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Taurine and/or inorganic potassium as dietary osmolyte counter the stress and enhance the growth of GIFT reared in ion imbalanced low saline water

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ABSTRACT

The effects of dietary osmolytes for alleviating osmotic stress and enhancing growth are not well elucidated in fish reared in inland saline water. The present study evaluated the effects of dietary taurine or potassium (K^+) individually or in combination on growth, ionic homeostasis, and stress response of GIFT tilapia reared in potassium deficient low saline water (PDLSW, 10 ppt salinity) mimicking inland saline water. Isonitrogenous and isoenergetic diets supplemented with five potassium concentrations (0, 0.3, 0.45, 0.6 and 0.75 %), two taurine (T) concentrations (0.5 and 1.0 %) and two combinations of both (K^+ 0.1 % + T 0.5 % and K^+ 0.2 % + T 0.5 %) were fed to GIFT juveniles (4.4 ± 0.02 g body weight) and reared in PDLSW for 45 days. The fish fed on the diet fortifying with K^+ 0.2 % + T 0.5 % showed the highest growth performance among the controls and other treatment groups. Dietary supplementation had no effects on PDLSW induced increase in osmoregulatory endpoints. The optimum dietary potassium requirement of GIFT reared in PDLSW was 0.57 and 0.599 g/100 g diet. Dietary K^+ down-regulated the PDLSW induced expression of NKAa1, AQP1, and ClC2, whereas inhibited taurine-induced up-regulation of AQP1 and CLC2, which is the first report in tilapia. In addition, dietary K^+ and taurine modulated antioxidant and metabolic enzyme activities for easing stress and balancing energy requirements. Thus, blending of potassium (0.2 %) and taurine (0.5 %) in the diet appears best to mitigate stress and enhance GIFT growth reared in inland saline water.

1. Introduction

The fastest growth of the aquaculture sector makes the industry most reliable in tackling the protein requirement of the growing population (FAO, 2020). Reclamation of a vast land of around 380 million ha (Lambers, 2003) through aqua-farming has immense potential for enhancing the expansion of aquaculture activities and production, livelihood generation and minimizing rural migration besides restoration of ecological balance (Allan et al., 2009). However, the potential use of inland saline water (ISW) for aquaculture has a significant limitation due to its sub-optimal ionic composition compared to seawater (Allan et al., 2009; Booth & Fielder, 2016). Despite variation in the composition of ISW across locations, most inland saline groundwater is deficient in potassium (Allan et al., 2009).

Potassium is the most abundant intracellular ion, playing crucial roles in osmotic, ionic, and acid-base regulations in aquatic animals (Booth & Fielder, 2016). Multiple shreds of evidence suggest that K^+ deficient groundwater causes morbidity and mortality of euryhaline/marine species, such as barramundi (*Lates calcarifer*), snapper (*Pagrus auratus*), mulloway (*Argyrosomus japonicas)*. The fish exhibit nearly optimal growth when the K^+ of water remains above 60 % of equivalent salinity seawater concentrations (Booth & Fielder, 2016). K^+ fortification through the addition of muriate of potash (KCl) and K-Mag for maintaining seawater equivalent K^+ in inland saline aquaculture has been accepted globally. It has several demerits such as high cost, unsustainable bioavailability and loss of ions during the harvest of fish, overflow and replenishing water (Allan et al., 2009). Hence, an alternate strategy for rectifying intracellular K^+ through dietary supplementation has gained more attention recently.

Studies on dietary supplementation of K^+ for maintaining ionic homeostasis and typical growth performance in fish are rare and contradictory. Shearer (1988) reported that juvenile Chinook salmon

Abbreviations: NKA, Sodium potassium ATPase; AQP, Aquaporin; CLC2, Chloride channel 2; GIFT, Genetically improved farmed tilapia.

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Table 1
Ionic composition (mg L^{-1}) of water in different treatments.

Experimental Groups[1]	Na^{+} [2]	K^{+} [3]	Ca^{2+} [4]	Mg^{2+} [5]
C	69.00 ± 1.00[a]	4.00 ± 1.00[a]	27.70 ± 1.10[a]	46.60 ± 1.30[a]
C1	3152.50 ± 4.50[c]	15.22 ± 1.07[b]	144.50 ± 4.50[b]	236.00 ± 4.00[b]
T1	3151.00 ± 6 .00[c]	15.65 ± 1.64[b]	144.00 ± 2.00[b]	249.20 ± 0.80[b]
T2	3149.00 ± 5.20[c]	16.00 ± 1.35[b]	147.00 ± 3.00[b]	248.00 ± 1.00[b]
T3	3149.50 ± 4.50[c]	17.80 ± 1.70[b]	146.50 ± 2.50[b]	248.00 ± 0.90[b]
T4	3152.50 ± 4.70[c]	18.80 ± 2.10[b]	147.50 ± 2.60[b]	247.50 ± 0.87[b]
T5	3151.00 ± 4.00[c]	15.65 ± 1.52[b]	144.00 ± 2.00[b]	249.20 ± 0.80[b]
T6	3151.00 ± 6.00[c]	15.65 ± 1.50[b]	144.00 ± 2.00[b]	249.20 ± 0.80[b]
T7	3149.50 ± 5.70[c]	15.50 ± 2.60[b]	146.50 ± 2.50[b]	248.00 ± 1.00[b]
T8	3149.00 ± 5.60[c]	15.60 ± 2.30[b]	145.60 ± 2.40[b]	248.50 ± 1.10[b]
ASW	3126.50 ± 3.50[b]	103.00 ± 2.00[c]	154.00 ± 4.00[c]	415.00 ± 5.00[c]
ISW	3290 ± 4.50[d]	17.20 ± 2.40[b]	144.00 ± 3.20[b]	244.00 ± 1.30[b]
p value	< 0.001	< 0.001	< 0.001	< 0.001

All values are expressed as Mean ± SE (n = 3); Mean values in the same column with different superscripts differ significantly ($p < 0.05$).[1]C, Potassium, K^{+}0 % in diet, freshwater; C1, K^{+} 0 % in diet, PDLSW; T1, K^{+} 0.3 % in diet, PDLSW; T2, K^{+} 0.45 % in diet, PDLSW; T3, K^{+} 0.6 % in diet, PDLSW; T4, K^{+} 0.75 % in diet, PDLSW; T5, taurine, T 0.5 % in diet, PDLSW; T6, T1.0 % in diet, PDLSW; T7, K^{+} 0.1 % + T 0.5 % in diet, PDLSW; T8, K^{+} 0.2 % + T 0.5 % in diet, PDLSW; ASW, Artificial Seawater (Kester et al., 1967); ISW, Inland Saline Water (10 ppt, Haryana).
[2]Na^{+}, sodium ion, is expressed as mg.L^{-1}; [3] K^{+}, potassium ion, is expressed as mg.L^{-1}; [4]Ca^{2+}, calcium ion, is expressed as mg.L^{-1}; [5] Mg^{2+}, magnesium ion, is expressed as mg.L^{-1}

benefitted from dietary supplementation of K^{+} when reared in freshwater, whereas K^{+} in feed does not ameliorate the adverse effects of K^{+} deficient saline groundwater on the survival of juvenile snapper (Booth & Fielder, 2016). However, fortifying water or diet with K^{+} ensures penaeids culture suitable in potassium deficient groundwater (Gong et al., 2004). Therefore, the evidence on dietary supplementation of K^{+} or dietary salts for the well-being of fish is sometimes speculative because studies were conducted in the water bodies not deficient with K^{+} and other ions to meet physiological needs (Sakamoto & Yone, 1978).

Organic osmolytes are small molecules, which do not perturb cell macromolecules, and often they protect macromolecules from perturbations caused by various stressors (Yancey, 2005). Among osmolytes, taurine has received more attention in teleost during acclimation to salinity. Taurine supplementation has been found essential for optimal performance of Nile tilapia (Goncalves et al., 2011). The accumulation of organic osmolytes in tissues and up-regulation of taurine transporter mRNA in response to salinity changes indicate that taurine may have some role in osmoregulatory acclimation in tilapia (*O. mossambicus*) (Takeuchi et al., 2000). Fluctuating salinity induces oxidative impairment by generating reactive oxygen species (ROS), and excessive ROS can lead to oxidative stress and cell malfunction, finally resulting in apoptosis or necrosis (Bal et al., 2021). An increase in SOD and CAT activity is a marker of ROS production overload (Bal et al., 2021). Taurine acts as a potent antioxidant and protects tissues from oxidative injuries (Zeng et al., 2010).

Teleosts can adjust in various salinities by secreting or absorbing ions using specialized "ionocytes" in the gill epithelium. A recent study reveals four types of branchial ionocytes in *O. mossambicus*; two ionocytes are involved in ion uptake; one is responsible for the salt secretion, and the other has an unknown function (Hiroai & McCormick, 2012). Several ion-transport proteins, sodium–potassium ATPase (NKA), Na^{+}/K^{+}/2Cl–cotransporter 1 (NKCC1a), and cystic fibrosis transmembrane conductance regulator (CFTR), apical Na^{+}/H^{+} exchanger 3 (NHE3), and Na^{+}/Cl^{-}cotransporter (NCC) in the branchial ionocytes are responsible for ion absorption or uptake in seawater or freshwater respectively (Hiroai & McCormick, 2012). In addition, a chloride channel family member, Clc-2c, has been demonstrated as a conduit for basolateral Cl-transport by Ncc2 expressing ionocytes contributes to the freshwater adaptability of Mozambique tilapia (Breves et al., 2017). The water channel, aquaporin1 (*AQP1*), regulates cell shrinkage in gills of tilapia exposed to seawater (Lam et al., 2014).

Tilapia, *Oreochromis niloticus,* notably genetically improved farmed tilapia (GIFT), has taken the lead as the principal species for culture in many parts of the world (Kumar & Engle, 2016). Salinity ranges from 10 to 20 ppt is optimal for the growth of Nile tilapia (Romana-Eguia & Eguia, 1999). The introduction of GIFT in inland saline aquaculture has immense potential, provided the cost of production remains within a limit. Nonetheless, studies considering the impact of feeding on hydromineral balance in fish are rare. There is little information available on the effects of PDLSW on the osmoregulatory ability and growth performance of GIFT. Thus, it is essential to gain insight into the scope of fortification dietary potassium (K^{+}) and taurine to ameliorate osmoionic perturbations and growth performance in the GIFT. We examined dietary K^{+} and taurine supplementation's interactive effects on osmoregulatory endpoints, growth, stress responses and transcriptional changes in *NKAa1, CLC2*, and *AQP1* in gills of GIFT reared in PDLSW.

2. Materials and methods

2.1. Experimental animals

The experimental fish, GIFT, was procured from Rajiv Gandhi Centre for Aquaculture (RGCA), Vijayawada, Andhra Pradesh, India and transported to the experimental site ICAR-Central Institute of Fisheries Education, Mumbai, India. After reaching the experimental location, the fish were transferred to circular water tanks containing 300 L freshwater and supplied with constant aeration. The fish were acclimatized for 15 days and were fed ad libitum using a commercial diet (35 % crude protein) twice daily.

2.2. Preparation of potassium deficient low saline water (PDLSW)

The artificial seawater (ASW, 10 ppt) was prepared by adding constitutions of artificial seawater (Kester et al., 1967) into fully aerated bore well water to eliminate the interference of imbalance from other ions. The inland saline water (ISW) composition was collected from the Baniyani unit at CIFE, Rohtak centre, India and was estimated for Na, K, Ca and Mg ions following the methods described earlier (data not shown). The salts, NaCl, KCl, $MgCl_2$, $CaCl_2$, Na_2SO_4, $NaHCO_3$, KBr and boric acid were added to well-aerated bore well water for mimicking the same ionic concentrations of ISW of 10 ppt salinity. The requirement of different salts to prepare ISW was calculated following the equations described earlier (Davis et al., 2005). All the ingredients were thoroughly mixed with constant agitation of water through aeration for 24 h. The prepared water was filtered using a filter bag (Spiryfy, India, mesh size, 5.0 μ) and was stored in 12 fibre reinforced plastic (FRP) tanks (1200 L) under constant aeration for 7 days. The water was disinfected

by applying bleaching powder (Calcium hypochlorite) at a rate of 15 mg L^{-1} and vigorously aerated for at least 48 h before use. The stored water had a salinity of 10 ppt which was constant in all treatments. Two types of water were used in this experiment (i) freshwater (0 ppt) for control (C) and (ii) PDLSW, 10 ppt for C1.

2.3. Preparation of diets

The basal and experimental diets (Table 2) were formulated by substituting an equal amount of cellulose either by KCl (ACS grade, Merck, India) or by taurine (Sigma chemicals, USA) and a combination of both. The seven purified diets were isoenergetic (406.7 kcal DE/100 g), isolipidic (6 %), and isonitrogenous (protein level of 35 %). The sources of different ingredients are mentioned in Table 2. The purified casein and gelatin (4:1) were used as a protein source, whereas dextrin and starch were used as a carbohydrate source. Fish oil and vegetable oil (1:1) served as a lipid source. Other ingredients, cellulose, choline chloride, butylated hydroxyl toluene (BHT), and potassium-free vitamins and minerals, were also used for preparing diets. The KCl as K^+ source and taurine levels in basal and experimental diets were as follows: C (potassium, K^+ 0 % in diet; freshwater), C1 (K^+ 0 % in diet; PDLSW), T1 (K^+ 0.3 % in diet; PDLSW), T2 (K^+ 0.45 % in diet; PDLSW), T3 (K^+ 0.6 % in diet; PDLSW), T4 (K^+ 0.75 % in diet; PDLSW), T5 (taurine, T 0.5 % in diet; PDLSW), T6 (T 1.0 % in diet: PDLSW), T7 (K^+ 0.1 % + T 0.5 % in diet; PDLSW), T8 (K^+ 0.2 % + T 0.5 % in diet; PDLSW).

Ingredients excluding oil and additives were weighed per the formulation and mixed well with water to make dough. Lukewarm water was used to dissolve gelatin to form a semi-liquid viscous substance and then mixed with other ingredients in the dough, which steam-cooked for 30 min in a pressure cooker. After cooking, the dough was smashed to cool rapidly and powdered well. Further, the rest of the ingredients, viz., oil, BHT, choline chloride, vitamin-mineral mixture and Stay C, were mixed uniformly along with it and tried to mix it uniformly. After adding all the ingredients, potassium chloride and taurine were correctly incorporated into the dough. The dough was pressed through a single screw mechanical pelletizer with 1 mm diameter dye (Uniextrude, S.B. Panchal & Company, India). The pelletizer was operated at 67 rpm at room temperature to make pellets, which were air-dried for 24 h and broken into pieces of 4–6 mm and stored in airtight containers at 4 °C until use.

2.4. Analysis of diets and feed intake

The proximate composition of the diets such as dry matter, crude protein, ether extract, crude fibre, nitrogen-free extract and total ash content were analyzed as per the standard method and depicted in Table 2 (AOAC, 1995). Digestible energy (DE) of the diet was calculated according to (Halver, 1976) as per the following formula:

Digestible Energy (kcal/100 g)
= {Crude Protein (%) × 4 Ether Extract (%) × 9 Nitrogen Free Extract (%) × 4}

The protein to energy ratio was calculated by using following formula:

P: E (mg CP/kcal DE) = (CP% × 1000)/DE

Feed intake was estimated by the modified method of Helland et al. (1996) for each treatment. The fish were fed ad libitum, and the uneaten feed was collected after an hour of feeding. The weight of uneaten feed was noted after drying it at 50 °C for 12 h.

Feed intake (g) = feed given (g) - feed collected after one hour (g)

Table 2
Formulation and proximate composition of different experimental diets.

Ingredient composition (%)	Diet (Experimental groups)[1]								
	C/C1	T1	T2	T3	T4	T5	T6	T7	T8
Casein[2]	33.12	33.12	33.12	33.12	33.12	33.12	33.12	33.12	33.12
Gelatin[2]	8.28	8.28	8.28	8.28	8.28	8.28	8.28	8.28	8.28
Dextrin[2]	15.30	15.30	15.30	15.30	15.30	15.30	15.30	15.30	15.30
Starch[2]	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00
Cellulose[2]	4.00	3.43	3.14	2.86	2.58	3.50	3.00	3.31	3.12
Fish oil[3]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Veg oil[4]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vit –min[5]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
CMC[6]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
BHT[7]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Cholin chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
KCl[8]	0	0.57	0.86	1.14	1.43	0	0	0.19	0.38
Taurine[9]	0	0	0	0	0	0.50	1.00	0.50	0.50
Total	100	100	100	100	100.01	100	100	100	100
Proximate composition (on dry matter basis)									
Dry matter (%)	90.99	91	91	91.04	91.05	91.29	91.3	91.07	91.17
Crude Protein (%)	35.07	35.1	35.06	35.03	35.04	35.03	35.04	35.04	35.07
Ether extract (%)	5.95	5.93	5.96	5.97	5.99	5.95	5.94	5.93	5.96
Crude fibre (%)	8.65	8.57	8.49	8.3	8.24	8.28	8.28	8.36	8.27
Nitrogen free extract (%)	47.7	47.3	47.28	47.27	47.21	47.14	47.18	47.2	47.3
Total ash (%)	2.64	3.13	3.17	3.4	3.52	3.6	3.48	3.47	3.44
DE[10] (kcal/100 g)	406.8	406.8	406.8	406.8	406.8	406.8	406.8	406.8	406.8
P:E[11] (mg protein/kcal DE)	86.2	86.2	86.2	86.2	86.2	86.2	86.2	86.2	86.2

[1] C/C1- control diet without taurine/potassium; T1, K^+ 0.3 % in diet, PDLSW; T2, K^+ 0.45 % in diet, PDLSW; T3, K^+ 0.6 % in diet, PDLSW; T4, K^+ 0.75 % in diet, PDLSW; T5, taurine, T, 0.5 % in diet, PDLSW; T6, T1.0 % in diet, PDLSW; T7, K^+ 0.1 % + T 0.5 % in diet, PDLSW; T8, K^+ 0.2 % + T 0.5 % in diet, PDLSW; ASW, Artificial Seawater (Kester et al.,1967); ISW, Inland Saline Water (10 ppt, Haryana).

[2] Purified ingredients procured from Hi Media Ltd., India; [3]Procured from Seacod Oil by Sanofi India Ltd., India; [4]Fortune Refined vegetable oil (Sunflower oil) procured from D Mart, Mumbai, India.

[5] 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; VitaminK, 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; [6]CMC, carboxymethyl cellulose; [7]BHT, butylated hydroxytoluene; [8]KCL, Potassium chloride procured from Merck, India; [9]Taurine procured from Sigma Aldrich, USA; [10]DE,digestible energy; [11]P:E, protein to energy ratio

2.5. Acclimation of fish and experimental procedures

The fish in freshwater were gradually acclimated to 10 ppt PDLSW by adding the aerated PDLSW to raise the salinity to 10 ppt by increasing 1 ppt salinity daily and acclimated for another 15 days. During this period, the fish were fed on the commercial diet containing 35 % protein as earlier. After that, acclimatized GIFT juveniles were randomly distributed into ten groups and were stocked in triplicate tanks. Each tanks of 100 L capacity (L 0.5 × H 0.47 × B 0.44) contained 15 fish (initial weight 4.4 ± 0.02 g) per tank. The fish were fed with the basal (C and C1) and test diets (T1 –T8) daily at approximately 5 % wet body weight twice a day (09.30 to 10.00 h and 16.30 to 17.00 h) and maintained in a photoperiod of 12 h light. The feed allocation was adjusted every week based on the total biomass in each tank. The feed residues were removed within 1.5 h of feeding. The faeces were siphoned out the everyday morning before starting the next day's feeding. The quantity of feed intake by fish was recorded accurately to calculate the dry matter intake (DMI).

2.6. Water quality assessment of rearing tanks

The temperature was checked twice a day using a water thermometer (Merck, Germany). The pH of all the tanks was measured using a pH probe (HI11310, HANNA Instruments, Singapore). Dissolved oxygen, free carbon dioxide, total hardness, calcium ions and magnesium ions concentration was measured per the standard methods (APHA, 2017). The water salinity of all the experimental tanks was checked every day by using a refractometer (Z741839, Merck Instruments, Germany). Ammonia-N and nitrite-N concentrations were measured using an ammonia-nitrite Test Kit (Spectroquant NOVA-MERCK, Germany). The potassium ions concentration was measured using Microprocessor Flame Photometer (RS232, Electronics India, India). The osmolality of the water was measured using a Cryoscopic Osmometer (Osmomat 030, Gonotec GmbH, Germany). The ionic profile of the water was examined while preparing the inland saline water and during the experiment. The average value of each ion in ASW, control and treatment tanks is depicted in Table 1. The physicochemical parameters of water (Table 3) were well within the optimum range suitable for tilapia culture (Singha et al., 2021). There were no significant ($P > 0.05$) differences in the physicochemical parameters of water among the control and treatment tanks. The survival of fish was 100 % in all the groups (Table 5).

Table 3
Physico-chemical parameters of water in control and treatment tanks.

Experimental groups[1]			
Parameters	C (FW)	C1 (PDLSW)	T1-T8 (PDLSW)
Temperature[1]	27.50 ± 1.10	27.50 ± 1.10	27.50 ± 1.10
pH[2]	7.10 ± 0.10	7.10 ± 0.10	7.10 ± 0.10
DO[3]	6.60 ± 0.30	6.60 ± 0.30	6.60 ± 0.30
NH_3-N[4]	0.05 ± 0.02	0.06 ± 0.02	0.06 ± 0.02
NO_2-N[5]	0.002 ± 0.001	0.003 ± 0.001	0.002 ± 0.001
NO_3-N[6]	0.026 ± 0.050	0.032 ± 0.007	0.025 ± 0.033
TA[7]	118.00 ± 12.00	189.00 ± 16.00	189.00 ± 16.00
TH[8]	125.00 ± 8.20	2430.00 ± 35.20	2430.00 ± 35.20

All values are expressed as Mean ± SE (n = 3); Mean values in the same column with different superscripts differ significantly ($p < 0.05$).

[1] C, potassium, K^+0 % in diet, Freshwater; C1, K^+ 0 % in diet, PDLSW; T1-T8, PDLSW with graded levels of potassium chloride and taurine and in combination of both in the diet.

[2] Temperature in °C; [3]DO, dissolved oxygen, is expressed as mg L^{-1}; [4]NH3-N, ammonia nitrogen, is expressed as mg.L^{-1}; [5]NO2-N, nitrite nitrogen, is expressed as mg. L^{-1}; [6]NO3-N, nitrate nitrogen, is expressed as mg.L^{-1}; [7] TA, total alkalinity, is expressed as mg.L^{-1}; [8]TH, total hardness, is expressed as mg.L^{-1}.

2.7. Sampling

Sampling was done after 45 days feeding trial, the number of the experimental animals in each experimental tank was counted for calculating the survival rate. The fish were starved overnight before sampling to measure the body weight for estimating the growth indices. Growth parameters, such as weight gain %, feed conversion ratio and protein efficiency ratio, were calculated as described previously (Singha et al., 2021). Fish were anaesthetized with clove oil (50 μL L^{-1}). Five fish from each replicate were weighed, and the samples of blood, gill, muscle and liver were collected and preserved for further analysis. The gill samples from control and treated fishes have collected aseptically in 1 ml cryo tubes containing RNAlater™ solution (Qiagen, Germany) and were kept at room temperature for 6 h and finally stored at −80 °C until RNA isolation. The number of samples used from each group was mentioned in the figures legends.

2.8. Plasma analysis

Approximately 1.0 – 1.5 ml blood was drawn from the caudal vein using heparinized 1 ml syringes fitted with 27 G needles. Blood samples were collected into heparinized 0.5 ml centrifuge tubes. After a centrifuge at 1000 g for 10 min at 4 °C, the plasma was stored in a refrigerator before analysis. The serum osmolality (mOsm kg^{-1}) was measured with a Cryoscopic Osmometer (Osmomat 030, Gonotec GmbH, Germany). Plasma concentrations of Na^+, K^+ and Cl^- in serum were measured by Eschweiler Combi Blood Electrolyte Analyzer (Diamond Diagnostics, USA).

2.9. Enzyme assays

2.9.1. Tissue sample preparation

The liver and muscle from controls and treatments were homogenized by using a tissue homogenizer (MICRA D-9, ART Prozess and Labortechnik, Germany) in chilled sucrose buffer (0.25 M, pH 7.4) containing protease inhibitor (Halt protease, Thermo Fisher Scientific, USA) to prepare 5 % tissue homogenates. The homogenates were centrifuged (Thermo Fisher Scientific, USA) at 5000 rpm for 10 min at 4 °C, and the supernatant was collected in 2 ml microfuge tubes and stored in −20 °C until used for enzyme assay.

2.9.2. Metabolic and oxidative stress enzymes activity

The lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activities were estimated by the method (Wrobiewski and LaDue, 1955). Oxidative stress enzymes, superoxide dismutase and catalase were assayed for the gill and liver. The SOD activity was assayed according to the method described earlier (Misra & Fridovich, 1972) based on the oxidation of epinephrine-adrenochrome transition by the enzyme. The catalase activity was assayed according to the method (Takahara et al., 1960), where 3 % hydrogen peroxide (H_2O_2) solution was used as substrate.

2.10. Analysis of gene expression

2.10.1. RNA extraction and cDNA synthesis

The total RNA content of gill samples was extracted using TRIzol reagent (Thermo Fisher Scientific, USA) following the manufacturer's protocols. Each sample's RNA purity (260/280) and concentration were determined with a Bioanalyser 2100 (Agilent Technologies, Wilmington, DE, USA). The isolated total RNA was treated with RNase-free DNase I (Invitrogen, USA) before cDNA synthesis. Reverse transcription was performed using the commercial cDNA Synthesis Kit (Thermo Scientific, USA) as per the manufacturer's protocol and kept at −80 °C until used.

Table 4
Primers for expression study of targeted genes in GIFT juveniles reared in potassium deficient low saline water of 10 g/l salinity and fed with graded levels of potassium and taurine and in combination of both 45 days.

Gene	Sequence	Accession number	Contig	Amplicon size (bp)
AQP-1[1]	FP 5′-AGGGTTCAAGTGTGCTCCAC-3′ RP 5′-GGAATAGGTCTGGGCAATGA-3′	EAW56645.1	66,976	129
CLC-2[2]	FP 5′-GTTATTGCTGGGTGGGTTTG-3′ RP 5′-TGATCCTGTTGGGTTCCATT-3′	NP_001164559	30	149
NKA-1[3]	FP 5′-TCCTGGCTGAACAGAACAAA-3′ RP 5′-AACAGATCCACAGCACAGCTC-3′	AAB31193	79,615	104
β-actin[4]	FP 5′-ACCCACACAGTGCCCATC-3′ RP 5′-CAGGTCCAGACGCAGGAT-3′	EU887951		140

[1] AQP-1, aquaporin 1; [2]CLC-2, Chloride channel 2; [3]NKA-1, Na^+,K^+-ATPase 1 α-subunit from the gills of GIFT; [4] β-actin for reference or housekeeping gene. The contigs have been selected from Lam et al., 2014.

Table 5
Growth, nutrient utilization and survival of GIFT juveniles reared in PDLSW of 10 ppt and fed with control and different experimental diets supplemented with graded levels of dietary potassium and taurine individually and incombination for the period of 45 days.

Experiment groups[1]	FW[2](g)	WG[3](%)	FI[4]	FCR[5]	PER[6]	Survival[7] %
C	12.70 ± 0.78^{a}	190.42 ± 19.0^{a}	15.70 ± 1.28^{a}	1.89 ± 0.02^{d}	1.51 ± 0.02^{a}	100
C1	14.56 ± 1.07^{a}	232.46 ± 21.13^{a}	18.56 ± 1.58^{b}	1.83 ± 0.01^{d}	1.56 ± 0.01^{a}	100
T1	18.80 ± 1.33^{b}	329.34 ± 22.20^{b}	15.38 ± 1.40^{a}	1.06 ± 0.07^{ab}	2.69 ± 0.15^{de}	100
T2	21.10 ± 0.80^{bc}	382.25 ± 19.24^{bc}	19.00 ± 0.43^{bc}	1.14 ± 0.02^{b}	2.51 ± 0.04^{d}	100
T3	22.45 ± 0.83^{c}	414.47 ± 18.67^{c}	19.70 ± 0.76^{bc}	1.09 ± 0.01^{a}	2.62 ± 0.03^{de}	100
T4	20.13 ± 0.67^{b}	360.54 ± 20.95^{bc}	17.78 ± 0.57^{b}	1.13 ± 0.02^{b}	2.52 ± 0.03^{d}	100
T5	18.87 ± 1.22^{b}	330.26 ± 18.90^{b}	20.42 ± 1.60^{c}	1.41 ± 0.02^{c}	2.02 ± 0.02^{b}	100
T6	19.00 ± 0.82^{b}	334.19 ± 18.62^{b}	19.14 ± 0.95^{bc}	1.31 ± 0.02^{c}	2.18 ± 0.02^{c}	100
T7	20.00 ± 0.76^{bc}	357.15 ± 18.80^{b}	19.27 ± 0.48^{bc}	1.08 ± 0.02^{a}	2.31 ± 0.03^{e}	100
T8	24.10 ± 0.50^{d}	481.28 ± 13.26^{d}	21.34 ± 0.87^{c}	1.07 ± 0.02^{a}	2.67 ± 0.04^{e}	100
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

All values are expressed as Mean ± SE (n = 3); Mean values in the same column with different superscripts differ significantly ($p < 0.05$).[1]C, potassium (K^+) 0 % in diet, freshwater; C1, K^+ 0 % in diet, PDLSW; T1, K^+ 0.3 % in diet, PDLSW; T2, K^+ 0.45 % in diet, PDLSW; T3, K^+ 0.6 % in diet, PDLSW; T4, K^+ 0.75 % in diet, PDLSW; T5, taurine, (T) 0.5 % in diet, PDLSW; T6, T1 0 % in diet, PDLSW; T7, K^+0.1 %+T 0.5 % in diet, PDLSW; T8, K^+ 0.2 %+T 0.5 % in diet, PDLSW. [2]FW, final weight; [3]WG, weight gain; [4]FI, feed intake;[5]FCR, feed conversion ratio; [6]PER, protein efficiency ratio

2.10.2. Primer designing and quantitative real-time PCR

The qPCR primer sequences for NKAa1, AQP1, CLC2 and β-actin have been previously described (Table 4, Lam et al., 2014). The primers were procured from Eurofins Genomics Pvt. Ltd. (Bangalore, India). Real-time PCR was performed with an Aria Mx Real-Time PCR system (Agilent, USA) using Maxima™ SYBR Green qPCR Master Mix 2x (Thermo Scientific, USA). Each reaction was prepared to10 µl per reaction volumes contains 5 µL Maxima™ SYBR Green qPCR Master Mix (2x), 1 µL forward and reverse qRT-PCR primer (10 picomols), 1 µL template cDNA and 2 µL nuclease-free water. The amplification reaction included an initial denaturation and polymerase activation step at 95 °C for 10 min, followed by forty cycles denaturation for 15 s at 95 °C, annealing, and extension at 59.3 °C for 15 s and 72 °C for 30 s respectively. After amplification, individual melting curves from 59 °C to 95 °C (0.5 °C every five seconds) were generated to confirm a single amplicon and the absence of primer-dimer artefacts. Melting curve analysis was used to verify the specificity of the reaction. The relative expression of NKAa1, AQP1, and CLC2 mRNA was estimated using the 2-ΔΔCT method (Livak & Schmittgen, 2002), where β-actin was used as a reference gene.

2.11. Statistical analysis

Data were expressed as mean ± standard error. All the parameters were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests to determine the significant differences between the means using IBM-SPSS statistics version 20. The polynomial regression method (Zeitoun et al., 1976) was applied to quantify optimum K^+ requirement based on weight gain and gill NKAa1 expression in fish reared in a different environment.

3. Results

3.1. Growth and survival of GIFT in ion imbalanced saline water

The growth performance and survival rate of the GIFT showed significant variations among the controls and treatments (Table 5). The final body weight (FBW) (24.1 ± 0.5 g) and weight gain percentage (WG %, 481.28 ± 13.26 %) were significantly ($p < 0.05$) higher in T8 compared to controls and all other treatments. The lowest final body weight and WG% (12.70 ± 0.78 g and 190.42 ± 19.0 %) were registered in freshwater (C), which was not significantly different from the group reared in PDLSW (C1, Table 5). The FBW and WG % in T3 were higher ($p < 0.05$) than all other treatment groups except T2 and T4. The feed intake (FI) was significantly ($p < 0.05$) higher in the T5 (20.42 ± 1.6) and T8 (21.34 ± 0.87) groups and not different from the FI in T2, T3, T6 and T7. The FI in C and T1 were significantly lower than C1 and T4 (Table 5). The highest food conversion ratio (FCR) was recorded in the C (1.89 ± 0.02) and C1 groups (1.83 ± 0.01), followed by T5 and T6, which were not different ($p > 0.05$). The FCR was lowest in the T3, T7, and T8 groups, and there was no significant difference among them ($p > 0.05$). The protein efficiency ratio (PER) was lowest in C (1.51 ± 0.02) and C1 (1.56 ± 0.01) groups and highest in the T7 and T8 (2.31 ± 0.03 and 2.67 ± 0.04), but not significantly different ($p > 0.05$) than the FCR in T1 and T3. The PER in T4 and T5 were higher ($p > 0.05$) than controls, otherwise lower than ($p < 0.05$) any treatment groups. Broken line regression analysis showed GIFT juveniles' optimum dietary potassium requirement was 0.574 g/100 g (Fig. 2a) and 0.592 g/100 g to weight gain percentage and branchial NKAa1 expression, respectively (Fig. 2b). However, second-order polynomial regression analysis revealed the K^+ requirement as 0.592 g/100 (Fig. 2a) and 0.608 g/100 (Fig. 2b), respectively, with WG % and branchial NKAa1expression.

Table 6
Serum osmolality and ionic concentrations in GIFT fed diets fortified with graded K^+ and taurine and in combination of both for 45 days.

Experimental groups[1]	Osmolality[2]	Na^+ [3]	K^+[4]	Cl^-[5]
C	276.43 ± 1.47^{a}	125.67 ± 1.45^{a}	1.66 ± 0.18^{b}	109.03 ± 0.73^{a}
C1	361.00 ± 2.10^{d}	144.00 ± 2.65 ^{cd}	2.69 ± 0.23^{e}	121.63 ± 0.82^{c}
T1	351.00 ± 2.00^{c}	145.10 ± 2.58^{d}	3.61 ± 0.30^{f}	122.60 ± 0.70^{c}
T2	346.67 ± 1.45^{bc}	147.60 ± 0.95^{d}	3.78 ± 0.15 ^{g}	122.64 ± 0.63^{c}
T3	351.33 ± 2.60^{c}	127.77 ± .13^{ab}	4.16 ± 0.20 ^{h}	120.50 ± 0.53^{c}
T4	349.13 ± 1.70^{bc}	130.17 ± 0.87^{b}	3.92 ± 0.18^{gh}	123.50 ± 1.60^{c}
T5	347.00 ± 0.58^{b}	145.33 ± 1.45^{d}	1.27 ± 0.11^{a}	115.00 ± 1.53^{b}
T6	346.33 ± 1.20^{b}	131.67 ± 2.73^{b}	1.38 ± 0.16^{ab}	115.33 ± 3.18^{b}
T7	344.00 ± 1.82^{b}	139.25 ± 0.85^{c}	1.58 ± 0.16^{b}	112.50 ± 2.10^{ab}
T8	345.00 ± 2.52^{b}	140.67 ± 1.00^{c}	1.95 ± 0.22^{b}	116.67 ± 1.76^{bc}
p value	< 0.001	< 0.001	< 0.001	< 0.001

All values are expressed as Mean ± SE (n = 3). Mean values in the same column with different superscripts differ significantly ($p < 0.05$).
[1] C, Potassium, K^+ 0 % in diet, freshwater; C1, K^+ 0 % in diet, PDLSW; T1, K^+ 0.3 % in diet, PDLSW; T2, K^+ 0.45 % in diet, PDLSW; T3, K^+ 0.6 % in diet, PDLSW; T4, K^+ 0.75 % in diet, PDLSW; T5, taurine, T 0.5 % in diet, PDLSW; T6, T 1.0 % in diet, PDLSW; T7, K^+ 0.1 %+ T 0.5 % in diet, PDLSW; T8, K^+ 0.2 % + T 0.5 % in diet, PDLSW
[2] Osmolality,expressed as mOsmol/kg; [3]Na^+, sodium ion, is expressed as mgL^{-1}; [4]K^+, potassium ion, is expressed as mgL^{-1}; [5]Cl^-, chloride ion, is expressed as mgL^{-1}

3.2. Serum osmolality and ionic composition

Serum osmolality and ionic concentration in GIFT fed different diets varied statistically among the control and treatments (Table 6). Serum osmolality was lowest in the C group, while the highest value (361.00 ± 2.10) was recorded in the C1 group. The serum osmolality was decreased ($p < 0.05$) in T1 to T4 groups compared to C1; the values were further significantly reduced ($p < 0.05$) in T5, which was not substantially different from the osmolality in T6, T7 and T8 groups. The serum Na^+ level was significantly ($p < 0.05$) higher in C1 than the C group, but there was a significant reduction of serum Na^+ in T3, T4, T6, T7 and T8 groups compared to the the C1 group. Serum K^+ levels were also increased significantly ($p < 0.05$) in the C1 group compared to the C group. The serum K^+ level was increased ($p < 0.05$) in T1 through T4 compared to C. The value was lowest in T5 than the values recorded in T6, T7 and T8, similar ($p > 0.05$). The serum Cl^- in C was least than any groups, otherwise the level was not different among C1, T1, T2, T3 and T4. The values were further decreased significantly in T5, which was similar to the values in 76, T7 and T8.

3.3. Na^+/K^+ ATPase

Gill NKAa1 expression was up-regulated significantly ($p < 0.05$) in the C1 group compared to the C group (Fig. 1). There was a gradual reduction ($p < 0.05$) of NKA a1 expression in the T1 through T3, which was lowest and similar to T8. The expression level in T4, T5, T6 and T7 was similar ($p > 0.05$) to T2. The mRNA abundance of NKAa1 subunit expression showed a negative linear correlation with the dietary potassium concentration ($Y = -10.81x + 7.94$). The requirement of dietary potassium was determined by the breaking point of the suitable derivatives of the line of best fit for upper and lower supplementation levels for NKAa1 expression to obtain a value of dietary K^+ between 0.599 and 0.608 g/100 g (Fig. 2 b).

3.4. Aquaporin 1

AQP1 mRNA expression in C was lower ($p < 0.05$) than C1 and was declined sharply ($p < 0.05$) in T1 through T4 (Fig. 1). The AQP1 expression markedly increased in T5 and reached a maximum in T6. The expression was significantly down-regulated in T7 than T6 and reached the lowest level in T8.

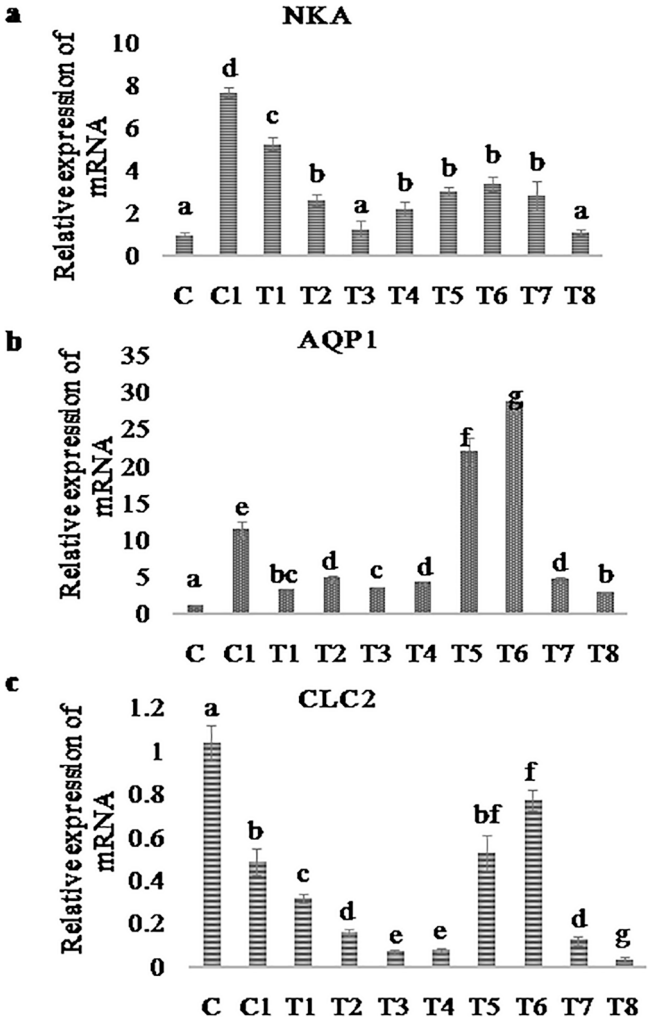

Fig. 1. Changes in branchial *NKAa1, AQP 1* and *CCL-2* mRNA expression in GIFT juveniles reared in FW and PDLSW of 10 ppt and fed with graded levels of dietary $K^+$and taurine for the period of 45 days. *NKAa1*, sodium–potassium ATPAase; *AQP1*, aquaporin 1; *CLC2*, chloride channel 2; FW, freshwater; PDLSW, potassium deficient low saline water. Values in the same mRNA expression with different superscript differ significantly ($p < 0.05$). Data expressed as Mean ± SE (n = 3).

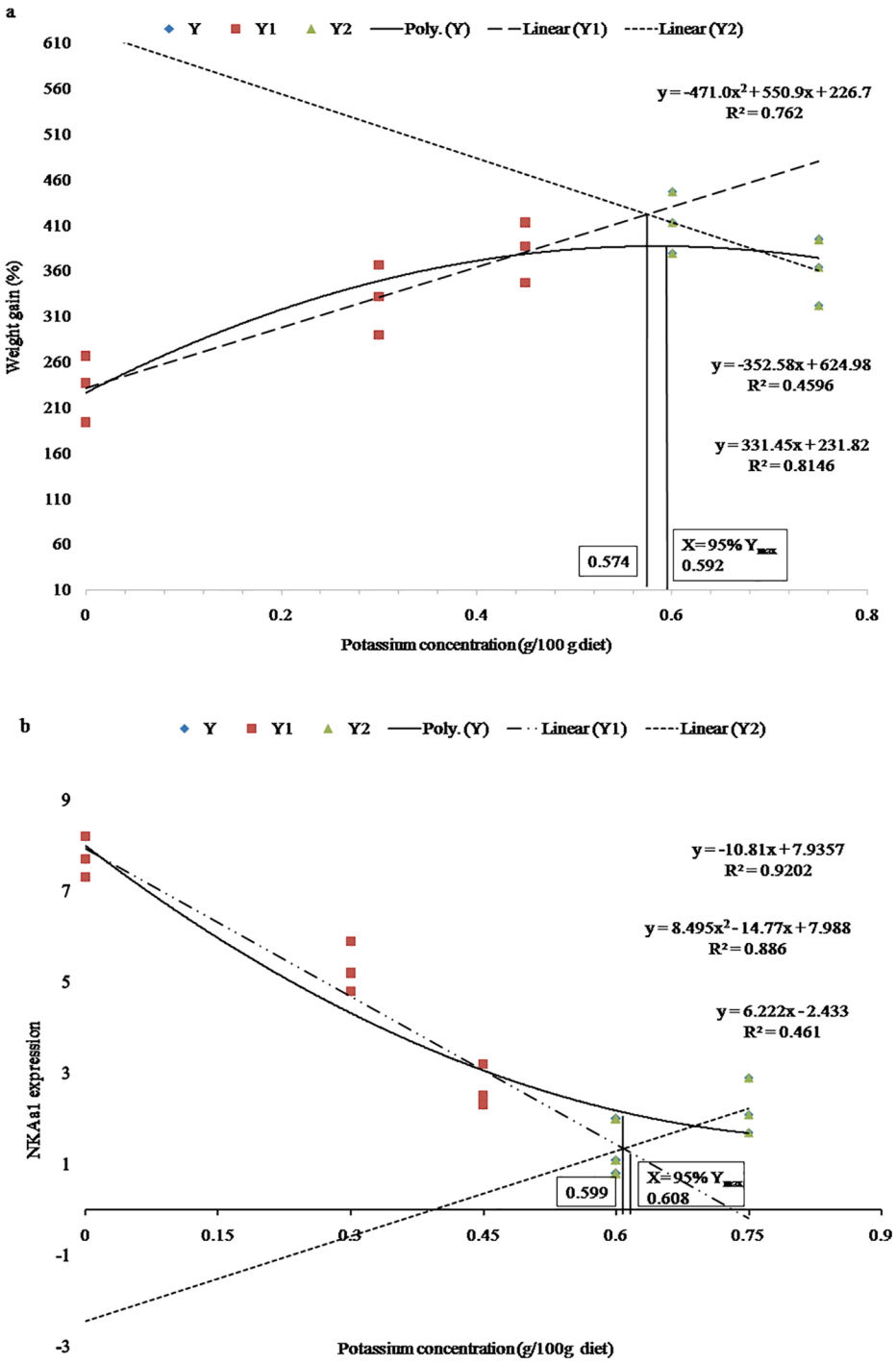

Fig. 2. The broken-line linear (dash line) and second-order polynomial (solid line) regression to the optimize dietary potassium requirement in relation to **a**. weight gain percentage (WG %) and **b.** to branchial *NKAa1* mRNA expression of GIFT juveniles reared in FW and PDLSW of 10 ppt and fed with graded levels of dietary potassium for the period of 45 days.

3.5. Chloride channel protein 2(CLC2)

Branchial ClC2 mRNA expression was highest in C and significantly down-regulated in C1 and further decreased in each treatment group such as T1, T2, T3 and T4. The level of expressions among these groups was quite different. The lowest value was recorded in T3 and T4. The ClC2 mRNA expression was significantly higher ($p < 0.05$) in T5 and T6 compared to other groups except for controls (Fig. 1). The expression was declined sharply in T7 and reached the lowest level in T8.

3.6. Oxidative stress enzymes

3.6.1. Superoxide dismutase (SOD)

The SOD activity in gill and liver varied significantly ($p < 0.05$) among the experimental groups (Fig. 3). The gill's SOD activity was significantly ($p < 0.05$) higher in C1, T1 and T2 groups than the C and other groups. There was no significant difference in SOD ($p > 0.05$) among C1, T1, and T2 groups. However, gill's SOD activity was lowest in the T8 compared to all the groups. In the liver, SOD activity was highest

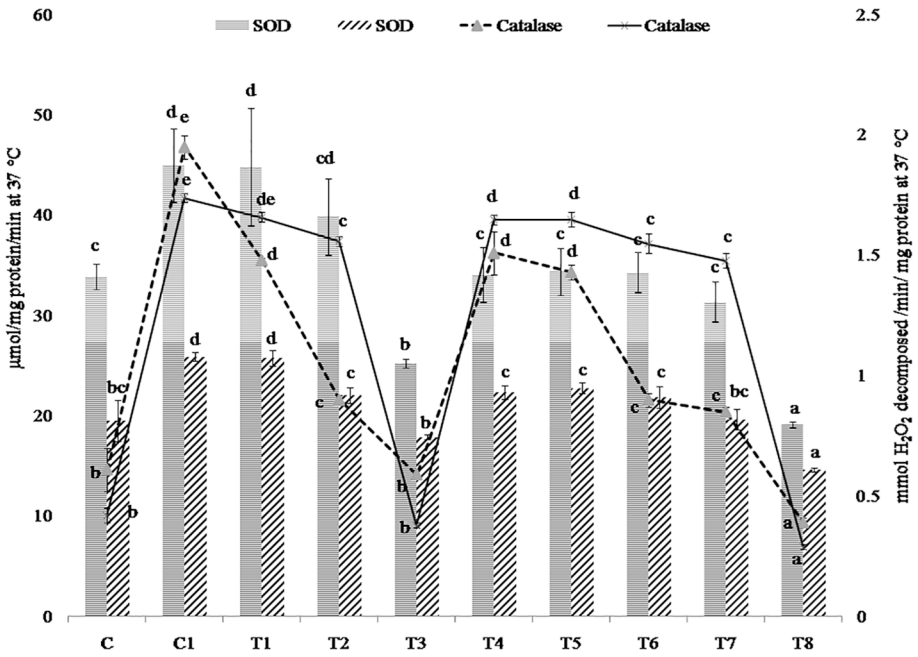

Fig. 3. Super oxide dismutase and catalase activities in liver and muscles in the control and experiment groups of GIFT fed on K^+ and taurine supplemented diets and reared in FW and PDLSW. Values of particular enzyme activities with different superscript differ significantly ($p < 0.05$). Data expressed as Mean ± SE ($n = 3$). SOD activity is expressed as µmol/mg protein/min at 37 °C; Catalase activity expressed as mmol H_2O_2 decomposed /min/ mg protein at 37 °C.

in C1 and T1 groups, and the values decreased significantly ($p < 0.05$) in all the groups with the lowest value in the T8 group. The SOD activity did not vary significantly ($p > 0.05$) among the C, T3 and T7.

3.6.2. Catalase

The catalase activity of gill was significantly higher ($p < 0.05$) in all the groups compared to C except T3. The highest value was recorded in C1, while the lowest was in T8 (Fig. 3). The liver catalase activity showed a similar pattern as exhibited by the gill catalase. It was highest in C1, and T1 groups, which did not vary significantly ($p > 0.05$) and the T8 group had the lowest activity in the liver. The T3 and C values were similar but lower ($p < 0.05$) than other treatments except for the T8 group.

3.7. Assay of metabolic enzymes

3.7.1. Lactate dehydrogenase

LDH activity was significantly ($p < 0.05$) higher in C1 than in all the groups in the liver. The lowest activity was recorded in C, T3 and T8 groups, followed by T2, T4 and T7 groups (Fig. 4). In the muscle, LDH

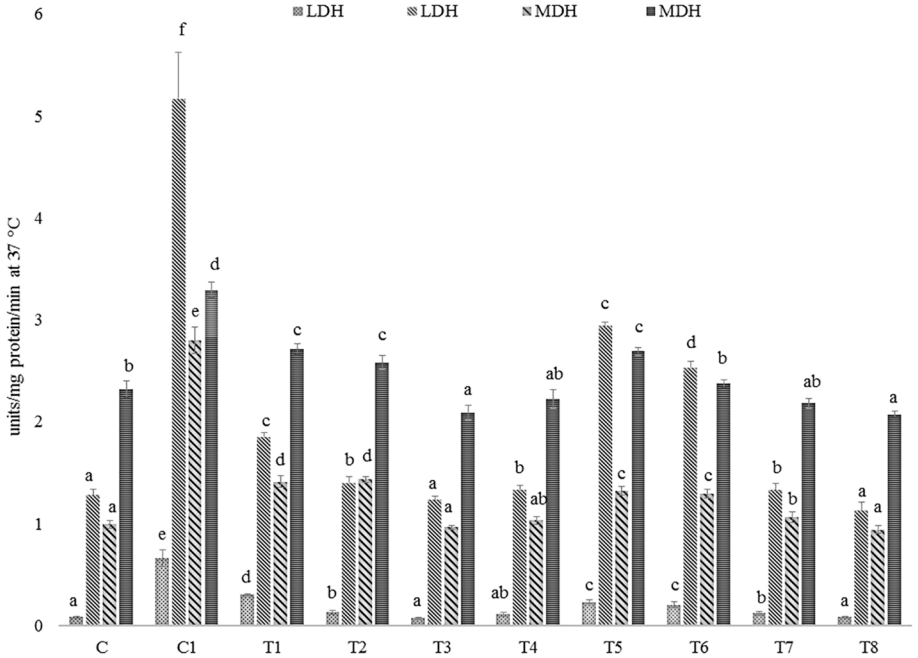

Fig. 4. LDH and MDH activities in the liver and muscle tissues in the control and experimental groups of GIFT fed on K^+ and taurine supplemented diets and reared in FW and PDLSW. Data expressed as Mean ± SE ($n = 3$). Mean values of particular enzyme activity with different superscripts differ significantly ($P < 0.05$). LDH Activities are expressed as: Units/min/mg protein at 37 °C; MDH: specific activity expressed as Units min^{-1} mg $protein^{-1}$ at 37 °C.

activity in C1 was highest, and the lowest was recorded in T3, T4 and T8, which was similar to that of the C group.

3.7.2. Malate dehydrogenase

The MDH activity in the liver was lowest in C, T3 and T8 and highest in C1 compared to all other treatments (Fig. 4). The liver's MDH activity in T1 and T2 was lower ($p < 0.05$) than C1 and the activity decreased further in T5, T6 and T7. The highest muscle MDH activity was found in the C1 group, and the lowest was noted in T3 and T8 groups, which were not significantly ($p > 0.05$) different. The T5 and T6 were different ($p < 0.05$) and higher than T4 and T7.

4. Discussion

The high cost of ameliorating inland saline water (ISA) bodies with potassium salts abates aquaculture potential. Thus, enriching K^+ through aquafeeds has gained much interest as an alternative (Booth & Fielder, 2016; Zaffar et al., 2021). Supplementation of K^+ or chelated K^+ in feeds resulted in a minor improvement in fish and shrimp growth (Saoud et al., 2007). Along with inorganic K^+, the addition of organic osmolytes like taurine, glycine, Myo-inositol, TMAO has also been attempted for combating osmotic stress (Yancey, 2005).

The present study evaluated the effect of K^+ and taurine fortified diets on the survival, growth, osmoregulatory and stress responses of GIFT in PDLSW instead of supplementing potassium in water. The PDLSW mimicked the inland saline water in their Na^+, K^+, Ca^{2+} and Mg^{2+} concentration and was deficient in K^+ compared to the seawater of 10 ppt salinity (Table 1). The survival of GIFT was 100 %, which did not vary with the dietary or environmental modifications employed in the experiment. The growth parameters except FCR were not influenced by PDLSW 10 ppt, although the optimal salinity range of their growth is 10–20 ppt (Romana-Eguia & Eguia, 1999). Probably imbalanced ionic constituents of PDLSW were the main reason for poor growth.

Consequently, dietary inorganic K^+ and taurine exhibited significant improvement in all the growth parameters irrespective of their doses. GIFT fed on 0.6 % potassium supplemented diet showed highest WG%, FI and FCR compared to the groups fed on basal diets or 0.3 % K^+ diets. However, an increase of K^+ over 0.45 % in the diet had no additive effect on WG %, FI and PER except FCR, which was lowest in the diet containing 0.6 % K^+. In comparison, the combination of 0.2 % K^+ and 0.5 % taurine in the diet showed the highest growth performance than the GIFT fed on a 0.6 % K^+ supplemented diet. The results indicate that the fish reared in PDLSW requires dietary K^+ for ameliorating osmoregulatory stress and improving growth better than the GIFT in freshwater (Shearer, 1988; Zhu et al., 2014). The requirement was higher than the optimum (2 %) reported in hybrid tilapia in freshwater (Shiau and Hsieh, 2001). Such difference in the need may be attributed to the species difference or/and difference in ionic constituents of the rearing medium (Shiau and Hsieh, 2001).

Dietary supplementation of essential ions deficient in the culture environment improves the growth and well-being of fish and shellfish (Shearer, 1988). A synergistic effect exists between the ratio of critical ions regulating physiological homeostasis and diets supplementation (Zhu et al., 2004). Dietary supplementation of NaCl effectively improved FCR and weight gain of the euryhaline red drum, *Sciaenops ocellatus* reared in fresh or brackish water, but not in seawater (Gong et al., 2004). In contrast, the addition of salt in the feed inhibited growth and increased FCR in Cobia reared in low-saline brackish water (Arockiaraj & Appelbaum, 2010). Tilapia have K^+ requirement through diet when there is a deficiency in K^+ in the rearing water; otherwise, they absorb K^+ directly from water as the diet lacks optimum K^+ (Shiau & Hsieh, 2001). The tilapia hybrid fed on 0.2 % dietary K^+ reached maximum WG %, and the body content of K^+ increased with the increase in K^+ in diet (Shiau & Hsieh, 2001).

Taurine is an essential nutrient for fish, and its deficiency causes retardation of growth and feed efficiency (Salze et al., 2012). Some freshwater fish do not require exogenous taurine supplementation as they can synthesize taurine (Espe et al., 2012). In contrast, some freshwater fish require taurine supplementation in the diet for their optimum performance of growth and welfare. Nile tilapia is one of them (Goncalves et al., 2011), requiring about 9.7 g/kg dietary taurine for optimum performance at the larval stage (Al-Feky et al., 2015). In the present study, the addition of taurine in diet exhibited the best growth performance of GIFT in PDLSW, suggesting the combination of taurine with K^+ had additive effects on growth. Al-Feky et al. (2015) reported taurine as an essential amino acid in Nile tilapia (*Oreochromis niloticus*). Thus, its addition might have improved growth in GIFT, which is an improved strain of Nile tilapia.

Hypersaline conditions cause cell shrinkage in FW fish unless osmolytes are accumulated cell milieu. As taurine is an organic osmolyte, its concentration would be higher in the intracellular milieu and may prevent cell volume change. In addition, taurine also modulates the level of other osmolytes, such as Na^+, which carries charges and regulates sodium–potassium pumps for maintaining ionic homeostasis (Schaffer et al., 2002). Also, the role of taurine as an antioxidant and metabolic modifier is well known elsewhere (Cheng et al., 2018; Yancey, 2005). Thus, taurine might act as an intracellular organic osmolyte and antioxidant to curb the salinity stress at 10 ppt, rather than rectifying the ionic imbalance, leading to higher growth performance.

Teleosts maintain the plasma osmolality relatively constant at approximately 300 mOsmol kg^{-1}, independent of their environmental salinity. However, in the acclimation process to a hyperosmotic environment, fish undergo two phases: first, there is a rapid rise in gill-ion fluxes followed by an increase in serum electrolytes and osmolality, followed by a regulatory period, and finally, restore serum ion homeostasis. GIFT reared in PDLSW showed higher osmolality than the FW group. Generally, the baseline plasma osmolality of saltwater-acclimated fish (~350 mOsm kg^{-1}) is slightly higher than that of freshwater-acclimated fish (~310 mOsm kg^{-1}) (Al-Feky et al., 2015). On supplementation of feed with either K^+ or taurine reduced the serum osmolality level but did not relapse to the similar level of the GIFT reared in freshwater. The exogenous supply of K^+ could activate the sodium–potassium pump to regulate the serum ion concentrations, including Na^+, K^+, and Cl^- concentrations, to yield an overall effect on osmolality. The influence of dietary potassium on osmolality is rare in teleosts. However, its impact on osmoregulation has been demonstrated in shrimps (Zaffar et al., 2021). In the present study, dietary taurine was efficient for improving the osmotic status of GIFT, suggesting its role as an organic osmolyte. Taurine efflux under hypotonic exposure of flounder erythrocytes and goldfish renal tissue (Fugelli et al., 1995) supports the osmotic role of taurine.

Conversely, hyperosmotic plasma increases taurine transporter mRNA in numerous tilapia tissues (Takeuchi et al., 2000). However, the actual tissue content of organic osmolytes and the possibility of other types of such solutes have received little attention in teleosts. The present study also revealed an upsurge of essential serum ions, such as Na^+, K^+, Cl^- in PDLSW, corroborating with the earlier reports in tilapia in the context of higher salinity (Fiess et al., 2007). Also, dietary K^+ and taurine reduced the serum ions in GIFT to the ranges optimum for tilapia (Fiess et al., 2007), indicating that the dietary osmolytes assisted in achieving ionic homeostasis in the fish reared in PDLSW. However, the osmotic and ionic concentrations could not be reduced to the level of freshwater even with dietary osmolytes, as the higher salinity in PDLSW demands more elevated baseline serum osmotic and ionic concentrations.

The transcriptome analysis of various ion transporters and channels entails molecular mechanisms underlying ionic homeostasis. One of the significant ion transporters in this context is Na^+/K^+ATPase (NKA). Moreover, the gill's NKA activity can estimate the potassium requirement (Shiau and Hsieh, 2001). Variation in environmental salinities and ion concentrations affect gill NKA activity in teleosts (Dutta et al., 2019). The present study reveals an increase ($P > 0.05$) in NKAa1 mRNA in the

PDLSW group compared to the FW group, probably owing to the imbalance ratio among Na^+, Cl^- and K^+ in the surrounding medium which ultimately elevated the activity of the NKA pump. The NKA expression matched the NKA pump's demand to cope with the ionic imbalance of PDLSW. Previous studies revealed that exposure of tilapia into 23–25‰ SW results in per-cell increases of Na^+/K^+ATPase (NKA) in gill mitochondria-rich cells (MRCs). Gill NKA protein levels increased in salmonids and most euryhaline fish on exposure to an environment of high salinity (Dutta et al., 2019). The enhanced NKA mRNA levels up-regulate NKA protein abundance to improve its activity, which may link to the increase in MRCs (Hwang et al., 1989).

The optimum K^+ and taurine supplementation minimized the NKA-α1 increase in PDLSW as the dietary inclusion of 0.6 % K^+ alone or 0.2 % K^+ combined with 0.5 % taurine decreased the NKAa1 expression level similar to the FW environment. An earlier study demonstrated that dietary K^+ enhanced the gill NKA activity in tilapia hybrid in freshwater (Shiau & Hsieh, 2001). In contrast, an increase in potassium fortification in the diets resulted in down-regulation of NKAa1 mRNA expression, clearly indicating that dietary potassium is much more efficient in reducing energy expenditure for operating the NKA pump in ion imbalanced environment. The standard diffusion gradient might have been achieved by K^+ supplementation, leading to low demand for energy-driven NKA pumps for potassium transport. Most of the genes related to metabolism and energy increased due to salinity change since energy supply is a prerequisite for iono-osmoregulation in GIFT (Qin et al., 2021). Such curtailment of energy towards the osmoregulatory process may be responsible for enhancing growth and feed conversion ratio. Moreover, taurine inclusion showed much more improvement compared to supplementation of K^+. The NKA expression decreased at a level even less than the freshwater suggests the taurine as an efficient osmolyte in GIFT reared under PDLSW.

Chloride is the most abundant anion and serves many different biological roles. As a counter ion for Na^+ and K^+, chloride (Cl^-) ensures electro neutrality both under steady-state and during transport across cellular membranes. Chloride channels are involved in cell volume regulation essential for maintaining osmotic and ionic homeostasis (Devuyst & Guggino, 2002). Hirose et al. (2003) documented that the CLC2 acts as the chloride channel in the gills of FW teleosts and higher expression of the chloride channels mRNA in osmoregulatory organs as an essential to transport Cl^-. The hormonal and osmotic control of branchial CLC-2c contributes to the freshwater adaptability of Mozambique tilapia (Breves et al., 2017). The present study shows that CLC2 was down-regulated in PDLSW, unlike NKAa1 mRNA, and the level of CLC2 expression was high in freshwater, as reported earlier (Tang & Lee, 2011). An increasing level of dietary K^+ decreased CLC2 expression in a dose-dependent manner in PDLSW. The combination of K^+ with taurine brought down the taurine-induced higher expression level of CLC2 at par with the level recorded in artificial seawater of 10 ppt salinity. The study reports for the first time on the role of dietary K^+ in abating taurine-induced CLC2 expression for maintaining ionic homeostasis in GIFT in PDLSW. However, the mechanism leading to this down-regulation warrants further investigation.

Aquaporins (AQPs) represent a new class of proteins extensively distributed on the cell membrane that forms pores and fundamentally acts in different transporting and trafficking processes. AQPs could be engaged in regulating cell volume (Compan et al., 2012). The AQP1 family has been studied in European eel (Martinez et al., 2005), black porgy (An et al., 2008), gilthead seabream, zebrafish (Fabra et al., 2005) and Atlantic salmon (Tipsmark et al., 2002). Recently transcriptomic analysis revealed the involvement of AQP1 in hybrid tilapia in response to osmotic stress (Su et al., 2020). The present study demonstrated that AQP1 transcript amounts were highest in gill under PDLSW acclimation. In contrast, taurine fortified diets further elevated transcript in the gill of GIFT indicates cellular volume correction in branchial cells. The present study showed that the expression profile of gill AQP1 is significantly altered during acclimation in PDLSW, although K^+ fortified diets have little effect on transcript abundance. Supplementation of taurine in diets enhanced osmoregulatory parameters and AQP1 mRNA transcript levels in a dose-dependent manner, requiring further investigation. There is a possibility that AQP1 may only play a partial role in regulating water exchange for controlling cell volume, and the importance of other AQPs need to be fully understood and investigated further.

An antioxidant defence system is present in the animal to neutralize free radicals, including enzymatic and non-enzymatic systems. The present study documented the role of superoxide dismutase (SOD) and catalase (CAT) in GIFT reared in PDLSW. If available antioxidants are insufficient to quench all free radicals, then the chances of DNA damage with concomitant tissue damage become higher. The antioxidant enzymes, SOD and catalase, were enhanced in PDLSW, suggesting oxidative stress due to the ionic imbalance in PDLSW, mimicking inland saline water. The antioxidant capacity of dietary taurine has been reported in *Cyprinus carpio* under salinity stress (Abdel-Tawwab & Monier, 2018). The GIFT reared on a diet fortified with 0.6 % of K^+ or 0.2 % K^+ and 0.5 % taurine showed the lowest activity of SOD and catalase in gills and liver compared to those recorded in other doses of K^+ and/taurine in diets indicating their vital role in alleviating oxidative stress in PDLSW.

LDH and MDH activities increase in the wake of high energy demand under stress. In the present study, LDH activities in liver and muscle were more elevated in PDLSW than in control (FW) and other K^+ fortified PDLSW groups. The increase in LDH activity of the PDLSW group may be due to higher lactate production under stress, which is the preferred substrate for gluconeogenesis in fish (Suarez & Mommsen, 1987). In the present study, higher LDH activities in liver and muscle in the PDLSW group than control (FW) and other groups indicate metabolic stress in fish reared in PDLSW. Dietary 0.6 % K^+ or 0.2 % K^+ and 0.5 % taurine reduced the LDH level in liver and muscle at a minimum level suggests a most relevant requirement of K^+ or/and taurine for improving metabolic depression in GIFT reared in PDLSW.

The synthesis and operation of various ion transporters and enzymes require enormous energy (Tseng & Hwang, 2008). The high activity of MDH ensures producing more energy for maintaining ionic homeostasis in high salinity (Zhang et al., 2017). A significant increase in liver MDH activity in fishes acclimated to PDLSW and fortified graded levels K^+ and taurine in diets reduced it. The MDH activity in the liver and muscle was lowest in a 0.6 % K^+ fortified diet-fed group. The addition of taurine in the diet at 0.5 % aided to keep the MDH activity most down even after reduction in the K^+ fortification at 0.2 % level, suggesting that taurine's inclusion has compensated the requirement of K^+ in ameliorating stress under PDLSW.

5. Conclusion

The fortification of the aquafeed with KCl and taurine was instrumental in improving the osmoregulatory and growth responses of GIFT. In the presence of taurine, there is no need of increasing K^+ above its optimum requirement in the diet. The serum osmoregulatory parameters were in the optimum ranges in the GIFT fed with either 0.6 % K^+ or 0.2 % K^+ combined with 0.5 % taurine, which supports such dietary supplementation in maintaining ionic homeostasis in fish in PDLSW. Higher growth rates of fish reared on potassium supplemented diets suggest that the potassium imbalance in PDLSW might have reduced the growth potential, which eventually compensated by feeding potassium. However, GIFT fed with a diet containing 0.2 % K^+ and 0.5 % taurine exhibited the best growth performance, ionic homeostasis and stress mitigation, which indicates a crucial role of taurine in improving the well-being of fish reared in PDLSW. Thus, the present study suggests that farmers growing GIFT in inland saline water with a low level of ambient K^+ should add both 0.2 % potassium and 0.5 % taurine in the diet to mitigate ionic homeostasis and stress for augmenting fish production. Incorporating 0.6 % inorganic potassium in diets is an efficient strategy to improve osmoregulatory stress and growth responses.

CRediT authorship contribution statement

Rajendran Velselvi: Investigation, Methodology, Formal analysis, Writing – original draft. **Subrata Dasgupta:** Conceptualization, Supervision, Writing – review & editing. **Tincy Varghese:** Methodology, Validation, Writing – original draft. **Narottam Prasad Sahu:** Conceptualization, Writing – review & editing. **Gayatri Tripathi:** Data curation, Validation. **Hougaina Panmei:** Methodology, Formal analysis. **Krishna Pada Singha:** Software, Formal analysis. **Gopal Krishna:** Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Subrata Dasgupta reports equipment, drugs, or supplies and travel were provided by Indian Council of Agricultural Research and World Bank.

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Data availability

Not applicable

Ethics approval/declarations

The study was undertaken with the approval of statutory authorities of the ICAR-Central Institute of Fisheries Education, Mumbai, India (University under Sec. 3 of University Grants Commission Act, and ISO 9001:2008 certified).

Consent for publication

All authors approved the final manuscript for publication.

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