Effect of Abiotic Salinity Stress on Haemolymph Metabolic Profiles in Cultured Tiger Shrimp *Penaeus monodon*

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Article History

Manuscript No. c92
Received in 30th August, 2012
Received in revised form 18th May, 2013
Accepted in final form 6th June, 2013

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Keywords

Salinity stress, shrimp, free amino acids, urea, ammonia

Abstract

Salinity is one of the most important abiotic stresses in aquaculture. Optimal salinity for growth and production efficiency is often species-specific. The objective of the present study is to find the variations in the metabolic profiles due to salinity stress in cultured tiger shrimp *Penaeus monodon*. Shrimp haemolymph samples were collected from culture ponds with wide range of water salinities (3, 8, 13, 17, 23, 30, 36 and 50 ppt (parts per thousand)). The free amino acid profiles of haemolymph were analysed by using LC 10A Shimadzu HPLC system. The experimental results indicated that glycine, proline, arginine taurine and alanine are the pre-dominant organic osmolytes in haemolymph across all the salinities. The concentration of non essential amino acid, glycine significantly (p<0.05) increased (72.99±2.99 to 162.07±14.31 nmoles ml^{-1}) with increase of salinity from 3 to 30 ppt and its level decreased thereafter. The peak concentration of essential amino acid, arginine (61.79±6.94 nmoles ml^{-1}) level was observed at 23 ppt. Significantly (p<0.05) higher concentration (µ moles ml^{-1}) of haemolymph ammonia (0.18±0.03 at 3 ppt; 0.46±0.07 at 50 ppt) and urea (0.22±0.03 at 3 ppt; 1.59±0.19 at 50 ppt) were observed in higher salinity. In hyper osmotic environments, the shrimp activates the urea cycle to produce a less toxic, osmolyte, urea to maintain high osmolality in the haemolymph and this is reflected in lower free amino acid level at high osmotic stress condition.

1. Introduction

Culture of *Penaeus monodon* is an ideal, because of some advantageous feature such as rapid growth, tolerance of high temperature, wide range of salinity and simple pond construction requirements (Liao et al., 1975). Growth and survival rate of *P. monodon* is influenced by number of ecological factors, among them important one is salinity (Chakraborti et al., 1986). Salinity influences the physiological status of aquatic animals and it is a masking factor that modifies numerous physiological response such as metabolism, growth, life cycle, nutrition and intra-inter specific relationships (Kinne, 1970; Fry, 1971; Venkataramiah et al., 1974). Though *P. monodon* is a euryhaline, it can tolerate wide range of salinity (Solis, 1988; Zhang et al., 1989; Chen, 1990; Pante, 1990), but only in a particular salinity [10-30 ppt (parts per thousand)] the growth is optimum called as optimum salinity or ambient salinity (Chanratchakool et al., 1995; Tsai et al., 2002). The absence of significant difference in growth between salinities of 15 and 30 ppt led to greater emphasis by shrimp farmers on maintaining low salinity levels in *P. monodon* ponds (New and Rabanal, 1985). In the course of farming, farmers have adopted black tiger shrimp to grow successfully in fresh and low saline water also. When ambient salinity has changed, the main challenge for aquatic animal is to regulate their osmotic pressure by a process called osmoregulation (Hochachka and Somero, 2002).

Seasonal variations in salinity were observed between various months in source waters (brackish water canals, estuaries, creeks, agricultural drains, etc) used for shrimp aquaculture. This variation becomes extreme on both sides due to extremely heavy rainfall as a result of cyclonic storms, floods and lack of rains due to severe drought. Besides extreme weather events, anthropogenic interventions like closure of bar mouth resulted in increased salinity of waters even upto 60 ppt. Kandaleru creek in Andhra Pradesh has essentially freshwater during the wet season (November to December), but during the dry

season (May and June) the salinity is around 25 ppt and for the remainder of the year the salinity varies from 10-18 ppt (Joseph et al., 2002, 2003).

A review of previous works on shrimp osmoregulation showed that most studies have focused on inorganic ions in the haemolymph (Castille and Lawrence, 1981; Ferraris et al., 1986). The effects of salinity (15 and 30 ppt) on water and haemolymph osmotic pressure were significant. *P. monodon* is an efficient osmoregulator in this salinity range and has an isosmotic concentration of 23-25 ppt (Allan and Maguire, 1992). Metabolic pools of free amino acids (FAA) are also known to play a major role in osmoregulation in shrimp (Cobb et al., 1975; Dalla Via, 1986). However, these studies examined the changes in FAA levels in tissues but not in haemolymph. Fang et al., (1992) studied the changes in FAA in haemolymph in experimental conditions by acclimatizing shrimp for short period (24 hours) at three salinity levels (15, 30 and 45 ppt). In the present study FAA profiles of haemolymph were analysed from samples collected from culture ponds with wide range salinities (3, 8, 13, 17, 23, 30, 36 and 50 ppt). Haemolymph ammonia and urea were also analysed to know the effect of salinity on nitrogen metabolism. The data generated will be useful for making suitable dietary modifications for amelioration of salinity stress in shrimp.

2. Materials and Methods

2.1 Sample collection

Shrimp samples were collected from culture ponds with a wide range of ambient salinities (3, 8, 13, 17, 23, 30, 36 and 50 ppt) from Andhra Pradesh and Tamilnadu for metabolic profile analysis. Haemolymph samples were collected from the ventral sinus of first abdominal segment of shrimp using a 26-gauge hypodermic needle on a 1 ml syringe.

2.2. Amino acid analysis

The free amino acid pool in the haemolymph was measured using the method outlined by Mente et al. (2002). The pooled haemolymph of 500 µl sample was collected and stored in micro-centrifuge tube containing 1.5 ml absolute ethanol. The homogenate was centrifuged to pellet the precipitated proteins. Triplicate samples of the supernatant were then taken to measure the free pool amino acid composition. A subsample of 100 µl was dried and reconstituted in 100 µl Sodium Citrate-Perchloric acid sample diluent (pH 2.20) and it is filtered through 0.2 µm membrane filter to determine FAA by HPLC model LC-10A (Shimadzu Corp., Japan). Separation of AA was done in a column (Shimpack ISC-07/S1504 Na) packed with a strongly acidic Na^+ type cation exchange resin (Styrene-divinyl benzene copolymer with sulfinic group) under gradient elusion at a flow rate of 0.3 ml minute^{-1} by using two buffers, (A) sodium citrate-perchloric acid (pH 3.2); (B) boric acid sodium citrate-sodium hydroxide (pH 10.0). The FAA were detected and quantified using a fluorescent detector (FLD-6A) after post column derivitization with O-pthaladehyde and 2-mercaptoethanol (Dayal et al., 2003). Amino acid standard solution (Sigma-Aldrich, Inc., USA) for fluorescent detection was used as external standard and for every ten-sample injections one standard run was carried out. All the analyses were carried out in triplicates. Amino acid results were expressed as nmol ml^{-1} for FAA.

2.3. Haemolymph ammonia and urea

Haemolymph ammonia was estimated using Sigma kit. Ammonia reacts with α-ketoglutaric acid (KGA) and reduces nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of L-glutamate dehydrogenase (GDH) to form L-glutamate and oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$). The decrease in absorbance at 340 nm, due to the oxidation of NADPH, is proportional to the ammonia concentration. L-Glutamate dehydrogenase reacts specifically with ammonia (Tulli et al., 2007). The values are expressed as µmoles ml^{-1}.

Haemolymph urea is converted quantitatively by urease into ammonia and carbon dioxide (Rajaram, 2010). In this method the ammonium ions react with hypochlorite and salicylate to give a green coloured complex (Berthelot reaction). This colour yield is enhanced by sodium nitroprusside. The colour intensity is directly related to the urea concentration and is measured spectrophotometrically at 578 nm. The values are expressed as µmoles ml^{-1}.

2.4. Statistical analysis

Statistical analysis was carried out by using SPSS-17 version of statistical software package for one way analysis of ANOVA after transforming the percent values to arcsine. The significance levels of treatments were compared using Duncan multiple range test.

3. Results and Discussion

The analytical results on FAA are presented in Figures 1 to 4. Among the non-essential amino acids, glycine, proline, taurine and alanine are the predominant amino acids (Figure 1-2). The concentration of glycine significantly (p<0.05) increased (72.99±2.99 to 162.07±14.31 nmoles ml^{-1}) with increase in salinity from 3 to 30 ppt and its level decreased thereafter to 145.11±11.83 at 36 ppt and 137.11±6.49 nmoles ml^{-1} at 50 ppt (Figure 2). Proline is the next predominant non-essential amino acid which also followed the same trend. Its concentration increased from 68.31±1.18 at 3 ppt to 142.32±15.53 at 30 ppt and decreased to 129.94±7.11 nmoles ml^{-1} at 50 ppt (Figure 1). Among the essential amino acids, arginine, leucine,

lysine, phenyl alanine and valine are the predominant FAA in the haemolymph across all the salinities (Figure 3). Except arginine, the concentration of essential amino acids are much lower than that of non-essential amino acids indicating that non-essential amino acids are the main organic osmolytes in shrimp haemolymph. The peak concentration of essential amino acid, arginine (61.79±6.94 nmoles ml^{-1}) level was observed at 23 ppt compared to 33.07±1.84 at 3 ppt and 25.46 ±4.26 at 50 ppt (Figure 4) whereas the non-essential amino acids, glycine and proline peaked at 30 ppt indicating the differences between essential and non essential amino acids (Figure 1 & 2).

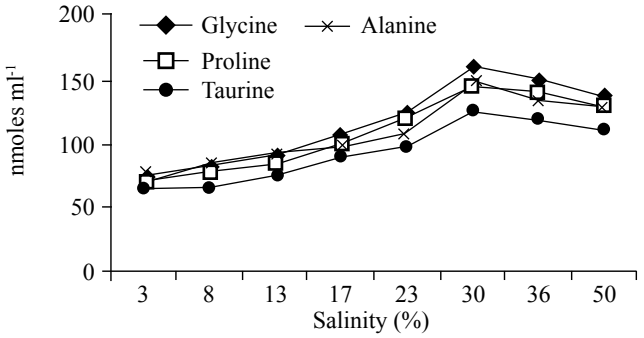

Figure 1: Effect of salinity on non-essential free amino acid levels in haemolymph of *Penaeus monodon* from culture ponds

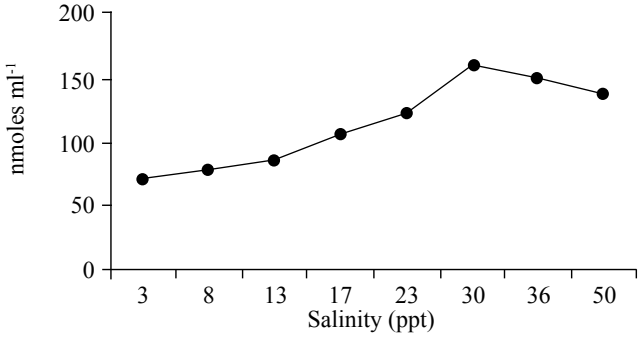

Figure 2: Effect of salinity on glycine FAA in haemolymph from culture ponds

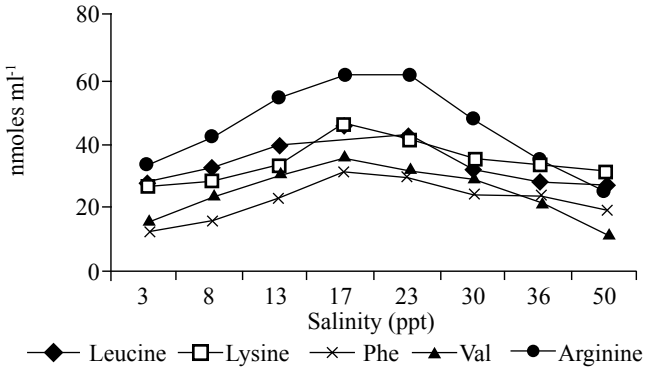

Figure 3: Effect of salinity on essential free amino acid levels in haemolymph of *Penaeus monodon* from culture ponds

Cobb et al. (1975) reported that in the white shrimp, *Penaeus stylirostris*, glycine, proline and alanine levels increased significantly in accordance with an increasingly saline environment. These authors further outlined that in penaeid shrimp, glycine and proline are main osmoeffectors under both increasing or decreasing salinity. The involvement of free amino acids in osmoregulation in juvenile *Penaeus japonicas* was studied by Dalla Via (1986) in shrimp adapted to sea water; the most abundant individual FAAs were found to be glycine, taurine, arginine, proline and alanine. During hyperosmotic stress the glycine concentration in the haemolymph decreased by 60% in *Chasmagnathus granulata* (Schein, 1999). Oliveira and Da Silva (2000) demonstrated that hepatopancreas gluconeogenesis is one of the pathways implicated in the metabolic adjustment of the amino acids pool during hypo-osmotic stress in *C. granulata.* The authors suggest that the amino acids released from the different tissues during the hypo-osmotic stress could be deaminated in the hepatopancreas, and the carbon chains used as substrates for the gluconeogenesis pathway (Oliveira and Da Silva, 2000). Lower concentrations of essential amino acids in haemolymph was earlier reported and it was inferred that these amino acids are better utilized in body building process at optimum salinity (Fang et al., 1992).

Significantly (p<0.05) higher concentration (µmoles ml^{-1}) of haemolymph ammonia (0.18±0.03 at 3 ppt; 0.46±0.07 at 50 ppt) and urea (0.22±0.03 at 3 ppt; 1.59±0.19 at 50 ppt) were observed in higher salinity (Figure 5). The increase in ammonia was moderate (100%) from brackishwater salinities (13-25 ppt) to higher salinities (50 ppt) whereas the urea increase was very high (500%) indicating that there is shift in nitrogen metabolism at hyperosmotic stress in shrimp. Chen and Chia (1996) observed that ammonia-N, urea-N and organic-N contributed 82%, 4% and 6% of total excreted nitrogen at 10 ppt, whereas at 40 ppt these contributions were 56%, 30% and 9% of total excreted nitrogen by *Scylla serrata*. In *Marsupenaeus japonius* ammonia-N, urea-N and organic-N contributed 90.9%, 3.1% and 4.2% of total excreted nitrogen at 18 ppt, whereas these contributions were 38.5%, 10.9% and

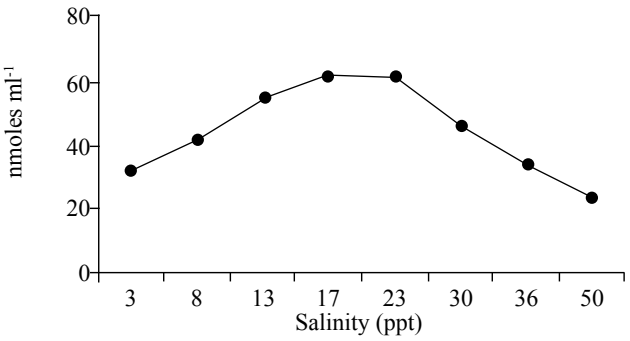

Figure 4: Arginine FAA in haemolymph from culture ponds

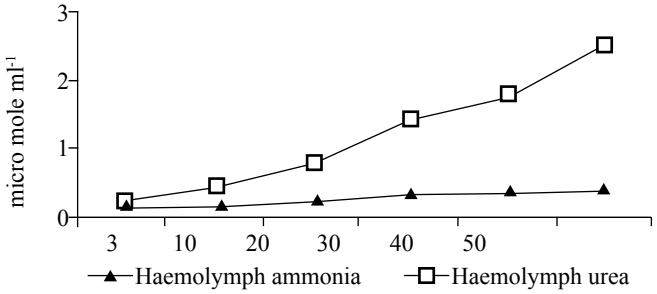

Figure 5. Effect of salinity on haemolymph ammonia and urea of *Penaeus monodon*

50.4% of total excreted nitrogen by the at 42 ppt. Significantly higher levels of haemolymph urea with an increase in arginase activity indicated that ureogenesis are activated for *M. japonicus* in hyperosmotic conditions. In hyperosmotic environments urea cycle can be activated so that the shrimp will produce a less toxic, by-product type osmolyte.

4. Conclusion

In hyper osmotic environments, the shrimp activates the urea cycle to produce a less toxic, byproduct type osmolyte. In order to maintain high osmolality in the haemolymph in hyper environmental stress conditions urea synthesis, a energy consuming process is much more efficient than expensive amino acids for osmoregulation. This is reflected in lower free amino acid levels at high osmotic stress condition.

5. Further Research

Osmoregulation is an energy depending process, adequate amount of energy should be provided through the diet, to meet their energy requirement. If not, shrimp may withdraw energy source from some of the tissues leads to the rapid reduction in the growth. So it is necessary to find out the optimum level of energy source to supply the adequate amount of energy according to the species and salinity. The changes in nitrogen metabolism and organic osmolytes like free amino acids indicated that there is need to study the dietary modification in shrimp.

6. Acknowledgements

Authors express their sincere thanks to Dr. A. G. Ponniah, Director, CIBA, Chennai for his intellectual support and encouragement to carry out this work.

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