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# Dietary Potassium Supplementation Improves the Growth and Physio-Biochemical Responses of Genetically Improved Farmed Tilapia (*Oreochromis niloticus*) Reared in Medium-Saline Inland Waters

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## Abstract

Genetically Improved Farmed Tilapia (GIFT) (*Oreochromis niloticus*) is a promising species for inland saline waters (ISW). However, ISW is potassium-deficient compared to seawater. The growth and physio-biochemical responses of GIFT tilapia to dietary potassium chloride (1%) supplementation were evaluated after a 60-day trial. The different treatments were freshwater without KCl (FW×K0), freshwater with KCl (FW×K1), 10 ppt without KCl (10×K0), 10 ppt with KCl (10×K1), 15 ppt without KCl (15×K0), and 15 ppt with KCl (15×K1). Results demonstrated significant enhancement in growth parameters across all KCl-supplemented treatments, with the most pronounced effects in FW×K1 treatment. Dietary KCl supplementation improved the average daily gain by 18.7% and 13.0% in 10×K1 and 15×K1 treatments. Biochemical analysis revealed that increasing salinity progressively elevated oxidative stress markers in K0 treatments, with 15×K0 showing the highest superoxide dismutase (SOD) and catalase (CAT) activities. Similarly, metabolic enzymes (lactate dehydrogenase, LDH; malate dehydrogenase, MDH) and transaminases (aspartate aminotransaminase, AST; alanine aminotransaminase, ALT) exhibited significantly higher activities in high-salinity K0 treatments, indicating increased anaerobic metabolism and protein catabolism. Branchial NKA (sodium potassium ATPase) activity increased at higher salinities with 15×K0 (15.53 ± 0.82), demonstrating the highest activity among all treatment combinations. K1 treatments exhibited significantly lower NKA activity than those of the K0 groups, irrespective of the rearing salinity. KCl supplementation effectively reduced osmotic stress, thereby lowering the energetic costs of osmoregulation, metabolism, and protein resources for growth. These findings suggest that dietary potassium supplementation offers a viable strategy for enhancing GIFT production in potassium-deficient inland saline environments.

**Keywords:** Specific growth rate, salinity, ion imbalance, potassium chloride, stress, sodium potassium, ATPase

## Introduction

Global food security and access to adequate dietary protein present significant challenges in today's world (McCarthy et al., 2018). As the global population continues to expand—projected to increase by 1.7 billion by 2050—addressing food shortages becomes increasingly urgent (Serraj, Krishnan, & Pingali, 2019). This situation is further complicated by the effects of global warming and climate change, with soil and water salinisation posing particular concerns for meeting future food demands (Singh, 2022).

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Approximately 833 million hectares of land worldwide are already affected by salt (Mustafa, Akhtar, & Abdullah, 2019). Intensive agricultural practices, relying heavily on fertilisers to maximise crop yields, have contributed to numerous environmental issues, including the loss of land productivity due to groundwater salinity (Majeed & Muhammad, 2019). Continuous irrigation with saline groundwater has transformed once-productive agricultural lands into unproductive wastelands (Rasool et al., 2013). These lands face two critical problems: they can no longer support traditional crops, and improper drainage has led to a rapidly rising saline water table. This situation creates an opportunity for the aquaculture sector. By utilising inland saline waters (ISW), aquaculture can serve a dual purpose: pumping water to reduce underground water salinity while simultaneously cultivating fish and shellfish, creating a win-win solution for food production and environmental management.

However, efficient aquaculture in ISW requires chemical fertiliser application to address ionic imbalances compared to the marine or brackish water of similar salinity. While ISW salinity typically ranges from 10 to 25 parts per thousand (ppt), these waters are generally deficient in potassium, regardless of their aquifer source (Zaffar et al., 2021). This deficiency occurs because potassium becomes trapped in clay sediments, making it unavailable in the water. Although potassium constitutes only a small proportion of total ions in ISW, it plays an indispensable role in the physiological functioning of aquatic animals (Marshall & Bryson, 1998; Evans, Piermarini, & Choe, 2005).

These ionic imbalances significantly impact aquatic organisms by altering the expression and function of ion transporters, increasing energy demands for osmoregulation, and consequently reducing the growth and survival rates of cultured fish (Marshall & Grosell, 2006). While direct mineral supplementation in water could address these imbalances, such approaches may be economically impractical at a commercial scale. A more viable alternative is the supplementation of deficient minerals through feed formulations, which can effectively support aquaculture production in inland saline environments (Aklakur, 2017).

For successful aquaculture operations in inland saline waters, proper species selection is a prerequisite. Euryhaline species such as shrimp, tilapia, and milkfish have proven beneficial in these environments. Particularly promising is the Genetically Improved Farmed Tilapia (GIFT)—an improved strain of *O. niloticus*—which can be effectively employed in ISW due to its faster growth rate, enhanced disease resistance, and strong market demand (Stickney, 2017).

While global aquaculture research frequently addresses protein and lipid requirements for optimal fish growth, the importance of minerals is often overlooked. ISW aquaculture specifically requires tailored diets with appropriate mineral supplementation, a consideration that is frequently missing even in the basic knowledge base of fish farmers. Previous studies have shown that dietary potassium levels above 0.6% are effective for fish maintained at 10 ppt salinity (Velselvi et al., 2022). Based on these findings, a higher inclusion level (1%) was selected in the present study to ensure adequate supplementation, particularly since the additive is inexpensive and its efficacy is more critical than cost. Therefore, we designed this study to investigate the effects of potassium supplementation in the form of potassium chloride (1% KCl) in the diet of GIFT tilapia reared in ISW of different salinities over 60 days. We aimed to evaluate changes in growth performance, nutrient utilisation efficiency, and antioxidant and metabolic enzyme status in GIFT tilapia fed with potassium-enriched diets.

## Materials and Methods

Approximately 1,200 GIFT fry (0.27 ± 0.03 g) were procured from MM Hatcheries, Chhattisgarh, India, and transported to the indoor wet laboratory of ICAR-Central Institute of Fisheries Education, Rohtak Centre, Haryana, India. The fish were carefully transferred to six circular, disinfected freshwater tanks (each with a 1000 L capacity) and left undisturbed overnight. After a week, the salinity of four tanks was increased at 1 ppt/day by adding 15 ppt water and draining equal amounts of freshwater until it reached 10 ppt (in two tanks) and 15 ppt (in two tanks). After reaching the desired salinity, the fish were acclimated to the respective salinity for another 7 days. Finally, the fish were transferred to respective treatment tanks (300 L capacity). During the entire acclimation period, the fish were fed with a commercial diet (35% protein and 6% lipid, Avanti Feeds, India) twice ad libitum.

Two hundred and seventy GIFT tilapia fingerlings (average body weight 2.73 ± 0.02 g) were randomly distributed to 6 treatments. Three different salinity ISW (Freshwater; FW, 10 ppt; 10, 15 ppt; 15) and two diets (K0; diet without KCl, K1; 1% KCl enriched diet) were employed to constitute the treatment as follows: FW×K0, FW×K1, 10×K0, 10×K1, 15×K0, 15×K1. In total, 18 fibre-reinforced circular plastic tanks (300 L capacity) were stocked with 15 fish per tank. The tanks were continuously aerated and covered. The fish were fed twice, at 3% of their biomass, daily at 9:00 and 17:00 hours. Fortnightly adjustment of the feed ration was done to match the biomass of the respective tanks. Thirty per cent of the water was exchanged twice a week. Bore well water of 15 ppt was pumped from the borewell (Banyani farm, Rohtak), and 10 ppt water was prepared by diluting 15 ppt with fresh water.

Table 1. Composition of experimental diets for GIFT fingerlings reared in inland saline water

| Ingredients | Percentage composition (%) | |
|---|---|---|
| | [1]K0 (0% KCl) | [1]K1 (1% KCl) |
| Fish Meal | 5.00 | 5.00 |
| Soybean meal | 28.50 | 28.50 |
| Groundnut oil cake | 21.50 | 21.50 |
| Mustard oil cake | 11.00 | 11.00 |
| Wheat flour | 9.46 | 8.46 |
| Corn flour | 7.00 | 7.00 |
| De-oiled Rice bran | 10.00 | 10.00 |
| Soybean Oil | 2.20 | 2.20 |
| Fish oil | 2.20 | 2.20 |
| Vitamin mineral mixture[2] | 1.50 | 1.50 |
| Carboxymethyl cellulose | 1.50 | 1.50 |
| Butylated hydroxy toluene | 0.02 | 0.02 |
| Choline chloride | 0.09 | 0.09 |
| Stay C[3] | 0.02 | 0.02 |
| Vitamin E | 0.012 | 0.012 |
| Potassium chloride[4] | 0.00 | 1.00 |
| Total | 100 | 100 |
| Proximate composition (on dry matter basis) | | |
| Dry matter (%) | 93.77 | 93.57 |
| Crude protein (%) | 36.29 | 36.16 |
| Ether extract (%) | 8.18 | 8.07 |
| Crude fibre (%) | 5.73 | 5.61 |
| Total Ash (%) | 8.37 | 8.29 |
| Nitrogen-free extract (%) | 41.43 | 41.87 |
| Digestible energy (Kcal/100 g) | 384.50 | 384.75 |
| P: E (mg CP/kcal DE) | 94.38 | 93.98 |

[1] Diet, K0 (Diet with no KCl), K1 (Diet with 1% KCl), [2]Composition of the Vitamin-mineral mixture (quantity/kg): Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Ascorbic acid, 2500 mg; Vitamin B6, 1000 mg; Vitamin B12, 6 mg; Calcium pantothenate, 2500 mg; Nicotinamide, 10 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 mg; Selenium, 125 mg

[3]Stay C-L ascorbate 2- triphosphate Calcium salt, DSM Firmeniech AG, Switzerland, [4]Potassium Chloride, Qualigens

A practical potassium-enriched diet with 36% CP and 8% lipid was prepared with the ingredients given in Table 1. All the ground ingredients were sieved, weighed and kept in the tray. All the ingredients except the additives and oil were mixed uniformly and then made into a dough with an adequate amount of water. The prepared dough was filled into autoclavable bags and placed under steam in a pressure cooker (120 °C) for about 20 min. The dough was taken out and kept in trays, cooled and again mixed with hands to prevent any clumps. The remaining ingredients, i.e. BHT, choline chloride, vitamin-mineral premix, vitamin E, ascorbic acid and KCl, were added to the cooked dough and given a proper mix with hands. Finally, oil was added to the cooked dough. After giving it a proper mix, the dough was finally pressed through a pelletiser (S.B. Panchal & Co., India) of 1.2 mm diameter. The obtained pellets were then air-dried overnight. On the very next day, the pellets were mechanically dried in the dryer (45 °C) till they reached less than 10% moisture level. After pelletising and drying, the feed was packed, labelled and stored at 4 °C until further use. The proximate composition of the experimental feed was determined using the standard procedure (AOAC International, 1995) and is presented in Table 1. The nitrogen-free extract (NFE) and digestible energy (DE) of the experimental diets were estimated following the method of Halver (1976).

***NFE*** (%) = 100 – [CP (%) + EE (%) + TA (%) CF (%)]

***Digestible Energy*** (kcal/100 g) = (CP (%) × 4+ EE (%) × 9 + NFE (%) × 4) (Halver, 1976)

The protein-to-energy ratio was estimated as follows:

$$\mathbf{P:E}\ \left(\mathrm{mg}\,\frac{\mathrm{CP}}{\mathrm{kcal}}\,\mathrm{DE}\right) = \frac{\mathrm{CP\%}\ \mathrm{x}\ 1000}{\mathrm{DE}}$$

The pH (8.09-8.26), temperature (28.2-29.1 °C) and dissolved oxygen (6.18-7.82 ppm) of the water were measured daily just before water exchange (18:00 hrs) with a multiparameter water analyser. Alkalinity, hardness, ammonia-N, nitrate-N, and nitrite-N were monitored twice a week and were in the range of 178-317, 243-310, 0.01-0.02, 0.01-0.04 and 0-0.003 ppm, respectively (American Public Health Association, 2017). Salinity varied according to the treatment (FW: 0-0.45 ppt, ISW: 10, 15: 10-10.25 ppt; 15.21-15.32 ppt). Water quality parameters were maintained throughout the experimental period and were found within the accepted range for tilapia culture (Stickney, 2017).

The average daily gain percentage (ADG) and Feed Conversion Rate (FCR) were calculated using the following formulas:

$$\mathbf{ADG} = \frac{\textit{Final body weight (g)} - \textit{Initial body weight (g)}}{\textit{Number of experimental days}} \times 100$$

$$\mathbf{FCR} = \frac{\textit{Feed consumed (g dry weight)}}{\textit{Weight gain (g)}} \times 100$$

The fish were starved for 1 night before the final sampling. The final wet weight of fish was recorded for every tank. Three fish per tank were taken and anaesthetised (50 μL $L^{-1}$ clove oil). The fish were dissected, and the liver (~0.5 g) and gill tissue (~0.2 g) were collected, weighed, and washed with normal saline. Liver tissue homogenate (5%) was prepared for enzyme analysis in sucrose solution (0.25 M, pH 7.4). After centrifuging it at 5000 rpm for 10 minutes at 4 °C, the obtained supernatant was collected and stored at -20 °C for further use. For branchial NKA measurement, the gill tissue was homogenised at 7000 rpm in 0.8 mL of SEI buffer using a hand homogeniser (Benchmark Scientific, D1000 Hand-held homogeniser). The homogenate was centrifuged for 5 minutes at 5,000 rpm at 4 °C. The supernatant was discarded, and the obtained pellet was resuspended in 0.5 mL SEID buffer (SEI with 0.1 g/L sodium deoxycholate) and then rehomogenised for another 30 s. Finally, the homogenate was centrifuged at 5000 rpm for 1 min at 4 °C, and the supernatant was collected and refrigerated at -20 °C. Bovine serum albumin (BSA) standard was used for tissue protein estimation (Bradford, 1976), and the estimated protein content was equalised in all samples with the homogenising buffer and used for all the enzyme analysis.

Superoxide dismutase (SOD) activity of the liver homogenate was assayed (Misra & Fridovich, 1972). Catalase (CAT) activity of the liver was measured using the method of Aebi (1974).

Hepatic aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were determined by measuring the amount of pyruvate and oxaloacetate produced, respectively and expressed as nanomoles pyruvate or oxaloacetate formed /min/ mg protein at 25 °C (Wootton, 1964).

Lactate dehydrogenase (LDH) enzymatic activity of the liver was estimated with sodium pyruvate as the substrate (Wroblewski & Ladue, 1955). For the estimation of malate dehydrogenase, the protocol given by Ochoa (1955) was used. The enzyme activity was expressed as units/mg protein/ min at 37 °C.

The branchial sodium-potassium ATPase (NKA, E.C. 7.2.2.13) activity was assessed by the method described by McCormick (1993) and was expressed as micromoles of ADP produced/ mg of protein/ hour at 25 °C.

Each treatment combination in the 2×3 factorial design (salinity × diet) was performed in triplicate tanks, and the tank was considered the experimental unit; values reported in tables represent tank means. The data was analysed using the statistical package SPSS version 22.0. A two-way analysis of variance (ANOVA) was performed to test the main effects of salinity and diet and their interaction (salinity × diet). When a significant interaction was detected, simple effects were examined within each level of the interacting factor, with Duncan's multiple range test (DMRT) to assess the individual effect. Comparisons were done at the 5% probability level ($p < 0.05$).

## Results and Discussion

The present research highlights that dietary potassium supplementation improves the growth of GIFT tilapia in inland saline water. Significant differences ($p < 0.05$) were observed in ADG across treatments (Fig. 1A). ADG of K0 groups decreased significantly with increasing salinity, with the highest value in FW×K0 and the lowest in 15×K0. Similarly, in K1 treatments, ADG decreased with increasing salinity, with the highest value in FW×K1 and the lowest in 15×K1. Between diets at each salinity level, K1 diets consistently showed significantly higher ADG values compared to K0 diets, with improvements becoming more pronounced at higher salinities (19% at FW, 27% at 10 ppt, and 42% at 15 ppt). The superior growth performance in potassium-supplemented treatments demonstrates this electrolyte's critical role in mitigating osmotic challenges, even when provided through the oral route. GIFT tilapia in FW×K1 treatment demonstrated the highest growth performance, suggesting potassium supplementation benefits are maximised in freshwater environments with lower osmoregulatory demands, underlining the indispensable role of potassium in osmoregulation (Griffith, 2017).

FCR values showed significant differences ($p < 0.05$) between treatments (Fig. 1B). Within K0 treatments, FCR increased significantly with increasing salinity, indicating declining feed efficiency. Within K1 treatments, a similar pattern was observed, though the magnitude of change was less pronounced. Between diets at each salinity level, K1 diets consistently showed significantly lower (better) FCR values compared to K0 diets. The improvement in FCR was approximately 15% in freshwater and increased to 23% at 15 ppt, indicating that potassium supplementation's benefits become more valuable as environmental osmotic challenges increase. Siqwepu, Salie, and Goosen (2020) also observed a better FCR and improved growth rate with dietary potassium chloride inclusion at 3.4% in African Catfish, *Clarias gariepinus,* reared in a RAS system. Lower FCR values in K1 treatments indicate that balancing potassium deficiency reduces metabolic costs of ionic regulation, directing more energy toward growth (Partridge & Lymbery, 2008). Potassium maintains intracellular osmotic balance and membrane potential in fish (Evans et al*.,* 2005). In potassium-deficient environments such as ISW, fish expend additional energy to maintain intracellular $K^+$ concentrations against steeper gradients (Partridge & Lymbery, 2008). Here, the significant improvement in ADG and FCR in K1 treatments aligns with the reports of Dersjant, Wu, Verstegen, Schrama, and Verreth (2001), who documented that optimal $K^+$ /$Na^+$ ratios in catfish diets enhance growth by reducing the energetic costs of ion regulation.

In K0 treatments, increasing salinity triggered elevated ROS production, shown by increased antioxidant enzyme activities from FW×K0 to 15×K0. Liver SOD (Fig. 2A) activity showed significant differences between treatments ($p < 0.05$). Within K0 treatments, SOD activity increased with increasing salinity, with the highest activity in 15×K0, representing approximately a 46% increase from FW×K0. Within K1 treatments, SOD activity at 15 ppt was significantly higher ($p < 0.05$) than all other treatments, while FW×K1 treatment showed the lowest enzyme activity. This response of K1 treatments suggests that potassium supplementation enhances cellular antioxidant activity in inland

Table 2. Sodium potassium ATPase (NKA) activity of GIFT tilapia fingerlings reared at different salinities and fed with dietary potassium for a period of 60 days

| Main effects | NKA[3] |
|---|---|
| **Effect of environmental salinity (ppt)[1]** | |
| FW | $2.80^{a} \pm 0.15$ |
| 10 | $8.71^{b} \pm 0.33$ |
| 15 | $11.67^{c} \pm 0.73$ |
| SEM | 0.41 |
| p-value | <0.001 |
| **Effect of dietary potassium[2]** | |
| K0 | $9.78^{b} \pm 1.17$ |
| K1 | $5.67^{a} \pm 0.93$ |
| SEM | 0.34 |
| p-value | <0.001 |
| **Interaction of environmental salinity X dietary potassium** | |
| **Interaction effects** | |
| FW×K0 | $3.48^{a} \pm 0.40$ |
| FW×K1 | $2.12^{a} \pm 0.15$ |
| 10×K0 | $10.33^{c} \pm 0.57$ |
| 10× K1 | $7.09^{b} \pm 0.56$ |
| 15×K0 | $15.53^{d} \pm 0.82$ |
| 15×K1 | $7.80^{b} \pm 0.73$ |
| p-value | *<0.001* |

All values are expressed as mean ± SE (n = 3). Means in the same column with different superscripts are significantly different ($p < 0.05$) among treatments. [1]Environmental salinity (FW: Freshwater; 10: 10 ppt ISW; 15: 15 ppt ISW); [2]Dietary potassium (K0: No potassium chloride, K1: 1% dietary potassium chloride); [3]Sodium Potassium ATPase (NKA) activity expressed as micromoles of ADP produced/ mg of protein/ hour at 25 °C

saline environments. Between diets at each salinity level, K0 treatments showed significantly higher SOD activity than K1 treatments at FW, 10 ppt and 15 ppt. CAT activity also showed significant ($p < 0.05$) differences between treatments (Fig. 2B). Within K0 treatments, CAT activity increased significantly with increasing salinity, with 15×K0 showing the highest activity, approximately 72% higher than FW×K0. Similarly, within K1 treatments, CAT activity increased with salinity, though the increase was more moderate (about 33% from FW×K1 to 15×K1). K1 treatments showed significantly lower CAT activity compared to corresponding K0 treatments at all salinities, with the difference becoming more pronounced at higher salinities (20% at FW, 25% at 10 ppt, and 33% at 15 ppt).

The pronounced SOD and CAT activities in 15×K0 treatment indicate that hydrogen peroxide generated through SOD activity necessitated increased CAT activity for neutralisation, creating a linked antioxidant response consuming cellular resources (Ighodaro & Akinloye, 2019). Elevated SOD activity suggests substantial superoxide production under high salinity, likely from increased mitochondrial electron transport during enhanced osmoregulation. This aligns with studies showing ionic imbalances trigger oxidative stress in fish (Martínez- Alvarez et al., 2002). Inadequate potassium in the body fluids leads to increased ROS generation (Hwang & Lee, 2007). In tilapia, $K^{+}$ plays a fundamental role in osmoregulation and cellular homeostasis beyond countering sodium imbalance. A similar increase in the antioxidant enzyme activity was also observed by Bhatt et al. (2024) in GIFT tilapia reared for 60 days under potassium deficiency in medium saline water.

Liver ALT and AST activity (Fig. 3A and Fig. 3B) showed significant treatment effects ($p < 0.05$). Within K0 treatments, ALT and AST activity increased significantly with increasing salinity, with approximately 85% and 93% increase from freshwater to 15 ppt, respectively. Within K1 treatments, a similar pattern was observed, though with more moderate increases (about 45% for ALT and 50% for AST across the salinity gradient). Between diets at each salinity level, K1 treatments showed significantly lower ALT and AST activity compared to corresponding K0 treatments, with differences becoming more pronounced at higher salinities.

AST and ALT activities increased in K0 treatments at higher salinities. In potassium-deficient environments, impaired cellular energy production increases reliance on protein as an energy source

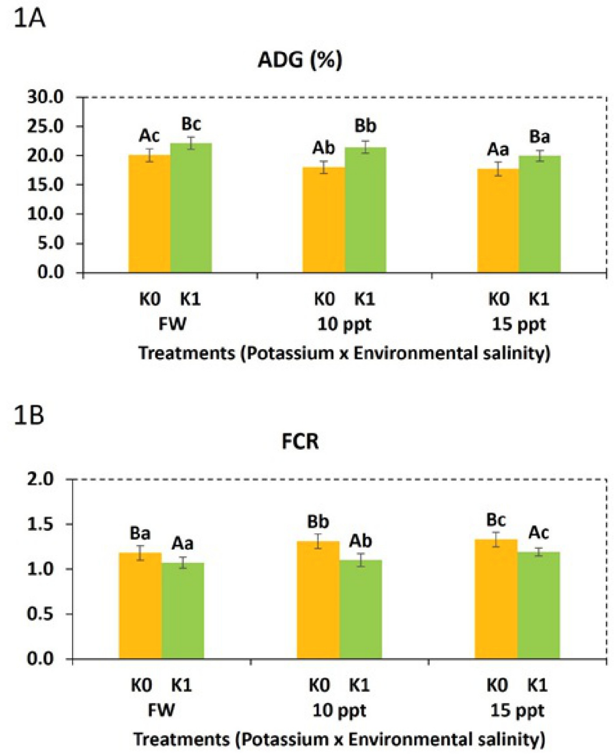


Fig. 1. Effect of environmental salinity (FW, 10 ppt, 15 ppt) and dietary potassium supplementation (K0: No KCl, K1: 1% KCl) on (A) average daily gain (ADG%) and (B) feed conversion ratio (FCR) in GIFT tilapia fingerlings. Data are presented as mean ± SE (n = 3). Different lowercase superscripts (a, b, c) above individual bars within a group indicate significant differences in environmental salinity ($p < 0.05$), while uppercase superscripts (A, B) denote significant differences due to dietary potassium supplementation within the same group ($p < 0.05$). A significant interaction between dietary potassium supplementation and environmental salinity was observed for both ADG% and FCR ($p < 0.05$).

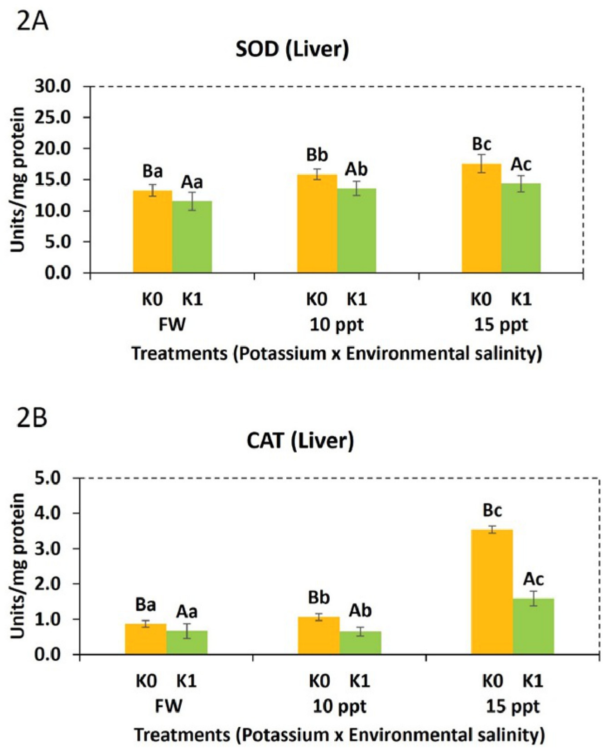


Fig. 2. Effect of environmental salinity (FW, 10 ppt, 15 ppt) and dietary potassium supplementation (K0: No KCl, K1: 1% KCl) on antioxidant enzyme activities in GIFT tilapia fingerlings: (A) Superoxide dismutase (SOD) and (B) Catalase (CAT). Values are expressed as mean ± SE (n = 3). Different lowercase superscripts (a, b, c) above individual bars within a group indicate significant differences in environmental salinity ($p < 0.05$), while uppercase superscripts (A, B) denote significant differences due to dietary potassium supplementation within the same group ($p < 0.05$). A significant interaction between dietary potassium supplementation and environmental salinity was observed for both SOD and CAT activities ($p < 0.05$).

*SOD, superoxide dismutase, 1U=50% inhibition of epinephrine auto-oxidation/mg protein/min

*CAT, catalase, 1U=nanomoles $H_2O_2$ decomposed/min/mg protein

(Hegazi, Attia, & Hegazi, 2014). With increasing osmotic stress, protein catabolism for energy and osmoregulatory purposes occurred, as transaminases facilitated the transfer of amino groups from amino acids to keto acids, thereby feeding into gluconeogenic and energy-producing pathways (Hou, Hu, Li, He, & Wu, 2020). This process generated aspartate and alanine as substrates for further metabolic processes (Holeček, 2023). Dramatically elevated AST and ALT activities in 10×K0 and 15×K0 treatments indicate substantial protein turnover. Dawood, Gewaily, and Sewilam (2023) showed elevated transaminase activities in tilapia under osmotic stress, indicating increased amino acid catabolism to meet energy requirements. Potassium-supplemented treatments lowered ALT and AST activities irrespective of salinity. Magnoni et al. (2019) reported similar protective effects of electrolyte balance on protein metabolism during osmotic adaptation, noting preserved protein synthesis as a key growth determinant under suboptimal conditions.

LDH activity differed significantly among treatments ($p < 0.05$). Within K0 treatments, LDH activity increased progressively with increasing salinity, reaching the highest in 15×K0, approximately 85% higher than in FW×K0 (Fig. 3C). Similarly, in K1

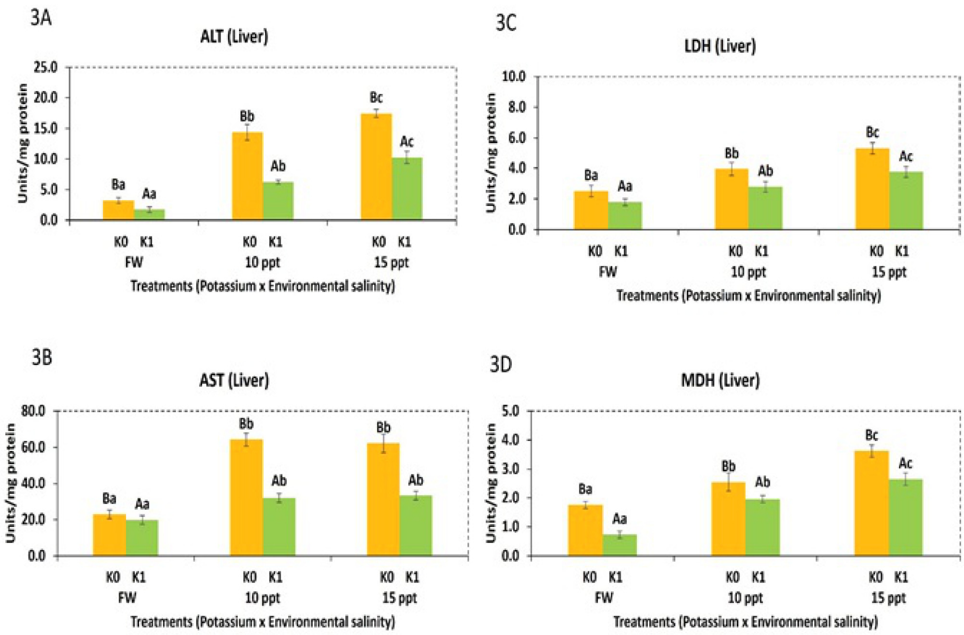


Fig. 3A. Effect of environmental salinity (FW, 10 ppt, 15 ppt) and dietary potassium supplementation (K0: No KCl, K1: 1% KCl) on hepatic and muscle enzyme activities in GIFT tilapia fingerlings: (A) Alanine transaminase (ALT), (B) Aspartate transaminase (AST), (C) Lactate dehydrogenase (LDH), and (D) Malate dehydrogenase (MDH). Values are expressed as mean ± SE (n = 3). Different lowercase superscripts (a, b, c) above individual bars within a group indicate significant differences in environmental salinity ($p < 0.05$), while uppercase superscripts (A, B) denote significant differences due to dietary potassium supplementation within the same group ($p < 0.05$). A significant interaction between dietary potassium supplementation and environmental salinity was observed for all enzymes: ALT, AST, LDH, and MDH ($p < 0.05$).

*ALT, alanine aminotransferase specific activity is expressed as nanomoles of sodium pyruvate released/min/mg protein at 25 °C.

*AST, aspartate aminotransferase specific activity is expressed as nanomoles of oxaloacetate released/min/mg protein at 25 °C.

*LDH, lactate dehydrogenase, activity is expressed as the amount of enzyme that catalyses the oxidation of 1 µmol of NADH /mg protein/min at 37 °C.

*MDH, malate dehydrogenase, activity is expressed as the amount of enzyme that catalyses the oxidation of 1 µmol of NADH /mg protein/min at 37 °C.

treatments, LDH activity increased with salinity, but the increase was less pronounced (about 43% from FW×K1 to 15×K1). At all the salinity levels, K1 diets significantly reduced LDH activity compared to K0 diets, with the protective effect becoming more pronounced as salinity increased (15% at FW, 24% at 10 ppt, and 36% at 15 ppt).

Elevated LDH activity in high-salinity K0 treatments suggests a shift toward anaerobic glycolysis, likely from mitochondrial dysfunction caused by oxidative damage. Tseng et al. (2008) observed LDH elevation in tilapia under osmotic stress, noting these alterations reflect compensatory mechanisms when aerobic metabolism is compromised. MDH activity (Fig. 3D) also exhibited significant differences across treatments ($p < 0.05$). In K0 treatments, MDH activity showed a clear salinity-dependent increase from FW×K0 to 15×K0, representing approximately a 103% increase. In K1 treatments, although MDH activity also increased with salinity, the magnitude of the increase was moderated to about 47% from FW×K1 to 15×K1. The protective effect of KCl

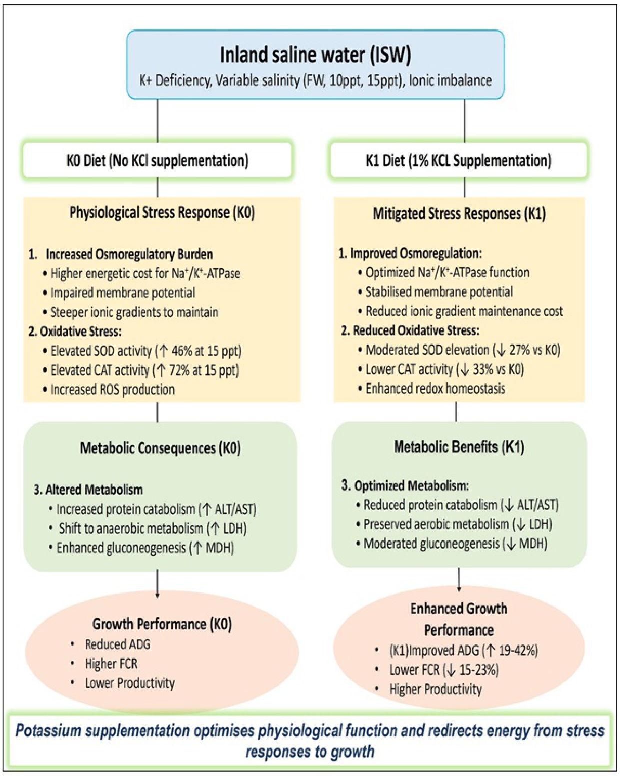


Fig. 4. Integrated physiological response due to dietary KCl supplementation in GIFT tilapia reared in inland saline water of varying salinities (FW, 10, 15 ppt) for a period of 60 days

supplementation was evident across all salinity levels, with K1 diets reducing MDH activity compared to corresponding K0 treatments at all salinities.

Increased MDH activity indicates enhanced gluconeogenic flux to meet elevated energy demands, as MDH provides oxaloacetate for the TCA cycle and serves in the malate-aspartate shuttle (Wolyniak, Frazier, Gemborys, & Loehr, 2024). Li, Li, Xing, Liu, and Li (2024) observed similar MDH activity elevation when exposing tilapia to higher salinities. The moderation of MDH activity by KCl supplementation suggests that potassium supplementation optimises metabolic function, highlighting its role in cellular metabolism in addition to osmoregulation (Hwang & Lee, 2007).

Environmental salinity and dietary potassium significantly affected NKA activity (Table 2) ($p < 0.05$ for both factors), with a significant interaction between these factors ($p < 0.05$). NKA activity increased with environmental salinity (2.80, 8.71, and 11.67 in FW, 10 ppt, and 15 ppt, respectively). Fish fed the K1 diet exhibited lower NKA activity (5.67) compared to those fed the K0 diet (9.78). The interaction analysis revealed that while both dietary treatments showed similar NKA activity in FW conditions, the difference between K0 and K1 treatments became more pronounced at higher salinities, with 15×K0 (15.53 ± 0.82) demonstrating the highest activity among all treatment combinations (Table 2). As a primary intracellular cation, $K^+$ maintains membrane potential, directly influencing NKA activity - the cornerstone of osmotic regulation (Copatti & Baldisserotto, 2021). Under elevated salinity, $K^+$ deficiency forces compensatory mechanisms that increase metabolic expenditure as cells maintain electrochemical gradients despite suboptimal ionic conditions (Marshall, 2003). Bhatt et al. (2024) also observed a similar increase in the NKA activity of the gills of GIFT tilapia reared for 60 days in sulphate-rich LPSW (Low potassium saline water) treatment group compared to the FW treatment, thereby reflecting the enhanced osmotic stress and reduced growth performance in LPSW treatment. Our results clearly revealed that KCl supplementation optimises NKA enzyme function, reducing metabolic and oxidative stress as evident in the K1 diet fed at all salinities. Velselvi et al. (2022) reported similar protective effects of potassium supplementation against oxidative damage in tilapia exposed to potassium-deficient waters. Overall, dietary potassium alters the physio-biochemical adaptations in GIFT tilapia reared in ISW, thereby enhancing growth performance and reducing hepatic and branchial stress responses (Fig. 4).

From an economic perspective, incorporating KCl into feed appear to be a viable mitigation strategy with substantial advantages for commercial tilapia production in inland saline environments. Supplementation of potassium in the water would require continuous application to maintain optimal levels, particularly in large-scale aquaculture systems with high water exchange rates. Our findings indicate that dietary supplementation with 1% KCl effectively delivers potassium directly to fish. Incorporating KCl into commercial feeds requires minimal modification to existing manufacturing processes and can be easily standardised. The cost increase of only 2-3% over the control diet led to superior performance of the cultured fish. Further research exploring optimal potassium supplementation across different life stages and in combination with other osmoregulatory compounds may further refine these strategies for sustainable aquaculture, especially in inland saline waters.

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