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IMPACT OF SALINITY ON PROLACTIN LEVEL IN INDIAN MAJOR CARP *LABEO ROHITA* (HAM.) AS A CLIMATE RESPONSE

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ABSTRACT
Inundation of saline water to inland water bodies of coastal areas during natural calamities is very common. It may result to an increase in salinity of inland waters which can hamper the fresh water farming activities. The present investigation was aimed to study the impact of salinity on prolactin level of Indian major carp. Advanced *Labeo rohita* fingerlings of average body weight ranging from 75g-110g and the average length of 19cm-22cm were exposed to different salinities (0ppt, 5ppt, 7ppt, and 10ppt). Blood sample was collected and analysed for prolactin after 1 hour, 3 hour, 6 hour, 12 hour, 24 hour, 168 hour and 360 hour interval from the exposure of fish to the salinity stress. Overall prolactin level recorded showed a decrease at 3 hours of exposure in comparison to 1 hour exposure and then steadily increased at subsequent sampling durations. The results showed that, the fishes were undergoing a stress condition in increased salinity. The present study concludes that, the salinity intrusion is unsuitable for sustenance and propagation of fresh water aquatic life.

INTRODUCTION

Aquaculture is competition for land and water resources, and is also expressing extreme events like flood, cyclone, surges, salinity intrusion to fresh water fish farms etc. In recent years, climate variability manifested by sea level rise, increased incidence of coastal flood and tropical cyclones, are responsible for salinity mediated water stress of fresh-water fisheries of coastal areas in various parts of the world (Cruz *et al.,* 2007 and Badjeck *et al.,* 2010). One of the biggest problems facing the world today is climate change and global warming. Many scientists believe that production of carbon dioxide and other greenhouse gases is having a heating effect on the atmosphere Kumar *et al.* (2013). According to Eddy, (2006) any stress factor may create an internal physiological imbalance in fish appears through disorder hormones and enzymes functions and changing in some blood picture characteristics, which need systemic physiological response by fish against stressors returning to homeostasis. Prolactin (PRL), also known as luteotropic hormone or luteotropin, is produced by pituitary gland in response to eating, mating, estrogen treatment, ovulation and nursing. It has been generally accepted that Prolactin is the dominant factor in regulating hydro mineral balance in fresh water Utida *et al.*(1972), Hirano (1986), Brown *et al.* (1987), Bern *et al.* (1992), McCormick, (1995) and McCormick (2001). Various studies have established that a major function of Prolactin in fish is the control of osmoregulation and adaptation to hypoosmotic environments Hirano (1986) and Prunet *et al.* (1990). Prolactin is a protein hormone that can cross the blood–brain barrier and act directly in the brain Walsh *et al.* (1990) and Pihoker *et al.* (1993). In fish, PRL controls water and electrolyte balance (especially in Freshwater conditions), growth and development.Consistent with Prolactins role in fresh water osmoregulation, PRL cell activity is higher in fresh water fish than in sea water animals Nagahama *et al.* (1975) and Nishioka *et al.* (1988). The objective of the work is to assess the effect of salinity on stress hormone Prolactin in the most preferred freshwater fish *Labeo rohita* exposed to different concentrations of salinity.

MATERIALS AND METHODS

Experimental setup

The advance fingerlings of *Labeo rohita* were procured from a commercial fish farm from Naihati, North-24-Parganas district of West Bengal, India and transported in oxygenated polythene bag (pH 7.6, alkalinity 110 ppm as $CaCo_3$, hardness 120 ppm as $CaCo_3$) to the laboratory. The average body weights of the fishes ranged from 75g to 110g and the average length ranged from 19cm to 22cm. Before experiment, healthy and active fingerlings (actively swimming) were segregated into 500 liter FRP (fiberglass reinforced plastic) tanks filled with freshwater under constant aeration and acclimatized for two weeks at ambient temperature of 27–29.5ºC. They were feed to satiation with a commercially available feed MFC (Mohan feed and chemicals) ltd. 22% finisher pellet feed (Condition 22% protein and 3% fat) once daily in the afternoon. All the fishes were fasted for 1 day before the beginning of the experiment to allow complete gastrointestinal evacuation. Different salinity concentrations (5ppt, 7ppt, and 10ppt) along with one control (0ppt) were prepared from brine solution (70 ppt) collected from Digha coast, West Bengal, India by the volumetric method. The experiments was set in triplicate in different treatment like

0ppt or control as T_0, 5ppt salinity as T_5, 7ppt salinity as T_7, and 10ppt salinity as T_{10}. Each tank was filled up with 250 liters water of different salinity concentration. Eight numbers of *Labeo rohita* advanced fingerlings were placed in each FRP tank. The experiment was set in three treatment group and one control group with triplicates.

Sampling and analysis

Salinity of each treatment was controlled daily by maintaining the volume of water by adding tap water. Other water qualities were monitored weekly. Temperature, pH, dissolved oxygen and salinity were determined directly by digital water analysis instrument (HANNA, HI 9828, Germany); Alkalinity and hardness were measured titrimetrically as per APHA (2012).

After experimentation, the fish were anaesthetized in 0.1% 2-phenoxyethanol (SRL, Mumbai) and blood was obtained by puncturing the heart using a heparinized syringe. The blood sample was collected at 1hour, 3hour, 6hour, 12hour, 24hour, 168hour, 360hour interval from the exposure of fish to the salinity stress. Plasma serum was separated (4,000 rpm for 4 min) immediately at 4ºC and stored at -20ºC until analysis.

The serum prolactin was determined using the commercially available kit as described here. [Serum Prolactin, with ACCU-binds ELISA micro wells, Prolactin test system product code: 725-300 (the quantitative determination of total Prolactin concentration in blood serum /plasma by a micro plate Enzyme immunoassay) kit].

The data were analyzed by one way analysis of variance (ANOVA) using statistical software Medcalc ® version 12.7.0. (MedCalc Software bvba, Ostend,Belgium). In all cases data were tested normality of variance homogeneity before analysis. All numerical data were represented as the mean ± standard deviation (SD) unless otherwise stated. These ANOVAs were followed by pair-wise multiple comparisons of individual treatments using Fisher (LSD) test. Statistical differences were considered significant at $P \leq 0.05$.

RESULTS

This experiment was designed to compare the extent of responses of PRL from *Labeo rohita* subjected to different salinities. After the onset of salinity stress, PRL release of *Labeo rohita* showed significant variation ($p < 0.05$) in T_0 as control (0ppt) as well as in different treatment groups *viz.*, T_5, T_7 and T_{10} saline gradient (5, 7, 10ppt) within 360 hour of exposure.

In control group the PRL value at first 1 hour was 2.67 ± 0.58ng/ml which is gradually increasing at 3 hour (3.0 ± 1.0ng/ml).Then at 6 hour the value was reached to 3.67 ± 0.58ng/ml and ultimately the PRL level reached its highest peak value at 360 hour (5.67 ± 0.58ng/ml) which was significant at $P < 0.05$.

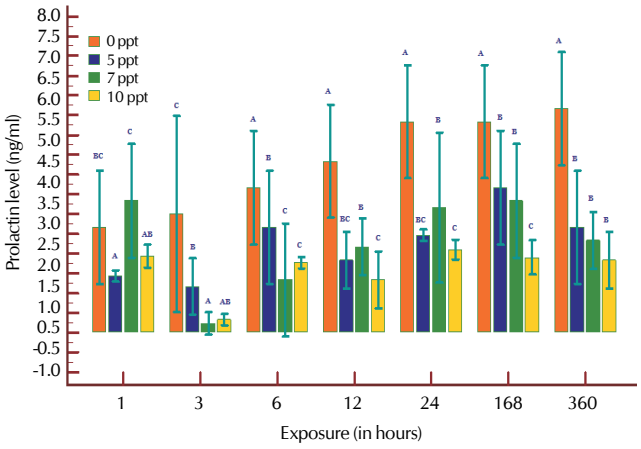

Figure1: Fluctuations of average overall prolactin level of *L. rohita* in different salinity concentrations at different exposure times during the experiment. Different superscripts indicate significant differences (ANOVA; $P < 0.05$).

In 5ppt group the PRL value at first 1 hour was 1.43 ± 0.58ng/ml which was decreasing at 3 hour 1.17 ± 0.29ng/ml. Then at 168 hour the value was reached the highest peak value at 3.67 ± 0.58ng/ml and then it was again decreased at 360 hour 2.67 ± 0.58ng/ml. In T_5, PRL level of *Labeo rohita* was fluctuated significantly ($P < 0.05$) in each sampling periods. In 7ppt group the PRL value at first 1hour was 3.33 ± 0.58ng/ml which was decreasing at 3 hour (0.23 ± 0.12ng/ml).Then at 168 hour the value was increased 3.67 ± 0.58ng/ml. In T_7, PRL level of *Labeo rohita* was fluctuated significantly ($P < 0.05$) in each sampling periods. In 10ppt group the PRL value at first 1hour was 1.93 ± 0.12ng/ml which was decreasing at 3 hour 0.33 ± 0.06ng/ml. Then at 6 hour the value was increased 1.77 ± 0.06ng/ml and then it was again decreased at 12 hour 1.33 ± 0.29ng/ml. But ultimately after 24 hour it was reached its highest peak value 2.1 ± 0.1ng/ml and at 360 hour it was again decreased than 24 hour. PRL level of *Labeo rohita* was fluctuated significantly ($P < 0.05$) in each sampling periods.

In each sampling hour (1, 3, 6, 12 and 24, 168, 360 hour), overall PRL level was significantly varied between all the salinities (T_0, T_5, T_7 and T_{10}) ($p < 0.05$). At 1hour no significant differences of PRL level was observed between T_0-T_{10} and T_5-T_{10}. In 3hour and 6 hour sampling period there was no significant differences between T_7-T_{10}. In 24 hour, there is no

Table 1: Hourly average values of serum Prolactin level (ng/ml) in *Labeo rohita* exposed to different salinity stress during study period

Exposure period (in hour)	0PPT (T_0)	5PPT(T_5)	7PPT (T_7)	10PPT (T_{10})
1H	2.67 ± 0.58^{aBC}	1.43 ± 0.58^{aA}	3.33 ± 0.58^{aC}	1.93 ± 0.12^{aAB}
3H	3.0 ± 1.0^{abC}	1.17 ± 0.29^{aB}	0.23 ± 0.12^{cA}	0.33 ± 0.06^{bAB}
6H	3.67 ± 0.58^{abA}	2.67 ± 0.58^{cB}	1.33 ± 0.58^{bC}	1.77 ± 0.06^{aC}
12H	4.33 ± 0.58^{bcA}	1.83 ± 0.29^{abBC}	2.17 ± 0.29^{abB}	1.33 ± 0.29^{cC}
24H	5.33 ± 0.58^{cC}	2.5 ± 0.06^{bcAB}	3.12 ± 0.76^{aB}	2.1 ± 0.1^{aA}
168H	5.33 ± 0.58^{cA}	3.67 ± 0.58^{dC}	3.33 ± 0.58^{aC}	1.9 ± 0.17^{aB}
360H	5.67 ± 0.58^{cA}	2.67 ± 0.58^{cB}	2.33 ± 0.29^{abB}	1.83 ± 0.29^{aB}

Capital letter (row wise), Small letter (column wise); Mean with different subscription letters are significantly different. ($P < 0.05$); Note: Values are mean ± S.D (3 replications)

Table 2: Fluctuations in water quality parameters (average) during experimental period when fishes were exposed to different salinity treatment

Parameters	T_0	T_5	T_7	T_{10}
DO (mg/l)	5.56 ± 0.30^A	5.69 ± 0.29^B	4.90 ± 0.32^C	4.91 ± 0.13^C
Temperature (°C)	28.14 ± 0.38^A	28.82 ± 0.89^B	28.84 ± 1.00^B	29.18 ± 1.04^C
pH	7.68 ± 0.12^A	8.34 ± 0.33^B	8.49 ± 0.47^C	8.62 ± 0.29^E
Alkalinity (mg/l)	222.29 ± 1.73^A	236.25 ± 4.25^B	168.33 ± 11.49^C	225.33 ± 9.79^E
Hardness (mg/l)	731 ± 10.96^A	1615 ± 37.29^B	2040 ± 165.86^C	2625 ± 83.61^E

significant differences of PRL level were observed between T_5 - T_7 and T_5 -T_{10} ($p > 0.05$) (Fig-1).

Assessment of physico-chemical parameters during experiment when fish exposed to saline water

The fluctuations in the dissolved oxygen in all the treatments were reflected in the (Table: 2). The DO was significantly varied ($p < 0.05$) in all the treatments. The fluctuations in the temperature in all the treatments were reflected in the table. The mean temperature (ºC) was significantly varied in all the treatments ($P < 0.05$) (Table-2). The mean maximum temperature recorded at T_{10} (30.1ºC) and minimum was recorded at T_0 (28.13ºC). The pH was significantly varied ($p < 0.05$) in all the treatments during experimental period. The mean maximum pH was recorded at T_{10} (8.62) and minimum was recorded at T_0 (7.6). The alkalinity was significantly varied ($p < 0.05$) in all the treatments during experimental period. The mean maximum alkalinity was observed (236.25mg/l) at T_5 and minimum was obtained at T_7 168.34mg/l. The hardness showed a gradual increasing trend in all salinity level and was significantly varied ($p < 0.05$) in all the treatments during experimental period. The mean maximum hardness was observed (2625mg/l) at T_{10} and minimum was obtained at T_0 (731mg/l).

DISCUSSION

A few works has been done related to present work and scanty of literature is available. Prolactin appers to act as a 'key hormone' for osmoregulatory capabilities in fresh water fish. Prolactin works as an antagonistic to growth hormone during saline water acclimation Madsen *et al*. (1992) and Bole-Feysot *et al*. (1998). According to Prunet *et al*. (1990) Madsen and Bern (1992) and Bole-Feysot *et al*. (1998) the hormone prolactin has a major involvement in osmoregulation in fish. According to Drago *et al*. (1989) Prolactin is also considered as a stress hormone. The regulation of water electrolyte balance seems to be the most prominent role of Prolactin in fish Ben-Jonatha *et al*. (1996). Number of significant alterations were marked in all the blood parameters like The TEC, Hb%, and Ht (PCV) values of *Channa punctatus* decreased significantly, whereas the TLC of the fish increased significantly during handling and transportation stress Mahananda *et al*. (2013). According to McCormick (2006) Prolactin is an important regulator of multiple biological functions in vertebrates, and has been viewed as an essential to ion uptake as well as reduction in ion and water permeability of osmoregulatory surfaces in fresh water and euryhaline fish. Prolactin has also a well known ion regulatory role in fresh water fishes and has been suggested to play a role in counteracting toxicant induced ionic disturbances. During this study it was seen that when freshwater fishes were transferred from freshwater to saline water, the Prolactin level was decreased. In 5ppt the level of Prolactin found to be 1.43ng/ml compared to the control value (2.67ng/ml) after 1 hour exposure. Similar trend was observed at 3 hour, 6 hour, 12 hour and 24 hour, 168 hour and 360 hour in 5ppt compared to control. Similar declining of Prolactin level was observed in other salinity treatments. Auperin *et al*. (1994) and Yada *et al*. (1994) also reported that when tilapia was transferred from freshwater to saline water there was a reduction of Prolactin level. In our study Prolactin level was decreased within 6 hour after transferred to saline water. Auperin *et al*. (1994) also reported a marked decrease in plasma Prolactin level in less euryhaline species *O.nilonticus*. A significant difference between Prolactin levels between different treatment (0ppt, 5ppt, 7ppt, 10ppt) was observed. Similarly Warming *et al*. (1996) also reported variations in plasma Prolactin levels in response to stressors. Wendlaar-Bonga *et al*. (1983) also told that severe stress may cause atrophy of pituitary Prolactin cells. Thereby inhibiting their biosynthetic activity as observed in trout, (*Savelinus fontalis*) under acute exposure.

According to Nishioka *et al*. (1985) the alteration in Prolactin level appears to be partly due to the direct actions of osmotic pressure. According to them when the osmotic pressure decreases the Prolactin activity also decreases. These may be the reason for declining Prolactin level during this study also. In this study it was seen that during first 1 hour the Prolactin level was increased then it's decreased. Similarly Andre P. Seale *et al*. (2002) also stated that during 7 days experiment the release of PRL_{177} and PRL_{188} was greater in hypo osmotic conditions only during the first 12 hour after which the rate was dropped to the same levels observed in hyper osmotic incubations. Reflecting the high levels of release during the first 12 hour the cumulative response of both Prolactins was greater in hypo osmotic medium than in hyper osmotic medium for the first 24 hour.

During experiment it was observed that on one occasion (7ppt at 1 hour) the Prolactin level increased very much (3.33ng/ml) compared to control (2.67ng/ml). Mancera *et al*. (1993) stated that when the increase in pituitary Prolactin expression is considered together with the reported increased Prolactin cells activity in *Sparus auratus* acclimated to 7ppt-8ppt it seems probable that the circulating Prolactin trend also increased. He also agreed and confirmed due to present result with previous studies that the role of Prolactin as a 'freshwater adaptive hormone' in *S.auratus*.

During one occasion in this study the Prolactin level was greater in 7ppt than 10ppt and control. Similarly Toshirio *et al*. (2004) also observed in his study that Prolactin cell volume to pituitary

volume was not significantly greater in 8ppt but was higher at 4ppt.These result indicate that Prolactin cell needed to be activated vigorously at 4ppt or less than half of its own osmoiarity but not at 8ppt, which is close to the iso-osmoiarity in spotted halibut juvelines. This seems to be associated with difference in low salinity tolerance between the species. Toshirio *et al*. (2004) after their experiment on Japanese flounder also opined that prolactin cells got activated at lower salinity (4ppt) but reduced at higher salinity (Hotta *et al.,* 2001).

According to McCormick (2006) the understanding of hormonal control of salinity acclimation process in fish shall determined how Prolactin and other hormones like Growth hormone, Cortisol, IGF_1 interact among themselves. They emphasized that further research on this needs to be carried out.

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