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Potassium amendment of inland saline groundwater: Insights into the physiological responses of silver pompano *Trachinotus blochii* (Lacepede, 1801) at different salinities

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ABSTRACT

A trial was conducted to evaluate the effect of graded levels of potassium (K^+) amended inland saline groundwater (ISW) on serum ionic composition, antioxidant and metabolic enzymes [Na^+/K^+ATPase, Catalase, Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Glutathione S-transferase (GST), Lactate dehydrogenase (LDH), aspartate aminotransferase (AST)] activities of silver pompano, *Trachinotus blochii* (Lacepede, 1801). The ISW was amended with 100% K^+ at 5 ppt (ISW5), 10 ppt (ISW10) and 15 ppt (ISW15) and reconstituted seawater (RSW) with similar salinities (RSW5, RSW10 and RSW15) was employed as a reference medium. Experiment was conducted using completely randomised design. *T. blochii* juveniles (1.5±0.5 g) were reared in 300 l capacity circular FRP tanks for 90 days. Fish were fed with commercial extruded floating feed. At the end of experimental period, serum ionic composition, enzymatic profiles and proximate composition were estimated. Results of the study showed that the serum ionic content (K^+, Na^+, Ca^{2+} and Mg^{2+}) varied dose-dependently with salinity ($p<0.05$). Na^+/K^+ATPase, antioxidant enzymes (Catalase and SOD) and metabolic enzymes (GPx, GST, LDH and AST) showed higher activities at lower salinity (ISW5 and RSW5) compared to fish reared at 10 ppt and 15 ppt ISW. Similarly, proximate composition exhibited significantly ($p<0.05$) lower protein and lipid content at lower salinity (ISW5 and RSW5) signifying the stress condition at lower salinity. Physiological responses of *T. blochii* in terms of serum ionic composition, enzymatic profile and proximate composition were optimum at 10 and 15 ppt amended ISW, implying that rearing at 10-15 ppt with 100% K^+ amendment of inland saline groundwater is appropriate.

Keywords: Antioxidant activity, Inland saline groundwater, Metabolic enzymes, Potassium amendment, Proximate composition, Salinity, Silver pompano

Introduction

In India, groundwater salinity has become a major issue in dry land and semi-arid zones where the soils are poorly drained. The inland saline groundwater (ISGW) is spread with an estimated area of 2.0 million ha at a salinity of over 2 ppt (CGWB, 2018) which has negative impact on food production by reducing agricultural productivity due to excess soluble or insoluble salts in water (Kumar and Sharma, 2020). Importantly, the application of inland saline aquaculture using ISW is limited due to varied composition of potassium (K^+), which regulates the osmoregulation in fish through Na^+/K^+ATPase enzyme activity (Marshall and Grosell, 2006). Several experimental and commercial cultures of brackish and marine water finfish and crustaceans with K^+ amendment have been attempted in India, USA, Australia and China (Mourad *et al*., 2012, Lakra *et al.,* 2014). However, considering the characteristics of the subtropical climate in north-western India, the species identified to investigate the feasibility of fish culture in ISW should be of shorter culture duration (Antony *et al.,* 2021).

The silver Pompano *Trachinotus blochii* (Lacepede, 1801) due to its euryhaline nature has emerged as a prospective species for mariculture with high market demand (Gopakumar *et al.,* 2011, Kalidas *et al.,* 2012). The efficacy of ISW at 5, 10 and 15 ppt on *T. blochii* juveniles found 100% mortality in potassium deficient ISW, whereas growth and survival were substantially improved upon the K^+ amendment of ISW at different salinities (Pathak *et al.,* 2019). Culture of fish in ISW at different salinities have several implications on the ionic regulation, Na^+/K^+ATPase activity, oxidative stress enzymes and proximate composition (Barman *et al.,* 2005, Partridge and Lymbery, 2008, Jahan *et al.,* 2017). Therefore, the feasibility of *T. blochii* culture in ISW would depend on the response of its physiological parameters to different salinities. Therefore, the present trial was carried out to evaluate the physiological responses of *T. blochii* reared in ISW amended with K^+ at 5, 10 and 15 ppt salinity.

Materials and methods

Experimental set up and design

The present study was carried out in the wet laboratory of ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Rohtak Centre, Haryana, India. The juveniles of *T. blochii* were procured from ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Mandapam. Experiment was planned in a completely randomised design under which treatments of K^+ amended inland saline water (ISW) equivalent to similar salinity seawater at 5 ppt (ISW5), 10 ppt (ISW10) and 15 ppt (ISW15) and similar salinity reconstituted seawater (RSW) treatments (RSW5, RSW10 and RSW15) used as reference. The 15 ppt source ISW was diluted to 10 and 5 ppt treatments. The K^+ concentration in ISW5, ISW10 and ISW15 before amendment was approximately 4.5, 8.1 and 11.5 ppm, which was modified to 55.33, 110.43 and 161.83 ppm respectively by supplementation of muriate of potash (KCl) to the level of 100% equivalent to similar salinity seawater. Quantity of K^+ for amendment was calculated by the formula of Davis *et al.* (2004):

Potassium supplementation in raw inland saline water (RSW) = 10.7 × Salinity of RSW - Potassium concentration of RSW.

Reference media for each salinity as control was artificially prepared by using sea salt composition of Instant Ocean® sea salt company, Blacksburg, Virginia, USA.

Experimental culture conditions

T. blochii juveniles (1.5±0.5 g) were acclimatised to desired salinities and randomly distributed to eighteen 300 l fibre-reinforced plastic tanks (3 tanks per treatment and 30 fish per tank). Fish were fed on extruded floating pellets (1.2/1.8 mm) (commercial feed 45% crude protein, 10% lipid and 2.5% fibre) @ 20% of the biomass for the first 15 days and 10% thereafter thrice a day till 90 days. About 50% of water exchange was done once a week and equal volume of treatment media was replenished.

Measurement of physico-chemical properties of water

Regular monitoring of potassium (K^+) concentration in each experiment was done using flame photometry (Esico, India). Handheld refractometer was used to measure salinity on daily basis. Temperature, pH, dissolved oxygen (DO), alkalinity and hardness were monitored daily as per the standard procedures (APHA, 2012). Metabolic wastes such as unionised ammonia (NH_3-N), nitrite and nitrate were estimated using Merck water quality test kits (Spectroquant®) once in 7 days. The ionic composition (Na^+, Ca^{2+} Mg^{2+} and Na^+/K^+ ratio) of inland saline water was found to be different at different salinities. However, potassium amendment at different salinities makes it suitable for rearing the fish (Pathak *et al.,* 2019). Water quality parameters of rearing tanks (Do-6.5-7.5 ppm, Temperature-27-28.5°C, pH-8-8.5, Alkalinity-170-250 ppm, Hardness-1300-3300 ppm, NO_2-N and NH_3-N below 0.002 and 0.02 ppm, respectively) were within the acceptable ranges for the culture of silver pompano (Hamed *et al.,* 2016).

Ionic composition

Serum ionic composition was analysed at monthly intervals (on the 30, 60 and 90 days of culture). Serum samples were collected and pooled from three fish of each replicate. Fish serum was collected by following the method explained by Kamble *et al.* (2018). Serum ionic concentrations (K^+, Na^+, Ca^{2+} and Mg^{2+}) were analysed in inductively coupled plasma atomic emission spectroscopy (ICP-AES, SPECTRO GmbH, Germany).

Tissue collection and homogenate preparation

Enzymatic assays were performed in liver and gill tissues at the end of the trial after anesthetising (clove oil @50 µl l^{-1}) fish randomly taken from each replicate. The gill filaments were frozen with ice-cold Tris buffer (pH 7.4) at -80°C for Na^+/K^+ ATPase analysis. Liver and gill tissues were merged in chilled sucrose (0.25 M) solution and homogenised in a mechanical tissue homogeniser (MICCRA D-9, ART Prozess and Labortechnik). Homogenised tissue samples were centrifuged for 25 min at 4°C (4000 rpm) to collect the supernatant and stored at -20°C for further analysis.

Na^+/K^+ ATPase activity

Na^+/K^+ ATPase trigger the release of free phosphate ion on decomposition of ATP into ADP. Na^+/K^+ ATPase Microplate Assay Kit (MBS8243226, My Bio Source, USA) was used for analysis. Na^+/K^+ ATPase activity was measured on a plate reader at 660 nm and expressed as micromole ADP mg protein^{-1} h^{-1}.

Oxidative stress and metabolic enzyme activity

Superoxide dismutase (SOD) estimation method explained by Misra and Fridovich (1972) based on the oxidation of epinephrine adrenochrome transition by the enzyme was used to check the SOD activity in liver and gill tissues. Absorbance was checked at 480 nm in an UV spectrophotometer (Analytical Technologies Ltd.). Catalase (CAT) assay estimation described by Takahara *et al.* (1960) by using phosphate buffer @50 mM (pH 7.0) was used to analyse the decrease in absorbance at 240 nm at 15 s intervals for 3 min. Glucose, Glutathione S-transferase (GST), Glutathione peroxidase (GPx) and Aspartate aminotransferase (AST) activities were estimated by spectrophotometry using Sigma-Aldrich

Assay kits. The lactate dehydrogenase (LDH) estimation method given by Wroblewski and Ladue (1955) was used to record the absorbance in unit mg protein^{-1} min^{-1} at 340 nm.

Proximate composition

At the end of the experiment, proximate composition analysis of fish muscles was carried out in percentage dry weight basis. Muscle samples were collected randomly from three fish per treatment and stored at -20°C before using it for the analysis. Standard methods by AOAC (2012), were followed for determination of proximate composition. Moisture content was determined by drying the samples in an oven at 102°C. Ash content was estimated using muffle furnace at 550°C for 18 h. Crude protein content was estimated by digestion and distillation method in nitrogen analyser system (Kelplus Classic dx, Pelican). Crude lipid content was estimated using ether extract in the Soxhlet apparatus (Model SD2, 1045, PELICAN).

Statistical analysis

One-way analysis of variance (ANOVA) in SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA) was used to statistically differentiate between the treatment groups and the Duncan's multiple range test was used to compare means at 5% level of significance ($p<0.05$).

Results and discussion

Fish show several physiological changes while acclimatising to varied salinities other than the regular environmental salinity. Therefore, investigation of physiological responses of fish at different salinities of ISW modulates ionic composition, enzymatic profile and proximate composition, which is directly related to growth, may provide the basis for determination of optimum conditions of fish in particular environments to increase production. Thus, in order to ascertain the feasibility of rearing *T. blochii* in ISW, a study was conducted to determine physiological responses. Growth performance of *T. blochii* juveniles in terms of mean final body weight, weight gain, specific growth rate and average daily growth was significantly higher at 10 and 15 ppt ISW compared to 5 ppt (Pathak *et al.,* 2019).

Serum ionic composition in all the treatments did not vary significantly at different time intervals. However, K^+ was substantially higher ($p<0.05$) in ISW10 and ISW15 than ISW5 (Table 1). This rise in serum K^+ concentration might be due to the buffering of plasma potassium near iso-osmotic salinity. Na^+ concentration was also significantly higher ($p<0.05$) in ISW10 and ISW15 than ISW5. Several studies have also reported serum sodium and potassium levels of fish reared in 100% K^+ amended inland saline groundwater of similar salinities. The serum Na^+ content was in the range of 166-172 mmol l^{-1} and the K^+ content was in the range of 5.11-6.71 mmol l^{-1} for juvenile cobia *Rachycentron canadum*, reared at 15 ppt ISW (Antony *et al.,* 2021). However, a similar trend of higher serum Na^+ (176 mmol l^{-1}) and K^+ (6.5 mmol l^{-1}) concentrations was reported in rabbit fish *Siganus rivulatus* (Mourad *et al.,* 2012). Also, similar observations were reported in Barramundi, *Lates calcarifer* and Mulloway, *Argyrosomus japonicus* juveniles reared at different salinities of ISW (Partridge and Lymbery, 2008, 2009).

Table 1. Serum ionic composition of *T. blochii* juveniles at different time intervals

Day of culture (DOC)	ISW 5	ISW 10	ISW 15	RSW 5	RSW 10	RSW 15
Sodium (mmol l^{-1})						
30^{t}	135.33±1.45^{b}	132.58±1.41^{b}	133.37±0.20^{b}	124.00±0.58^{a}	125.82±1.26^{a}	124.91±0.06^{a}
60	133.33±0.88^{b}	134.17±2.3^{b}	132.67±0.67^{b}	123.97±0.55^{a}	123.50±1.04^{a}	121.03±0.48^{a}
90	132.27±0.93^{b}	131.33±1.33^{b}	131.60±0.99^{b}	121.27±0.71^{a}	127.55±0.40a	123.98±1.02^{a}
Potassium (mmol l^{-1})						
30	5.38±0.09^{a}	6.54±0.06^{b}	6.59±1.66^{c}	5.66±0.06^{a}	6.71±0.15^{d}	6.72±0.09^{d}
60	5.33±0.12^{a}	6.52±0.04^{c}	6.45±0.02^{b}	5.67±0.07^{a}	6.79±0.12^{d}	6.85±0.08^{e}
90	5.66±0.59^{a}	6.51±0.02^{c}	6.43±0.03^{b}	5.68±0.14^{a}	6.74±0.06^{d}	6.88±0.07^{e}
Calcium (mmol l^{-1})						
30	249.70±0.85^{d}	281.89±1.45^{e}	359.07±1.37^{f}	201.05±1.35^{a}	225.17±0.87^{b}	237.61±1.2^{c}
60	250.43±0.51^{c}	280.73±1.57^{d}	356.07±1.69^{e}	200.65±0.67^{a}	224.02±1.18^{b}	286.39±1.38^{d}
90	249.94±1.08^{c}	281.05±1.98^{d}	358.24±1.27^{e}	202.07±1.03^{a}	224.04±0.85^{b}	286.28±0.84^{d}
Magnesium (mmol l^{-1})						
30	74.68±0.42^{a}	97.89±1.19^{b}	128.33±0.38^{c}	75.87±0.27^{a}	96.06±2.51^{b}	127.89±0.91^{c}
60	74.72±0.80^{a}	97.29±0.86^{b}	129.06±0.21^{c}	76.18±0.75^{a}	95.21±2.60^{b}	128.61±0.99^{c}
90	74.00±0.84^{a}	97.24±0.70^{b}	128.95±0.41^{c}	76.85±0.15^{a}	95.85±2.00^{b}	127.85±0.60^{c}

Values are expressed as mean±standard error (n=3)

Superscripts (a, b, c, d, e and f) in the columns indicates significant difference ($p<0.05$) between treatments at different time intervals

Serum Ca^{2+} and Mg^{2+} concentration also showed dose-dependent increase with salinities and considerably higher levels ($p<0.05$) were observed in ISW15 followed by ISW10. The present finding corroborated with the previous studies on *Penaeus monodon* in ISW (Antony *et al.,* 2015, Tantulo and Fotedar, 2006, Tantulo and Fotedar, 2007). Ionic regulation has strong relation with iso-osmotic point, wherein fish does not have to spend more energy to maintain homeostasis. Therefore, Pathak *et al.* (2019) suggested that fish regulated osmotic balance at ISW10 and ISW15 as the iso-osmotic point of *T. blochii* was in between 10-15 ppt, which was reconfirmed in the present study with higher serum K^+ concentration at ISW10 and ISW15. Also, previous study reported higher uptake of potassium at near-isosmotic salinities in *Argyrosomus japonicas* reared in saline ground water supplemented with 100% K^+ of similar salinities (Partridge and Lymbery, 2008; 2009). Furthermore, gill Na^+/K^+ATPase levels showed considerably higher ($p<0.05$) activity at ISW5 and ASW5 (Table 2). Similar rise in Na^+/K^+ATPase activity at low salinity (6 ppt) associated with other osmoregulatory parameters shows that gill Na^+/K^+ATPase activity can modify the energy requirement for osmoregulation (Laiz-Carrion *et al.,* 2005).

Superoxide dismutase (SOD), catalase, glucose, GST, GPx, AST and LDH enzymes activities were substantially higher ($p<0.05$) at ISW5 than ISW10 and ISW15 (Table 2). These results corroborated with low salinity rearing conditions for *T. ovatus* and *Sebastes schlegeli* (Wang *et al.,* 2005; Liu *et al.,* 2013). Nevertheless, increased levels of antioxidant enzyme activities in response to low salinity condition might explain the self defence mechanism for maintaining the ionic balance and reduce oxidative stress. Higher activities of AST and LDH could be correlated with the modulation of energy metabolism due to changes in osmotic and ionic regulation of fish reared at low salinity. Similar changes have been reported in *P. vannamei* and *Labeo rohita* during different salinity rearing in ISW (Tseng and Hwang, 2008, Jahan *et al.,* 2017, Murmu *et al.,* 2020).

The moisture content decreased with the increase in salinity from ISW5 to ISW15. Significantly higher ($p<0.05$) moisture content was observed at ISW5 than at ISW10 and ISW15. The results from the present study are in agreement with several others, as this change was also observed in the carcass composition of milkfish *Chanos chanos*, whereby moisture content decreased with an increase in salinity from 0-15 ppt (Barman *et al.,* 2012). Inverse relationship of moisture content with crude lipid content was observed in this experiment at different salinities which can be correlated with several species such as Europian seabass, *Dicentrachus labrax* and salmonids (Dendrinos and Thrope 1985; Shearer, 1994). Fallah *et al.* (2013) while studying the proximate analysis of *Capoeta damascina* revealed that moisture content increased while total lipid content decreased, with increase in salinity, as the fishes were of freshwater orgin and the fish requires lower energy for osmoregulation in freshwater than in brackishwater. Therefore, reduction in lipid content at ISW5 might be due to the fact that *T. blochii* is a marine fish, which requires lower energy for osmoregulation at iso-osmotic salinity *i.e.* at 10 ppt followed by 15 ppt, resulting in more deposition of lipids in fish muscles when reared at intermediate salinities than at 5 ppt. The crude protein and crude lipid content were considerably higher ($p<0.05$) at ISW10 and ISW15 than ISW5 (Table 3), which may be correlated to the utilisation of secondary energy sources such as crude protein and crude lipid content for osmoregulation rather than the growth at lower salinity (ISW 5) (Xu *et al.,* 2010). Additionally, an increase in oxidative stress as exhibited by increased activities of catalase and SOD at ISW5 might have caused lower crude protein and crude lipid in *T. blochii*.

Table 2. Enzymatic profile of *T. blochii* juveniles reared in different treatment media at the end of the trial

Parameters	Treatments					
	ISW 5	ISW 10	ISW 15	RSW 5	RSW 10	RSW 15
Na^+/K^+ATPase (μ mol ADP mg protein^{-1})	10.48±1.08^b	4.68±0.74^a	4.94±1.21^a	13.14±0.55^b	3.95±0.78^a	4.67±0.73^a
CAT (Liver)	20.34±2.41^{ab}	11.60±1.68^a	11.52±2.64^a	32.80±10.28^b	10.99±1.74^a	11.05±3.12^a
CAT (Gills)	2.94±1.32^b	1.25±0.20^a	1.19±0.17^a	2.04±0.34^{ab}	1.88±0.21^a	1.87±0.18^a
SOD (Liver)	18.24±1.26^b	8.14±1.82^a	7.35±1.72^a	13.77±1.54^b	7.82±1.46^a	8.21±1.87^a
SOD (Gills)	12.09±2.12^b	3.91±0.56^a	4.54±1.01^a	14.08±1.12^b	5.65±1.18^a	4.59±0.92^a
GPx (μ mol mg protein^{-1})	9.55±0.43^b	4.07±0.79^a	4.09±1.03^a	8.97±1.71^b	4.44±0.85^a	3.46±0.79^a
GST (μ mol mg protein^{-1})	9.52±0.81^b	5.51±0.74^a	6.28±1.48^a	9.47±0.99^b	6.36±1.00^a	5.35±0.09^a
Glucose (mg dl^{-1})	82.22±3.07^c	40.26±5.36^a	36.06±2.66^a	77.99±3.47^c	37.92±4.06^a	32.77±5.49^a
LDH (μ mol mg protein^{-1})	5.67±0.10^d	2.28±0.49^{ab}	3.56±0.61^{bc}	4.44±0.39^{cd}	2.15±0.13^a	2.08±0.54^a
AST (n mol mg protein^{-1})	27.37±1.12^b	19.92±1.31^a	17.91±1.68^a	24.22±1.38^{a,b}	16.98±2.22^a	15.60±2.30^a

Values are expressed as mean±standard error (n=3)

Superscripts (a, b, c, d) in the columns indicates significant difference ($p<0.05$) between treatments at different time intervals

Table 3. Proximate composition of *T. blochii* juveniles reared in different treatment media at the end of the trial

Treatments	Moisture (%)	Ash (%)	Crude lipid (%)	Crude protein (%)
ISW 5	69.34±0.21[b]	2.12±0.22[a]	4.10±0.59[a]	10.67±0.43[a]
ISW 10	66.55±0.64[a]	3.05±0.13[b]	7.23±0.38[b]	15.23±0.22[b]
ISW 15	66.58±0.38[a]	3.08±0.15[b]	8.20±0.13[c]	14.86±0.11[b]
RSW 5	69.16±0.29[b]	2.04±0.25[a]	5.84±0.56[a]	11.45±0.57[a]
RSW 10	66.69±0.65[a]	3.16±0.17[b]	8.74±0.57[c]	15.41±0.29[b]
RSW 15	66.78±0.19[a]	3.21±0.13[b]	9.55±0.29[c]	15.62±0.25[b]

Values are expressed as mean±standard error (n=3)
Superscripts (a-b) in the columns indicates significant difference ($p<0.05$) between treatments

This can be explained by the fact that ROS production by the cells increases during environmental stress and an uncontrolled rise in ROS damages lipids and proteins and disturbs the homeostasis in fish (Peng *et al.,* 2017). Similarly, *T. blochii* reared in seawater had significantly higher crude protein and lipid content with lower moisture content at intermediate salinities (15-25 ppt) (Hamed *et al.,* 2016). Garg *et al.* (2012) found high protein and fat accumulation in milk fish (*C. chanos*) maintained at 25 ppt, while moisture content remained significantly lower at this salinity than at lower salinities. Overall, this study confirms the suitability of *T. blochii* culture in K^+ amended inland saline water of 10-15 ppt based on the response of physiological attributes. Furthermore it is recommended that K^+ amendment of ISW10 would be more economical in terms of lower potassium amendment requirement than ISW15 without affecting growth (Pathak *et al.,* 2019) and other physiological parameters, and therefore the culture of *T. blochii* could be undertaken in ISW10.

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