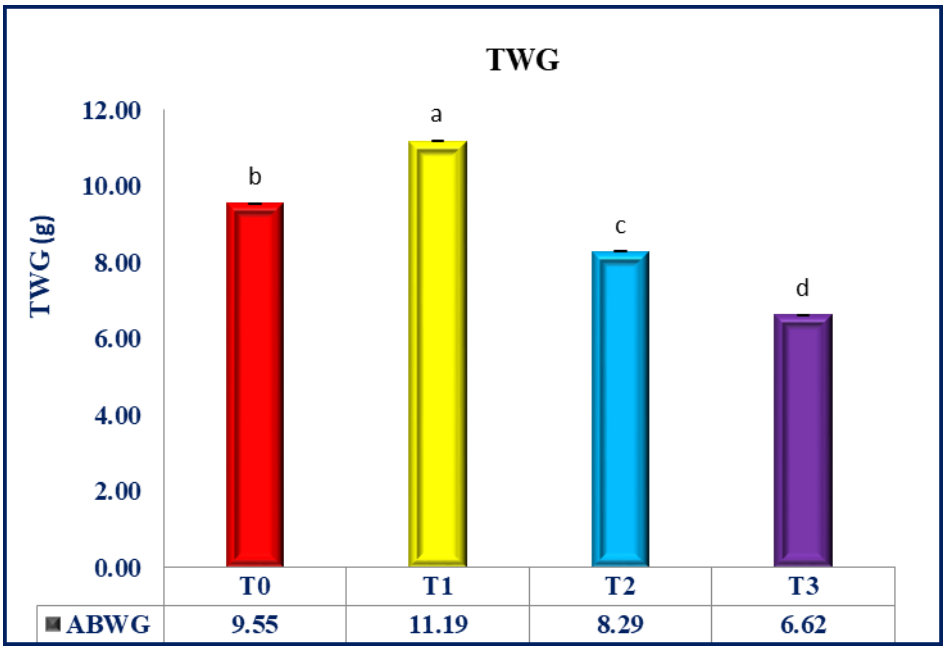

Fig 1: Total weight gain (TWG) during the culture period (T_0; 0 ppt), (T_1; 5 ppt) (T_2; 10 ppt) and (T_3; 15 ppt), (*Mean±SE; significantly different, p<0.05).

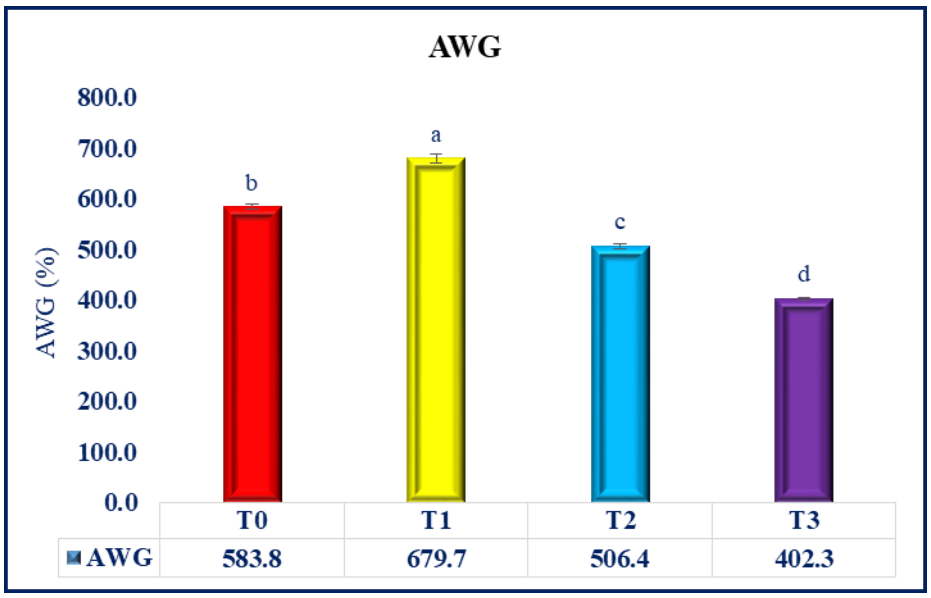

Fig 2: Average weight gain (AWG) during the culture period (T_0; 0 ppt) (T_1; 5 ppt) (T_2; 10 ppt) and (T_3; 15 ppt), (*Mean±SE; significantly different, p<0.05)

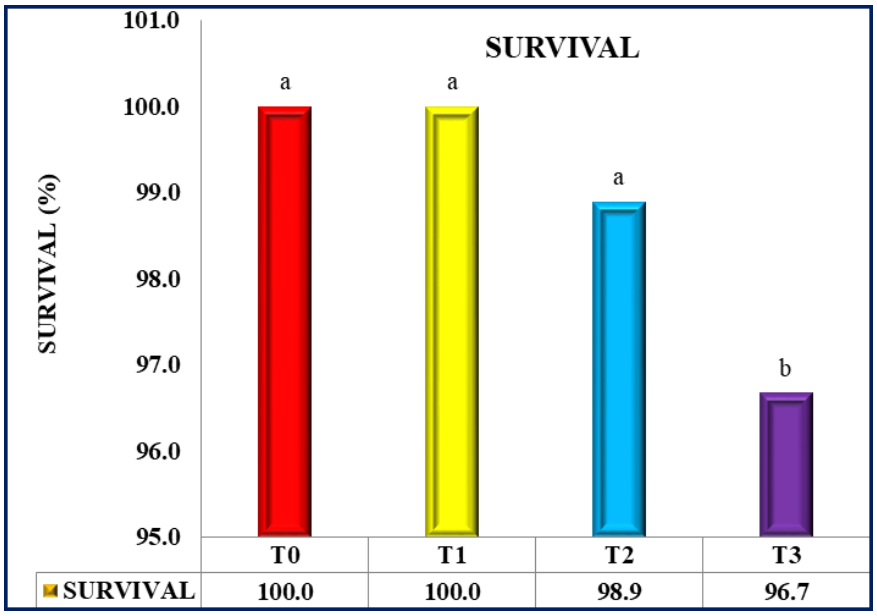

Fig 3: Survival during the culture period (T_0; 0 ppt), (T_1; 5 ppt), (T_2; 10 ppt) and (T_3; 15 ppt), (*Mean±SE; significantly different, p<0.05)

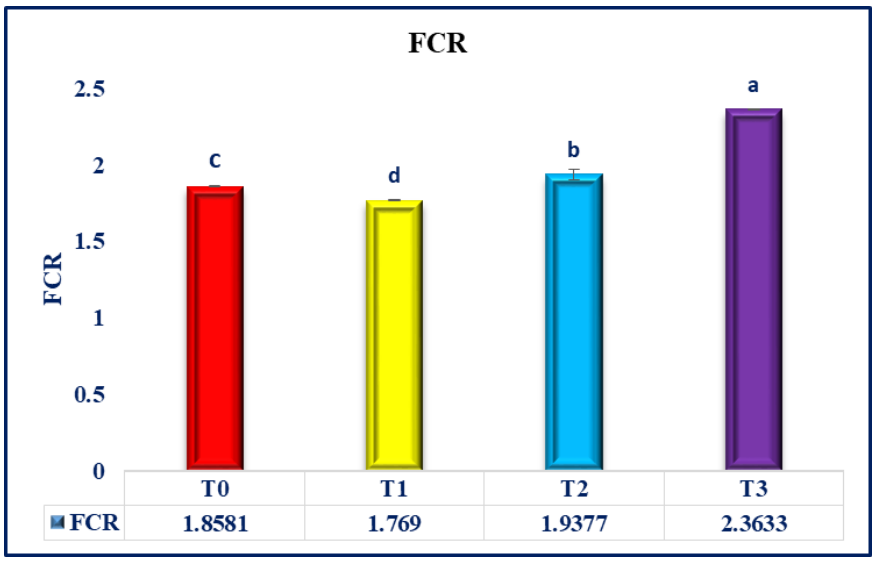

Fig 4: Food conversion ratio during the culture period. (T_0; 0 ppt), (T_1; 5 ppt), (T_2; 10 ppt) and (T_3; 15 ppt), (*Mean±SE; significantly different, p<0.05)

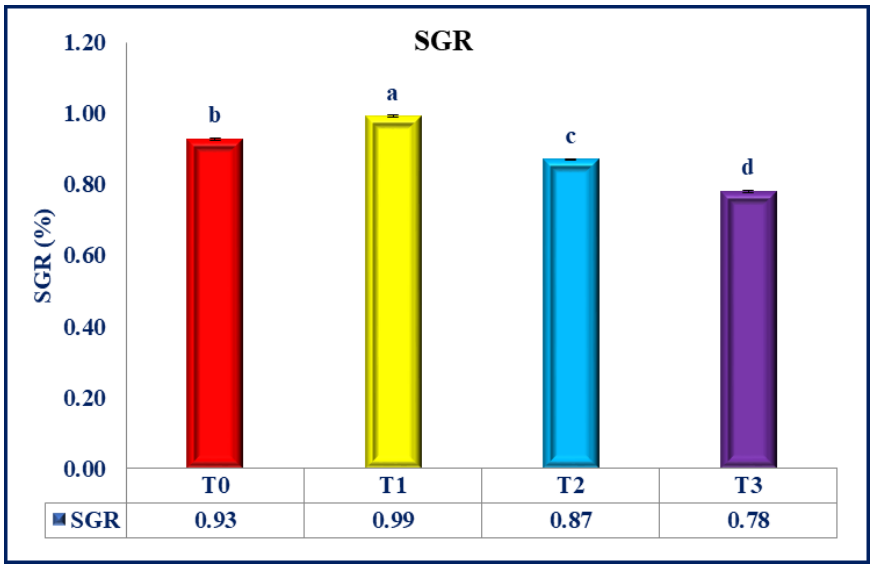

Fig 5: Specific growth rate during the culture period. (T_0; 0 ppt), (T_1; 5 ppt), (T_2; 10 ppt) and (T_3; 15 ppt), (*Mean±SE; significantly different, p<0.05)

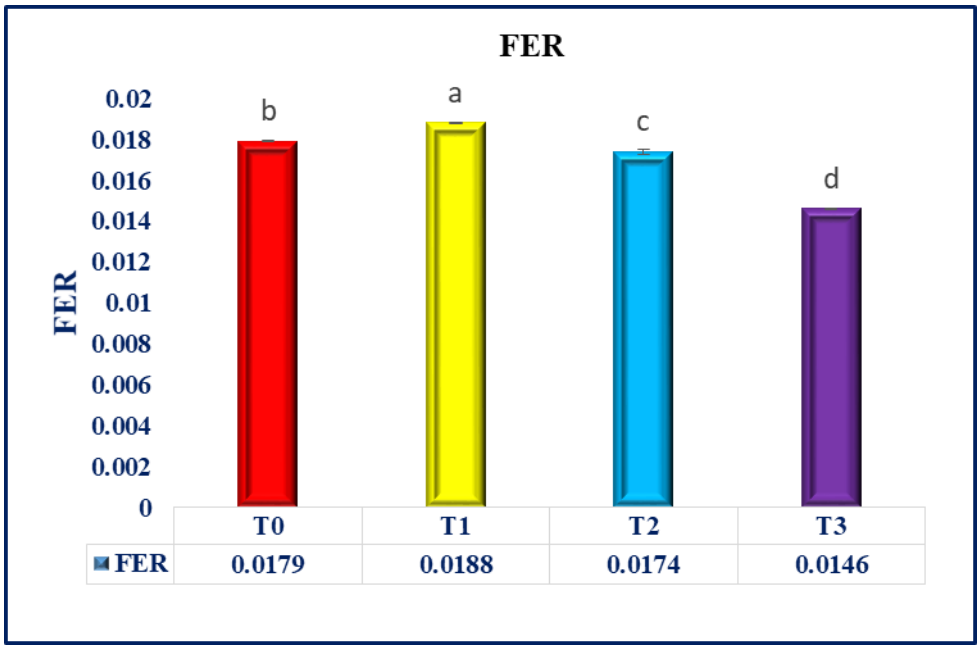

Fig 6: Feed efficiency ratio during the culture period. (T_0; 0 ppt), (T_1; 5 ppt), (T_2; 10 ppt) and (T_3; 15 ppt), (*Mean±SE; significantly different, $p<0.05$

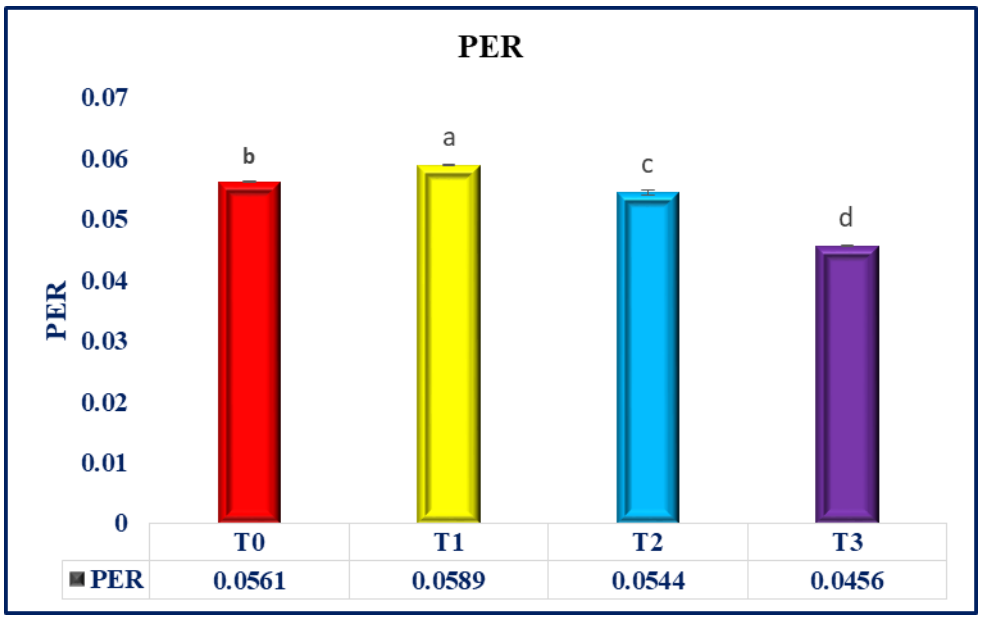

Fig 7: Protein efficiency ratio during the culture period. (T_0; 0 ppt), (T_1; 5 ppt), (T_2; 10 ppt) and (T_3; 15 ppt), (*Mean±SE; significantly different, $p<0.05$).

3.3 Effect of salinity on physiological response

Various external environmental factors, including salinity, influence fish growth potential and survival (Buckel, 1995; Deane, 2009; Iqbal, 2012) [3, 9, 19]. Salinity, an abiotic factor can significant influence on the physiological and behavioural responses of aquatic organisms, such as fish and shellfish species (Peterson, 1994) [32]. The degree of tolerance to salinity fluctuations varies among different aquatic organisms during different stages of their life cycle (James *et al.*, 2003; Nielsen *et al.*, 2003; Huang *et al.*, 2022) [20,29,17]. Osmoregulation maintains the balance of ionic concentration in the internal environment of an organism in response to changes in the surrounding media. Fish species capable of adapting to fluctuating salinity levels undergo multiple physiological adjustments to maintain osmotic balance (Whitfield, 2015) [38]. Disruption of osmotic homeostasis can lead to physiological stress, reduced survival rates, and impaired growth performance (Skomal, 2012) [34].

In case of stenohaline freshwater fish undergo various physiological adaptations when exposed to salt stress, leading to an increased energy demand to maintain internal homeostasis (Bailey *et al.*, 2002) [1].

In our study, the mean values for ALT ranged from 3.1±0.03 to 5.1±0.02 in T_0 and T_3, respectively (Table 3, Fig. 8), while AST activity ranged from 19.10±0.04 to 27.10±0.01 in T_0 and T_3, respectively (Table 3, Fig. 9). Both ALT and AST values showed significant differences ($p<0.05$) between the treatments and control. Das *et al.* (2004) reported that ALT levels are increased under stressful conditions, which can be influenced by environmental parameters such as temperature and salinity. Our study shows an increase in the enzymatic activities of both ALT and AST with increasing salinity. This increase may be indicative of stress or liver damage, as previously suggested by Huang *et al.* (2006) [18] who found that

damaged or diseased organs release additional ALT and AST into the bloodstream, causing enzyme levels to rise. Knox and Greengard (1965) [23] also reported elevated transaminase activity during stress, as response of higher AST and ALT activity, leads to the mobilization of free amino acids to produce glucose to cope with stress (Chatterjee *et al.*, 2006) [4]. Fluctuations in environmental conditions i.e. temperature and salinity can affect blood enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST), which can serve as reliable biomarkers for assessing the health status of fish and can indicate various pathological conditions and disorders (Das *et al.*, 2004; Ghelichpour *et al.*, 2020) [5,15] . Elevated concentrations of alanine aminotransferase (ALT) in fish liver indicate hepatocellular abundance and injury, causing the enzyme to leak into circulation. ALT levels is a reliable biomarker of liver health and functionality (González JD, *et al.*, 2012) [16]. Aspartate aminotransferase (AST) shows similar results and can be used as a biomarker for assessing histopathological injuries (Taheri Mirghaed A, Fayaz S, Hoseini SM., 2019) [36]. The significant differences in ALT and AST values between the treatments and control suggest that salinity is a significant environmental factor affecting liver health and function in the fish.

Table 3: Physiological response of *O. niloticus*

Treatment	(ALT) (U mg protein-1 min^{-1})	(AST) (U mg protein-1 min^{-1})
Beginning	2.4±0.04^{e}	14.2±0.1^{e}
T_0 (0 ppt)	3.1±0.03^{d}	19.1±0.04^{d}
T_1 (5 ppt)	5.1±0.02^{c}	20.1±0.01^{c}
T_2 (10 ppt)	6.2±0.03^{b}	24.2±0.05^{b}
T_3 (15 ppt)	7.4±0.07^{a}	27.1±0.01^{a}

*Values are presented as mean±SE in the same column within each classification bearing

Different letters are significantly (p<0.05) different at different treatments during the sampling''

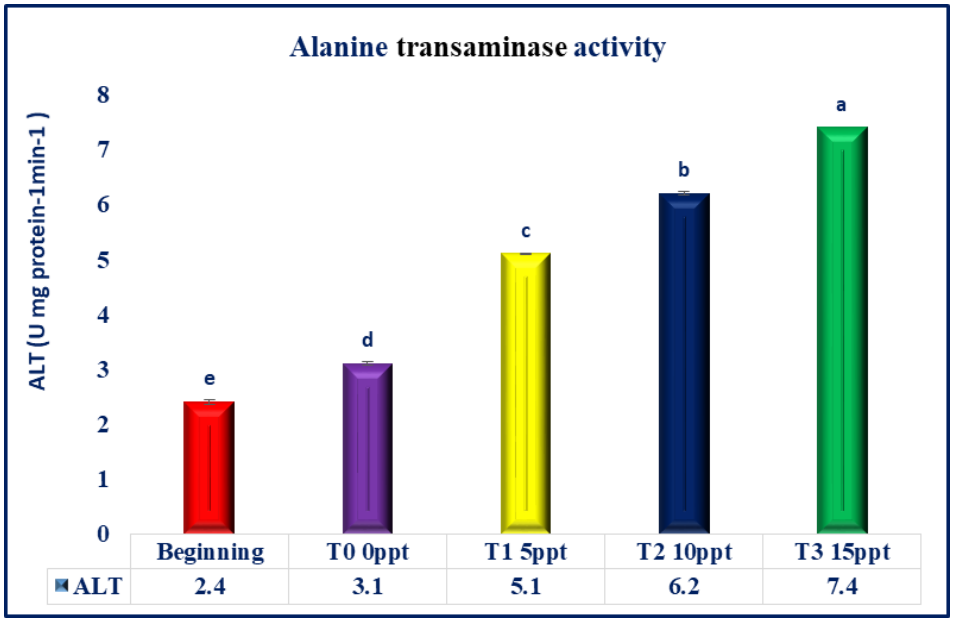

Fig 8: Alanine transaminase activity (ALT) during the culture period. (T_0; 0 ppt) (T_1; 5 ppt) (T_2; 10 ppt) and (T_3; 15 ppt) (*Mean±SE; significantly different, p<0.05)

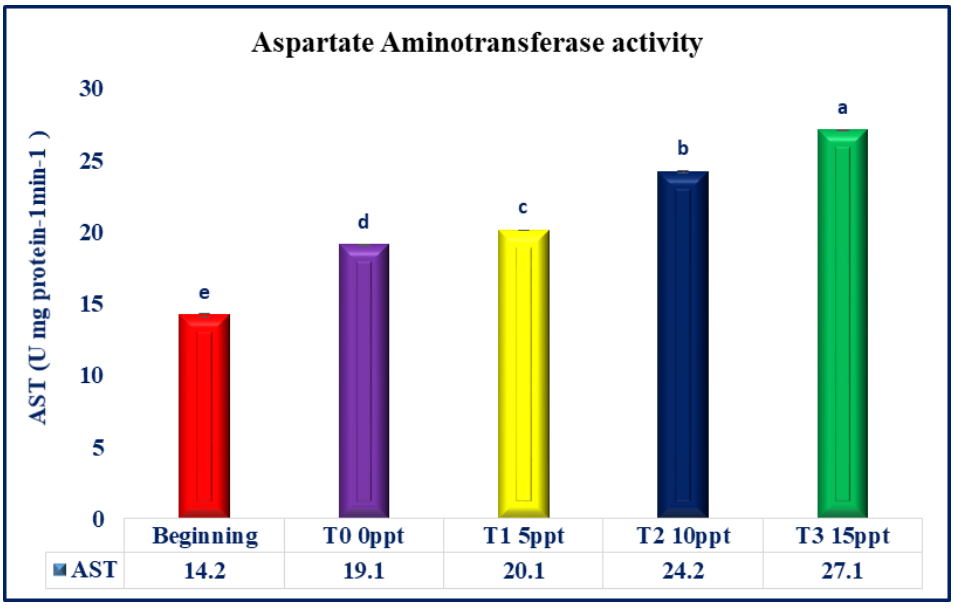

Fig 9: Aspartate aminotransferase activity (AST) during the culture period. (T_0; 0 ppt), (T_1; 5 ppt), (T_2; 10 ppt) and (T_3; 15 ppt), (*Mean±SE; significantly different, p<0.05)

Conclusion

Fish and fish products offer a cost-effective source of animal protein, particularly relevant in regions with vast salt-affected lands and ground saline water like India's arid and semi-arid areas. The study underscores the suitability of Oreochromis niloticus for saline water culture, citing its popularity, growth performance, ease of maintenance, and disease resistance. The global freshwater scarcity and competition with other sectors intensify the need for aquaculture in brackish and seawater environments. The research experiment, conducted with varying salinities, revealed that O. niloticus performs best at 0 and 5 ppt salinity levels, making it suitable for inland saline culture. Additionally, enzyme activity analysis indicated a correlation between higher salinity and increased ALT and AST levels. The study suggests future research directions, including exploring microbiota in saline soil and water, assessing Indian Major Carp's adaptability to varying salinities, and identifying new species for inland saline water culture.

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