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Glutamate dehydrogenase and Na^+-K^+ ATPase expression and growth response of *Litopenaeus vannamei* to different salinities and dietary protein levels

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Abstract:

Improvement in the osmoregulation capacity via nutritional supplies is vitally important in shrimp aquaculture. The effects of dietary protein levels on the osmoregulation capacity of the Pacific white shrimp (*L. vannamei*) were investigated. This involved an examination of growth performance, glutamate dehydrogenase (GDH) and Na^+-K^+ ATPase mRNA expression,, and GDH activity in muscles and gills. Three experimental diets were formulated, containing 25%, 40%, and 50% dietary protein, and fed to the shrimp at a salinity of 25. After 20 days, no significant difference was observed in weight gain, though GDH and Na^+-K^+ ATPase gene expression and GDH activity increased with higher dietary protein levels. Subsequently, shrimp fed diets with 25% and 50% dietary protein were transferred into tanks with salinities of 38 and 5, respectively, and sampled at weeks 1 and 2. Shrimp fed with 40% protein at 25 in salinity (optimal conditions) were used as a control. Regardless of the salinities, shrimp fed with 50% dietary protein had significantly higher growth performance than other diets; no significant differences were found in comparison with the control. Shrimp fed with 25% dietary protein and maintained at salinities of 38 and 5 had significantly lower weight gain values after 2 weeks. Ambient salinity change also stimulated the hepatosomatic index, which increased in the first week and then recovered to a relatively normal level, as in the control, after 2 weeks. These findings indicate that in white shrimp, the specific protein nutrient and energy demands related to ambient salinity change are associated with protein metabolism. Increased dietary protein level could improve the osmoregulation capacity of *L. vannamei* with more energy resources allocated to GDH activity and expression.

Keywords: *Litopenaeus vannamei* – gene expression – glutamate dehydrogenase – Na^+-K^+ ATPase – protein – salinity

1. Introduction

Among all the environmental factors, salinity could be the most important factor to influence the physiological status of the aquatic animals since ambient salinity change would directly relates to their osmoregulation capacity (Fry, 1971; Kinne, 1971). However, for many cultured aquatic species, it is cultured often at different salinities. Though many literatures had reported the physiological response to different salinities, different aquatic animals had different physiological status at deferent salinities (Tsuzuki et al., 2007; Tantulo and Fotedar, 2006; Li et al., 2007).

When ambient salinity changes, the main challenge for aquatic animals is to regulate their osmo pressure, and in the process, they need to regulate the cell volume and maintain the stability of cellular macrolecules such as enzymes (Hochachka and Somero, 2002). Na+-K+ ATPase has been believed to be of central importance in ion regulation and cellular water balance for aquatic vertebrates and invertebrates by pumping Na+ ions into the hemolymph, and its activity is directly related to their osmoregulation capacity (Towle, 1981; 1984). Many free amino acids called compatible osmolytes also had crucial important roles in this process (Hare et al., 1998; Kempf and Bremer, 1998; Hochachka and somero, 2002), including proline, alanine, glutamic acid, glycine and taurine (Lockwood, 1956; Somero, 1983). Glutamate dehydrogenase (GDH) involves into the production of glutamate from α-ketoglutarate since it catalyses the reversible oxidative deamination of glutamate to α-ketoglutarate (Plaitakis and Zaganas, 2001). Because alanine is produced by transamination of pyruvate with glutamate, and proline is synthesized from glutamate via a pyroline-5-carboxylate intermediate (Willet and Burton, 2003), GDH would play important roles in osmoregulation by regulate the synthesis of both proline and alanine.

The pacific white shrimp, *Litopenaeus vannamei*, is a tropical species that has been widely cultured in extensive, intensive, and semi-intensive systems (Frías-Espericueta et al., 1999), and it has become an attractive cultivar for inland saline water farming in many parts of the world including the United States (McGraw et al., 2002), Thailand (Saoud et al., 2003) and China (Cheng et al.,2006) in these year. Although L. vannamei is euryhaline and able to tolerate a wide range of salinity from 1 to 50 ‰ (Pante, 1990), a salinity change does alter its growth performance and physiological responses which directly relates to its osmoregulation capacity. Regarding the important role of Na+-K+ ATPase in osmoregulation of aquatic animals, its study in L.vannamei was only focused on the activities measurement (Pan et al., 2007; Hurtado et al., 2007). And for GDH, only some biochemistry characteristics and the activities were determined in some crustacean species (Roustiau et al., 1985; Rosas et al., 2001). Till now, information about GDH and Na+-K+ ATPase gene expression in L.vannamei is still limited.

Although attempts have been made through dietary manipulation of macro-nutrients to improve the growth performance and physiological condition of *L. vannamei* at low salinity, little success was achieved. Hurtado et al. (2007) reported that the increase of

highly unsaturated fatty acids in the diet did not change the ion regulation at low salinity. Roy et al. (2006) also found that the inclusion of cholesterol and lecithin in the diet did not improve the growth performance of L.vannamei at low salinity. In contrast, Cuzon et al. (2004) reported that high protein diet improved the growth of *L. vannamei* at low salinity because the dietary amino acids could serve as osmotic regulators to reduce the energy loss from muscle and promote growth. Therefore, the role of dietary protein levels and associated mechanisms on osmoregulation in *L. vannamei* warrants further investigation.

In this study, we determined the GDH and Na+-K+ ATPase gene expression together with the GDH activity in both gill and muscle of *L.vannamei* fed diets with different dietary protein levels, and evaluated the growth responds of these shrimp to ambient salinity challenge.

2. Materials and methods

2.1 Experimental diets

Three isocaloric diets with three dietary protein levels were formulated, which were 20%, 40% and 50% respectively. All the experimental diets were prepared by thoroughly mixing dry ingredients with oil and then adding water until a stiff dough resulted. The dough was then passed though a meat-mincer equipped with a 2 mm die, and the resulting spaghetti-like strands were air dried at 60°C. After drying, the material was broken up into regular pieces sieved to a convenient pellet size and stored at -20°C.

2.2 Experimental design

Juvenile white shrimps (body weight 3.43±0.32g) obtained from the Multidisciplinary unite of research and education in SISAL of Facultad de Ciencias, Universidad Nacional Autónoma de México, were held in five fiberglass tanks (12.5 m^2) with a density of 12 shrimps per m2 at a salinity of 25 ‰ ,and adapted to the experiment diets for 1 week prior to start the experiment. After 20 days, 10 shrimps were sampled in each tank, and then at once, the ambient salinity in tanks with shrimp fed diets with both 20% and 50% were changed to 38 ‰ and 5‰ respectively. And 10 shrimps were sampled randomly in each tank at 1week and 2 week after the salinity change. All samples were firstly frozen rapidly in liquid nitrogen, and then transferred to -80 refrigerator for further analysis. Water quality parameters were monitored throughout the feeding trial, and the growth performance was also recorded and evaluated. The indexes for the assessment of growth performance were calculated as follows:

Weight gain (%) =100× (Wt − W0)/W0, where, W0 is the initial weight and Wt is the final weight

Survival (%) =100× (final shrimp number)/ (initial shrimp number)

Hepatosomatic index (HSI,%) =100×(liver weight/body weight)

2.3 GDH activity assay

Enzyme assays were performed individually on crude homogenates of each tissue following the method proposed by King et al. (1985) and Regnault (1993). Conditions of these assays were 850µl 80 mM imidazole buffer, pH 8.0, and 10µl 1M NaCl. We used 40 µl enzyme extract and 50µl 20 mM NAD (final volume=1 ml). Enzyme activity was determined from the slope of NAD reduction to NADH recorded at 340 nm (=6.22×10−3) at room temperature using a GESYS spectrophotometer. Supernatant protein was estimated by the Bradford method using bovine albumin as a standard (Bradford, 1976; Stoscheck, 1990). Results were expressed as mIU (µmol NADH formed min^{-1} mg^{-1} protein).

2.4 Total RNA extraction and reverse transcription

Total RNA extractions from gill were carried out according to the TRIZOL protocols (Invitrogen). RNA was quantified at 260 and 280 nm using UV-240 spectrophotometer (SHIMAZU Corporation, Kyoto, Japan). Before reverse transcription, RNA was treated with DNAase (PROMEGA) at 37 for 15 minutes and washed two times. Samples of polyadenylated RNA were reverse-transcribed from 1µg of total RNA denatured at 70 for 5 min. Reactions were carried out in a total volume of 25µl and the volumes of the reaction components were as follows: 1µl dNTP(25mM), 0.5µl Oligo dT,5µl Tp MMLV, 2.5ul DTT, 0.325µl RNAsine, and 0.675 µl MMLV. The protocol to realize reverse transcription was at 25 for 10 minutes, 42 for 50 minutes, and 94 for 5 minutes.

2.5 Quantitative real time PCR (qPCR) analysis

mRNA expression of the target genes were measured by qPCR. The specific primers for all test genes were designed based on published *L. vannamei* and *P. monodon* cDNAs (Table 2). All primers were produced by EURO GENTEC Ltd. The qPCR was carried out in the iCycler iQ real-time PCR system (Bio-Rad Laboratories, Richmond, CA) using SYBR Green. The amplifications were performed in a 96-well plate in a 15 µl reaction volume containing 7.5 µl of SYBR Green Master Mix (Bio-Rad), 0.5 µl (each) gene-specific forward and reverse primers, 1.5 ul nuclease-free water, and 5 µl of cDNA. The thermal profile for SYBR Green PCR was 95 °C for 3 min followed by 40 cycles of 95 °C for 30 s and 60 °C for 1 min. After PCR amplification, melt-curve analysis was conducted using the iCycler iQ Optical System Software (Bio-Rad) to confirm that there was one amplified product. The amplified fragments by qPCR were controlled both by sequencing and electrophoresis on 1.2 % agarose gels with TAE buffer in 1X TAE buffer (Tris–acetic acid–EDTA) with one drop of ethidium bromide.

2.6 Data analysis

Each numerical measurement was expressed as mean±standard error, and data of weight gain and HSI at each sample time was subjected respectively to one-way analysis of variance (ANOVA, SPSS for Windows, version 14.0) to determine

significant differences among all treatments. The level of significant difference was set at $P < 0.05$.

3. Results

All shrimp survived in this study. The mRNA transcripts to β- actin ratio of both Na^+-K^+ ATPase and GDH of *L.vannamei* fed diets with three dietary protein levels are presented in table 3. Shrimp fed diet with 20% dietary protein had the lowest value. Though no significant difference was observed in these treatments, there was a tendency of increasing both in Na+-K+ ATPase and GDH mRNA transcripts toβ- actin ratio of *L.vannamei* when dietary protein increased. GDH activities in both gill and muscle of *L.vannamei* were similar to the results of mRNA transcripts of Na+-K+ ATPase and GDH with the lowest values observed in shrimp fed diet with 20% dietary protein (Table 3).

Figure 1 presents the weight gain of the shrimp fed the three experimental diets after 20 days at 25 ppt, and its change after ambient salinity changes for 1 and 2 weeks. No significant differences were observed in the weight gains of shrimp after food acclimation of 20 days among the treatments at 25 ppt. After ambient salinity change one week, significantly higher weight gains were observed in shrimp fed diet with 50% dietary protein at 5 ppt and with 40% dietary protein at 25 ppt. And after two weeks, shrimp fed diet with 50% dietary protein both at 38 and 5 ppt and fed diet with 40% protein at 25ppt were significantly higher than those fed diet with 20% dietary protein both at 38 and 5 ppt.

Figure 2 shows the heptosomatic index (HSI) of *L.vannamei* food acclimation of 20 days and its change after the ambient salinity change. No significant difference was observed in HSI of *L.vannamei* fed diets with different dietary protein levels after 20 days. When ambient salinity changed, shrimp HSI tented to increase significantly in the first week, and significantly higher values were observed in *L.vannamei* fed diet with 20% dietary protein at 5 ppt and diet with 50% dietary protein at 38 ppt. While after two weeks, the alerted higher HSI values began to recover to a normal level controlled as the treatment of shrimp fed diet with 40% dietary protein at 25 ppt, and relatively higher HSI value was only observed in shrimp fed diet with 20% protein level at 5 ppt.

4. Discussion

In this study, though no significant difference was observed in both GDH mRNA level and activity of *L.vannamei* fed diets with different dietary protein levels, an increasing tendency appeared to be induced in *L.vannamei* by higher dietary protein. As a control, similar changes in Na^+-K^+ ATPase mRNA level was also found in this study. Since Na+-K+ ATPase had been proved to be a key enzyme for osmoregulation by pumping Na+ ions into the hemolymph (Towle, 1984), and higher activity usually indicate higher osmoregulation capacity, this finding together with that in GDH would indicate that GDH might be a practical parameter reflecting the osmoregulation capacity by

regulating the alanine and proline synthesis (Plaitakis and Zaganas, 2001), which is in consistent with the finding of Arena et al. (submitted) demonstrating that GDH activity increased with low salinity adaptation, and meanwhile was amplified by dietary higher protein in L.vannamei. While in T.californicus, willet and Burton (2003) demonstrated GDH transcription and enzyme activity did not appear to function in the regulation of alanine and proline accumulation during hyperosmotic stress, and their previous work suggested that the regulation of proline accumulation was not mediated by GDH enzyme activity (Burton, 1986,1991). The conflicts of these findings between L.vannamei and T.califonicus might be explained by the species difference, but since there is a lack of definitive evidence demonstrating the general correlation between GDH and osmoregulation capacity, especially in macro crustacean species, whether the increasing GDH expression and activity in *L.vannamei* could reflect higher free amino acids metabolism in attempt of shrimp to obtain higher proline and analine to meet the osmoregulation demands still need further study.

The dietary protein requirement of *L.vannamei* for optimal growth has been obtained by many researchers which ranged from 30-36% (Colvin and Brand, 1977; Smith et al., 1985; Kureshy and Davis, 2002), but no significant higher weight gain was observed in *L.vannamei* fed diet with 40% dietary protein due to the food acclimation time was only 20 days which was not enough long for determine the difference. However, after ambient salinity changed, shrimp fed diets with higher protein showed significant higher growth performance compared with that fed diet with 20% dietary protein indicating that increasing dietary protein level in the diet of *L.vannamei* would be a practical nutrient modulation method to increase the production of *L.vanname*i at extreme high and low salinity aquaculture, and this finding together with the results of GDH and Na^+-K^+ ATPase confirmed the important function of dietary protein or amino acids in osmoregulation of L.vannamei (Cuzon et al., 2004). However, the dietary protein requirement of L.vannamei at low salinity less than 5 ppt wasvery limited and contravertial. Huang et al.(2004) found that at 2 ppt the dietary protein requirement of L.vannamei was 26.70%, while Liu et al (2005) concluded that at 1-3 ppt the optimal dietary protein was around 40% which was collaborated by the results in the present study in certain degree, but the optimal dietary protein requiement of L.vannamei should be further studied.

In this study, ambient salinity change alerted the HSI which increased after ambient salinity change in the first week, and then recovered to a relatively normal level after two weeks. A similar increase in digestive gland weight was also reported in M.japonicus after a salinity change (Cuzon et al., 2004). The digestive gland index in shrimp could reflect the nutrition accumulation and utilization status since the hepatopancreas is the major digestive gland and also a sensitive indicator for metabolism and nutritional status in various shrimp species (Al-Mohanna and Nott, 1989; Bautista et al., 1994; Rosas et al., 1995). Therefore, this change indicate that shrimp was stimulated by the acute ambient salinity stress to accelerate the nutrient metabolism to meet the special nutrient and energy demand, such as hemocyanin (Rosas et al., 2001), and the finding of Li et al. (2008) could collaborate this which found an escalation of digestive enzyme activities, especially the trypsin activity of

L.vannamei occurred at extreme salinities providing direct evidence showing the possibility of deriving extra food energy to compensate the energy loss for osmoregulation when salinity sways from the physiological optimum.

Overall, acute ambient salinity change could lead to metabolism acceleration, and increase dietary protein level could provide the shrimp with extra energy or amino acids to improve osmoregulation capacity and to obtain the maximum growth. Glutamate dehydrogenase might be of important function in osmoregulation of *L.vannamei*, but further study should be conducted to confirm the underlying mechanism.

References

Al-Mohanna, S. Y., Nott, J. A., 1989. Functional cytology of the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda) during molt cycle. Mar. Biol. 102, 535–544.

Arena, L., Cuzon, G., Pascual, C., Gaxiola, G., Chimal, M.E., Lopez, N., Van Wormhoudt, A., Rosas, C. Glutamate dehydrogenase activities of the pacific white shrimp, *Litopenaeus vannamei,* fed diets with different dietary protein levels at different salinities. Unpublished.

Bautista, M. N., Lavilla-Pitogo, C., Subosa, P. F., Begino, E. T., 1994. Aflatoxin B1 contamination of shrimp feeds and its effect on growth and hepatopancreas of preadult *Penaeus monodo*n. J. Sci. Food Agricult. 65, 5–11.

Bradford, M.M., 1976. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 7, 248-254.

Burton, R.S., 1986. Incorporation of 14C-bicarbonate into the free amino acid pool during hyperosmotic stress in an intertidal copepod. J. Exp. Zool. 238, 55–61.

Burton, R.S., 1991. Regulation of proline synthesis during osmotic stress in the copepod *Tigriopus californicus*. J. Exp.Zool. 259, 166–173.

Cheng, K.M, Hu, C.Q, Liu, Y.N., Zheng, S.X, Qi, X..J., 2006. Effects of dietary calcium, phosphorus and calcium / phosphorus ratio on the growth and tissue mineralization of *Litopenaeus vannamei* reared in low-salinity water. Aquaculture 251,472– 483.

Conklin, D.E., 1997. Vitamin. In: D'Abramo, L.R., Conklin, D.E., Akiyam, D.M. (Eds), Crustacean Nutrition. The World Aquaculture Society, Baton Rouge, p.130-131.

Cuzon, G., Guillaume, J., 1997. Energy and protein: energy ratio In: L.R. D'Abramo, D.E. Conklin and D.M. Akiyama, Editors, Crustacean Nutrition, Advances in World Aquaculture Society vol. 6, World Aquaculture Society, Baton Rouge, LA, 51–70.

Cuzon, G., Lawrence, A., Gaxiola,G., Rosas, C., Guillaume, J., 2004. Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture 235, 513-551.

Frías-Espericueta, M.G., Harfush-Melendez, M., Osuna-López, J.I., Páez-Osuna, F, 1999. Acute toxicity of ammonia to juvenile shrimp *Penaeus vannamei* Boone. Bull.Environ. Contam.Toxicol. 62, 646-652.

Fry, F.E.J., 1971. The effect of environmental factors on the physiology of fish. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology Vol.VII: Environmental Relations and Behavior. Academic Press, New York, pp. 1–98.
Hare, P.D., Cress, W.A., Van Staden, J., 1998. Dissecting the roles of osmolyte accumulation during stress. Plant, Cell, Env. 21, 535-553.
Hochachka, P.W., Somero, G.N., 2002. Biochemical adaptation. Oxfor University Press, New York, NY,217-289.
Huang, K., Wang, W., Lu, H., Dai, X., Zhou, J., 2004. Salinity effects on growth and biochemical composition of *Penaeus vannamei*. Marine Sciences 9,20-25.
Hurtado, M.A., Racotta, I.S., Civera, R., Ibarra, L., Hernandez-Rodriguez, M., Palacios, E., 2007. Effect of hypo- and hypersaline conditions on osmolality and Na+/K+-ATPase activity in juvenile shrimp (*Litopenaeus vannamei*) fed low- and high-HUFA diets. Comp. Biochem. Physiol. 147A, 703-710.
Kempf, B., Bremer,E., 1998. Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolaity environments. Arch. Microbiol. 170, 319-330.
King, F.D., Cucci, T.L. and Bidigare, R.R., 1985. A pathway of nitrogen metabolism in marine decapod crabs. Comp. Biochem. Physiol. 80A, 401–403.
Kinne, O., 1971. Salinity: Animal invertebrates. In: Kinne, O. (Ed.), Marine Ecology Vol.1: Environmental Factors. Wiley Interscience, London, pp. 821–995.
Kureshy, N., Davis, D.A., 2002. Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 204,125-143.
Li, E., Chen, L., Zeng, C., Chen, X., Yu, N., Lai, Q., Qin, J.G., 2007. Growth, body composition, respiration and ambient ammonia nitrogen tolerance of the juvenile white shrimp, *Litopenaeus vannamei*, at different salinities. Aquaculture, 265,385-390.
Li, E., Chen, L., Zeng, C., Yu, N., Xiong, Z., Chen, X., Qin, J.G., 2008. Comparison of digestive and antioxidant enzymes activities, haemolymph oxyhemocyanin contents and hepatopancreas histology of white shrimp, *Litopenaeus vannamei,* at various salinities. Aquaculture, 274,80-86.
Liu, D., He, J., Liu, Y., Zheng, S., Tian, L., 2005. Effects of Dietary Protein Levels on Growth Performance and Immune Condition of Pacific White Shrimp *Litopenaeus vannamei* Juveniles at Very Low Salinity. Acta Scientiarum Naturalium Universitatis Sunyatseni 44,21-223.
Lockwood, A P. M. Aspects of the physicalogy of Crustacea. Aberdeen University Press.1956, 88.
McGraw, W.J., Davis, D.A., Teichert-Coddington, D., Rouse, D.B., 2002. Acclimation of *Litopenaeus vannamei* postlarvae to low salinity: influence of age, salinity, endpoint and rate of salinity reduction. J. World Aquac. Soc. 33, 78–84.
Pan, L.Q., Zhang, L.J., Liu, H.Y., 2007. Effects of salinity and pH on ion-transport enzyme activities, survival and growth of *Litopenaeus vannamei* postlarvae. Aquaculture 273,711-720.
Pante, M.J.R., 1990. Influence of Environmental Stress on the Heritability of Molting Frequency and Growth Rate of the Penaeid Shrimp, *Penaeus vannamei.* University of Houston-Clear lake, Houston, TX, USA, M.Sc. Thesis.

Plaitakis, A., Zaganas, I., 2001. Regulation of human glutamate dehydrogenases: implications for glutamate ammonia and energy metabolism in brain. J. Neurosci. Res. 66, 899-908.
Regnault, M., 1993. Effect of severe hypoxia on some aspects of nitrogen metabolism in the crab *Cancer pagurus*. Mar. Behav. Physiol. 22, 131–140.
Rosas, C., Bolongaro-Crevenna, A., Sa´nchez, A., Goriola, G., Soto, L., Escobar, E. 1995. Role of digestive gland in energetic metabolism of *Penaeus setiferus*. Biol. Bull. 189, 168–174.
Rosas, C., Cuzon, G., Gaxiola, G., Le Priol, Y., Pascual, C., Rossignyol, J., Contreras, F., Sanchez, A. and van Wormhoudt, A., 2001. Metabolism and growth of juveniles of *Litopenaeus vannamei*: effect of salinity and dietary carbohydrates levels. J. Exp. Mar. Biol. Ecol. 259 1, 1–22.
Rosas, C., López, N., Mercado, P. and Martinez, E., 2001. Effect of salinity acclimation on oxygen consumption of white shrimp *Litopenaeus vannamei j*uveniles. J. Crustac. Biol. 21 4, pp. 912–922.
Rosas, C., Martinez, E., Gaxiola, G., Díaz, E., Brito, R. and Soto, L., 1998. Effect of dissolved oxygen on the energy balance and survival of *Penaeus setiferus* juveniles. Mar. Ecol., Prog. Ser. 174, pp. 67–75.
Roustiau, S., Batrel, Y., Bernicard-Peron, A., Le Gal, Y., 1985. Effect of thermal acclimation on subunit cooperativity in *Palaemon serratus* glutamate dehydrogenase. Biochem. Syst. Ecol. 13,45-50.
Roy, L.A., Davis, D.A., Saoud, I.P., 2006. Effects of lecithin and cholesterol supplementation to practical diets for *Litopenaeus vannamei* reared in low salinity waters. Aquaculture 257, 446–452.
Saoud, I.P., Davis, D.A., Rouse, D.B., 2003. Suitability studies of inland well waters for *Litopenaeus vannamei* culture. Aquaculture, 217, 373– 383.
Smith, L.L., Lee, P.G., Lawrence, A.L., Strawn, K., 1985. Growth and digestibility by three sizes of *Penaeus vannamei* Boone: Effects of dietary protein level and protein source. Aquaculture 46,85-96.
Somero, G. N, Bowlus, R. D., 1983. Osmolytes and metabolic end products of molluscs: the design of compatible solute systems. In: Hochachka PW (ed.). Environmental Biochemistry and Physiology,the Mollusca. London:Academic Press. 77–100.
Stoscheck, C.M.,1990. Quantitation of Protein. Methods in Enzymology 18, 50-69.
Tantulo, U., Fotedar, R., 2006. Comparison of growth, osmoregulatory capacity, ionic regulation and organosomatic indices of black tiger prawn (*Penaeus monodon* Fabricius, 1798) juveniles reared in potassium fortified inland saline water and ocean water at different salinities. Aquaculture 258,594-405.
Towle, D.W., 1981. Role of Na+-K+-ATPase in ionic regulation by marine and estuarine animals. Mar Biol Lett.2, 107-122.
Towle, D.W., 1981. Role of Na+-K+-ATPase in ionic regulation by marine and estuarine animals. Mar. Biol. Lett. 2, 107-122.
Towle, D.W., 1984. Membrance-bound ATPase in arthropod ion-tranporting tissues. Am. Zool. 24, 177-185.

Tsuzuki, M.Y., Sugai, J.K., Maciel, J.C., Francisco, C.J., Cerqueira, V.R., 2007. Survival, growth and digestive enzyme activity of juveniles of the fat snook (*Centropomus parallelus*) reared at different salinities. Aquaculture 271, 319-325.
Willett, C.S., Burton, R.S., 2003. Characterization of the glutamate dehydrogenase gene and its regulation in a euryhaline copepod. Comp. Biochem. Physiol., B, 135, 639-649.

Tables

Table 1. Experimental diet compositions (g/kg)

Ingredients	Diets		
	CP20	CP40	CP50
Fish meal	100	300	500
Soybean meal	50	95	140
Squid meal	80	90	100
Fish soluble protein concentrate	50	70	80
Wheat starch	610	340	70
Cod liver oil	40	40	40
Soybean lecithin	10	10	10
Vitamin Stay C[a]	10	10	10
Vitamin and mineral premix[b]	30	30	30
CMC	20	20	20
Proximate nutritional components			
Crude protein %	24	39	52
Crude lipid %	9.6	9.4	9.8
Crude carbohydrate %	49	28	6
Digestible energy[c]	16.3	16.5	16.5

[a] Ascorbyl phosphate (Stay-C-35% DSM S.A de C.V.).

[b] Vitamin and mineral premix provided by DSM, S.A. de C.V.

[c] Digestible energy estimated using the following coefficients: 15 kJ for carbohydrates, 35 kJ for lipids and 23 kJ for protein according to Cuzon and Guillaume, (1997).

Table 2. Primers for quantitative real time PCR of GDH and Na^+-K^+ ATPase of *L.vannamei*.

Target gene	Gene Bank #	Forward/ Reverse sequence (5'→3')	Product (bp)
Na^+-K^+ ATPase	DQ399796	AGCAAGGCCATCAACGATCT GCCCACTGCACAATCACAAT	122bp
GDH B	EU496492	CTTTCCAGGATCGCATTTCT AAGCAGCAGTACGGAGATCAA	141bp
β-actin	AF300705	CGCGACCTCACAGACTACCT GTGGTCATCTCCTGCTCGAA	140bp

Table 3. Na+-K+ ATPase and GDH gene expression and GDH activity in both Gill and muscle of *L.vannamei* fed diets with different dietary protein levels after 20 days.

Index	Dietary protein levels			ANOVA
	20%	40%	50%	P value
Na+-K+ ATPase	**0.31±0.04**	**0.34±0.02**	**0.33±0.03**	0.235
GDH	**0.16±0.02**	**0.20±0.05**	**0.22±0.04**	0.587
Gill GDH activity	**2.30±0.31**	**3.00±0.20**	**2.48±0.50**	0.249
Muscle GDH activity	**14.30±1.92**	**16.69±0.49**	**18.43±2.37**	0.151

Figures

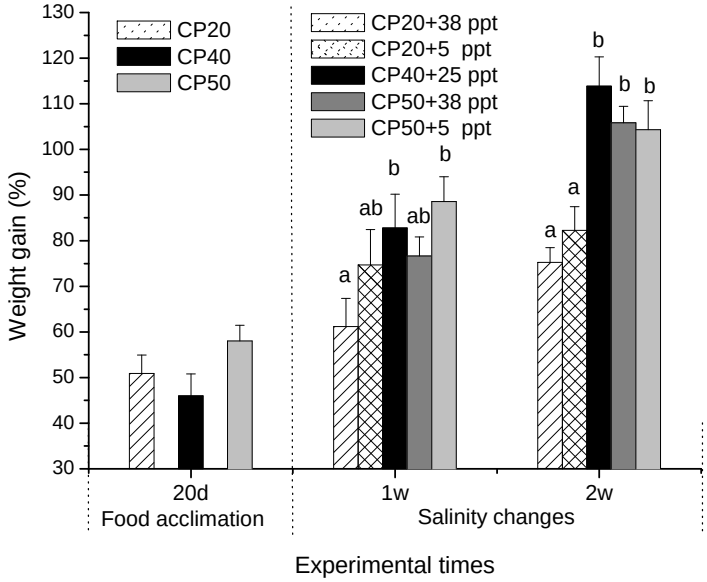

Figure 1. Weight gain of *L.vannamei* fed the three experimental diets after 20 days at 25 ppt, and its change after ambient salinity changes for 1 and 2 weeks. Bars sharing different letters in each sampling time are significantly different ($P < 0.05$).

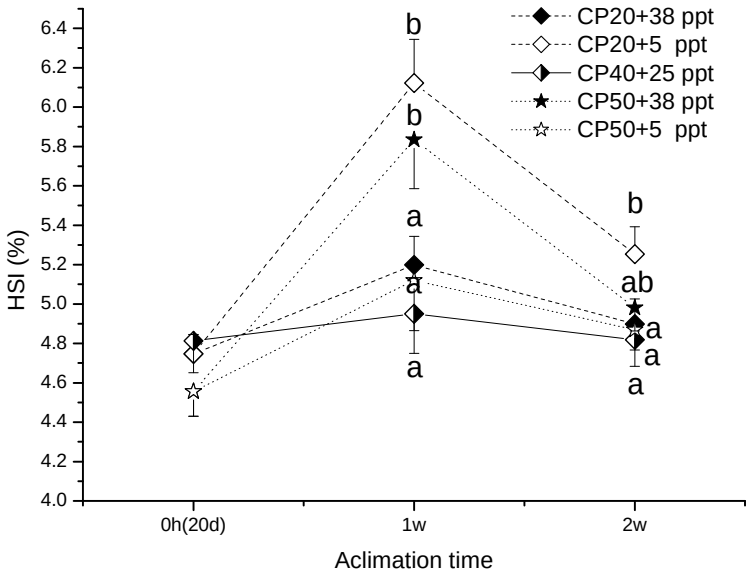

Figure 2. HSI of *L.vannamei* fed the three experimental diets after 20 days at 25 ppt, and its change after ambient salinity changes for 1 and 2 weeks. Bars sharing different letters in each sampling time are significantly different ($P < 0.05$).