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Effect of Mineral Suplement in the Diet for *Penaeus monodon* F. Shrimp Culture in a Low Salinity Medium

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ABSTRACT

Shrimp culture in a low salinity medium has been developed widely such as in Thailand, China, Indonesia and some Federal country of USA. This new approach of shrimp culture of the tiger prawn *Penaeus monodon* Fab was done as low salinity shrimp farming; low-salinity inland system, inland shrimp farming, and inland culture. Water source was then treated with a step-wise dilution process with river waters to the salinity range of 25-30 ppt to be used for post-larval rearing and growing. The process of dilution will continue to salinity of 5 ppt to be used for the growing ponds until harvest. The relocation of conventional brakish water ponds for shrimp culture from coastal area to inland area was initially caused by the problems of desease, marine pollutions, environmental issues such as mangrove destructions issues and the change of spatial planning in coastal area. The aim of the study is known the effect of K^+ and Mg^{2+} addition and its combination to the weekly growth rate (based on weight) of the shrimp and Osmotic Capacity. The study using addition of mineral in the diet with assumtion that this approach will have more effective effects to the specific growth rate (SGR), Absolute Growth (ΔG), survival rate (SR) and osmotic capability (OC) of the shrimp. The experiment design was Complete Random Design with 3 replicates for each treatment. Treatment A: standard diet added with 1% K (1 gr KCl/100 gr diet), treatment B: standard diet adde with 1% Mg^{2+} (1 gr dolomite/100 gr diet), treatment C was a combined of 0.5% K^+ and 0.5% Mg^{2+}, and treatment D was standard diet without mineral (as control). Result of data analysis using ANOVA revealed that all mineral diet treatment had no significant effect ($P > 0.05$) to SGR and ΔG for *Penaeus monodon* cultured in low salinity. Although a combined mineral (K and Mg) treatment C had shows the highest of absolute growth (0.7023 gr) then followed by treatment A (0.6421 gr), B (0.5303 gr), and D (0.4951 gr). The treatment of C with combination of 0.5% K and 0.5% Mg had gave the highest absolute growth and provide the best result. The weekly specific growth rate (SGR) had shown a steady decreasing pattern, with the lowest rate in week-5 and increase again in week-6. All mineral suplement in the diet had revealed a significant effect to the osmoregulatory capacity (OC) with (P value < 0.05) with seawater medium variabel as already mentions earlier. All treatments had gave high survival rate (SR) of the *Penaeus monodon* post-larvae where treatment A: 95.83%; B: 5.83%; C: 91.67% and control D : 85.50%.

Key words: *Penaeus monodon* F., Mineral, supplements, diet, low-salinity culture

INTRODUCTION

Shrimp culture in a low salinity medium had been developed widely such as in Thailand, China, Indonesia and some Federal country of USA. The new approach of shrimp culture was done and known as low salinity shrimp farming; low-salinity inland system, inland shrimp farming, and inland culture. The low salinity ponds should not always located near to the coast. The case in Thailand and Indonesia, the inland commercial scale growing ponds of tiger prawn *Penaeus monodon* Fab, the shrimp culture medium was using a concentrated coastal seawater with additions of salt to reach salinity upto 250 ppt. This source of water-medium for the inland shrimp culture was then transported to the inland ponds upto 150 km from the coastal area using trucks. In the inland ponds, this water source was then treated with a step-wise dilution process with river waters to the salinity range of 25-30 ppt to be used for post-larval rearing and growing. The process of dilution will continue to salinity of 5 ppt to be used for the growing ponds until harvest.

The relocation of conventional brakish water ponds for shrimp culture from coastal area to inland area was initially caused by the problems of desease, marine pollutions, environmental issues such as mangrove destructions issues and the change of spatial planning in coastal area. The shrimp deseases out-break 1990-1994 such as *monodon baculo virus* (MBV), *yellow head baculo virus (YHB),* white-spot *systemic ectodermal and nesodermal baculo virus (SEMBV), white spoted syndrome virus (WSSV)* (Kokarkin, 1999; Rengpipat *et al*., 2003). The species found of capable with wide salinity tolerance (0.5-40 ppt) *Penaeus monodon* Fab, and make this species found suitable for healthy postlarvae (PL) and thus become excellent candidate for low salinity shrimp culture.

The application of low salinity shrimp farmer at Lampung, Brebes, and Sidoharjo had been using bared padi-fileds. Eventhough, some problems such as moulting-failure, disoriented-swim, slow-growing rate and high mortality were appeared (Lockwood, 1989; Davis *et al*., 2002; Davis and Saoud, 2004). These problems were also encountered in other countries with the same method. Most of those problems were assumed related with the low concentration and imbalance ratio of some important seawater ionic compounds, and affecting the shrimp physiological processes (Lockwood, 1989; Davis *et al*., 2002; Davis and Saoud, 2004; Roy, 2006). The deficiency of main ionic compounds (such as K, Na, Ca^{2+}, Mg^{2+}) had seriously affecting to the survival and the growth of shrimp in the low salinity ponds (Collins *et al*., 2005).

The low concentration of K, Na, Ca^{2+}, Mg^{2+} in the low salinity medium will affect the osmotic imbalance of the shrimp. Evenmore, when this ionic concentration in the diet was also low, will evenmore affecting to the shrimps physiological processes and growth. Davis *et al.* (2005) had proved that low mineral content in the shrimp diet in the low salinity as well as in the high salinity culture ponds had decreased significantly the growth rates of shrimp in the culture. The most significant decrease had happened in the low salinity ponds. According to Saoud *et al*. (2003) and Davis *et al*. (2005) the imbalance of important ionic ratio of K^+ and Mg^{2+} had also limited the growth and survival rate of the shrimp. The earlier findings suggest that addition of important water mineral in the diet of the shrimp with suitable ratio will significantly support the growth and survival of the shrimp in low salinity medium (Davis *et al*., 2002; and Davis *et al*., 2005).

The aim study to know the effect of the K^+ and Mg^{2+} addition and its combination to the weekly growth rate (based on weight) and Osmotic Capacity. The study using addition of mineral in the diet with assumtion that this approach will have more effective effects to the specific growth rate (SGR), Absolute Growth (ΔG), survival rate (SR) and osmotic capability (OC).

MATERIAL AND METHOD

The study was carried out at the Marine Culture Laboratory (BBPBAP), Jepara 2009-2010. The main material is standard shrimp diet with addition of mineral K^+ and Mg^{2+}. Mineral K from KCl, where concentration of K in KCl was 1.47% x 100 gr; and mineral Mg from dolomite CaMg $(CO3)_2$, where concentration of Mg was 0.41% x 100 gr. Combined diet of K=1.21 % x 100 gr and Mg = 0.345% x 100 gr. Total dosage of mineralisez diet was 5% of total shrimp weight.

Design of experiment was Complete Random Design with 3 replicates for each treatment. Treatment A: standard diet added with 1% K (1 gr KCl / 100 gr diet), treatment B: standard diet adde with 1% Mg $^{2+}$ (1 gr dolomite/100 gr diet). Treatment C was a combined of 0.5% K^+ and 0.5% Mg^{2+}, and treatment D was standard diet without mineral (as control). All of mineral suplements were using white egg yolk as bider for the feed pellets.

The main test organisms is post larva of tiger shrimp *Penaeus monodon* Fabricius of 16 days (PL-16) originated from one batch of spawning. Shrimp post larvae had passed through a morphometric, stress test, and PCR test (FAO, 2003), then hold in a fibre tank for four days to become PL-20. Holding tanks for PL-16 was cilider tanks with 130 cm diameter and volume 500 L. Starting salinity of 25 ppt was then decreased to become 5 ppt to be used for the whole experiment. The initial weight of the 20 days post-larvae (PL-20) were ranged 0.0208-0.0212 gr, mean weight 0.021 grams and standard d eviation 0.0001128. The experiment using PVC tanks size: 60 x 32 x 35 cm with 48 litre of seawater and salinity of 5 ppt. Total length of experiment was 44 days (Buckle *et al*., 2006).

Experiment variables were Specific Growth Rate (SGR), Survival-Rate (SR), Absolute Growth (ΔG) and Osmotic Capacity (OC) of the post-larva.

RESULT AND DISCUSSION

The seawater medium variabels during the study are as follows, salinity 5 ppt, temperature 28.7 °C, dissolved oxygen 6.6 ppm, total amonia-nitrogen 0.15 ppm and nitrite nitrogen 0.05 ppm which was regarded as suitable seawater medium for shrimp culture in general. Result of experiment data consist of absolute growth, specific growth rate and osmotic capacity was presented in Table 1.

Result of data analysis using ANOVA revealed that all mineral diet treatment had no significant effect (F cal < F tab; $P > 0.05$) to SGR and ΔG for *Penaeus monodon* cultured in low salinity. Although a combined mineral (K and Mg) treatment C had shows the highest of absolute growth (0.7023 gr) then followed by treatment A (0.6421 gr), B (0.5303 gr), and D (0.4951 gr). The best treatment of C with combination of 0.5% K and 0.5% Mg had gave the highest absolute growth. This result based on the assumption that the mineral combination in the diet had supported the mineral interactions to reduce osmotic stress associated with uniqe environments (Roy, 2006). All treatment had gave a normal growth for the post-larva as indicated with a countinued weight increase during the study as in Table 1. The highest SGR was given by treatment C = 8.03%, A = 7.73%, B = 7.37% and the lowest was treatment D = 7.23% (no mineral suplement). While weekly growth rate data had shown a steady increase of the three mineral diet treatment from week 1 to week 7, where treatment C (combined K and Mg) gave the highest growth rate as in Figure.1**.**

More specific statement that despite the ability of shrimp to tolerate wide range of environmental salinities, farmers still have to adress problems associated with variation of important ionic profiles/ ratio among ponds (Soud *et al*., 2003) which lead to poor growth and survival of the shrimp (Davis *et al.,* 2004). Better shrimp survival and growth were observed when K^+ was added to the culture waters to improve the Na: K or Cl: K ratio (Saoud.IP *et al*., 2007). Levels of K^+, Na^+, Mg^{2+}, and Ca^{2+} in the hepatopancreas were not affected by dietary suplemen of K^+, Mg^{2+} or NaCl in the trials (Roy *et al*., 2007).

The wekly specific growth rate (SGR) had shown a steady decreasing pattern as in Figure 2**,** with the lowest rate in week-5 and increase again in week-6. All mineral suplement in the diet had revealed a significant effect to the osmoregulatory capacity (OC) with (Fcal > F tab and P value < 0.05) with seawater medium variabel as already mentions earlier. All treatments had gave high survival rate (SR) of the *Penaeus monodon* post-larvae where traement A: 95.83%; B: 5.83%; C: 91.67% and control D: 85.50%.

Farmers in West Alabama have been successfully in raising *Litopenaeus vannamei* in inland low salinity waters by raising the K^+ and Mg^{2+} concentration of the pond waters to more ideal levels (Roy *et al*., 2009). McNevin *et al*. 2004 *in* Roy *et al*., (2009) observed increased shrimp production in Alabama low salinity waters by raising the levels of K^+ (6,2 mg L^{-1}) and Mg^{2+} (4,6 mg L^{-1}) to 40 mg L^{-1} and 20 mg L^{-1}. According to Cheng *et al*., 2005 in Roy *et al*., (2009) to obtain an optimum growth in the culture of shrimp in low salinity medium will need range of 2.60-3.46 gr kg^{-1} Mg^{2+} in the diets. The study of Roy *et al*. (2009) had shows that mineral Mg^{2+} suplement in the diets had no significant effect to the growth and survival of *Litopenaeus vannmei* in medium with low Mg^{2+}. While in other cases some authors have reported that dietary mineral supplementation can affect haemolymph osmolality of shrimp (Gong *et al*., 2004 in Roy *et al*., 2009). At low salinity levels, the prawns maintain their osmotic and ionic balance by decreasing the rate of water transport across the gut, as well as the rate sodium (Na) and chloride (Cl) extrusion through the gills (Manik *et al*., 1979 *in* Navas and Sebastian, 1989). The prawns can survive only when the cells are able to tolerate the decreased haemolymph sodium and chloride. An alternative aprroach to counteract low Na: K and Mg: Ca ratios in the water by suplementing K and Mg to the feed, thus allowing the shrimp to absorb these ions from the digestive tract. Supplementation of K and Mg in excess of the dietary requirement has been proposed as a means to improve the osmoregularity capacity of shrimp, thus increasing growth and survival (Gong *et al*., 2004; Cheng *et al*., 2005; Saoud and Davis, 2005; Roy *et al*., 2006; Saoud *et al*., 2007).

The origin of brakish-water or seawater resource used for the experiment was analysied with result as in Table 2 and Table 3. Brakish water-pond water samples content of Ca: 26.16; Na: 1749.8; Na:K = 8.6:1; Mg:K = 0.1:1 while the seawater samples contents are Na^+: 9160; Ca^{2+}: 80; Na:K = 183.20:1; Mg:K = 4.80:1.

Table 1. Absolute Growth (ΔG), Specific Growth Rate (SGR) and Osmoregulatory Capacity (OC) of *Penaues monodon* treated with K and Mg minerals in low salinity medium (5 ppt) at BBBAP Jepara

R	A			B			C			D		
	ΔG	SGR Final	OC	ΔG	SGR Final	OC	ΔG	SGR Final	OC	ΔG	SGR Final	OC
1	0.4348	6.98	508.07	0.5761	7.6	507.05	0.7474	8.2	506.07	0.5812	7.62	509.08
2	0.9162	8.61	508.07	0.3978	6.78	507.04	0.5899	7.66	506.07	0.3888	6.73	509.08
3	0.5753	7.59	508.07	0.617	7.74	507.06	0.7697	8.24	506.07	0.5154	7.35	509.08
X	0.6421	7.73	508.07	0.5303	7.37	507.05	0.7023	8.03	506.07	0.4951	7.23	509.08

Where: R: replicates (3)
Treatment: A: 1 % K; B: 1 % Mg; C: 0.5% K and 0.5% Mg; D: control without mineral suplement;
SGR Final: Ln (Total growth / 44 days); ΔG: absolute growth (Wt – Wo); OC: Osmoregulatory Capacity (haemolymph osmolality) = mOsm L^{-1}

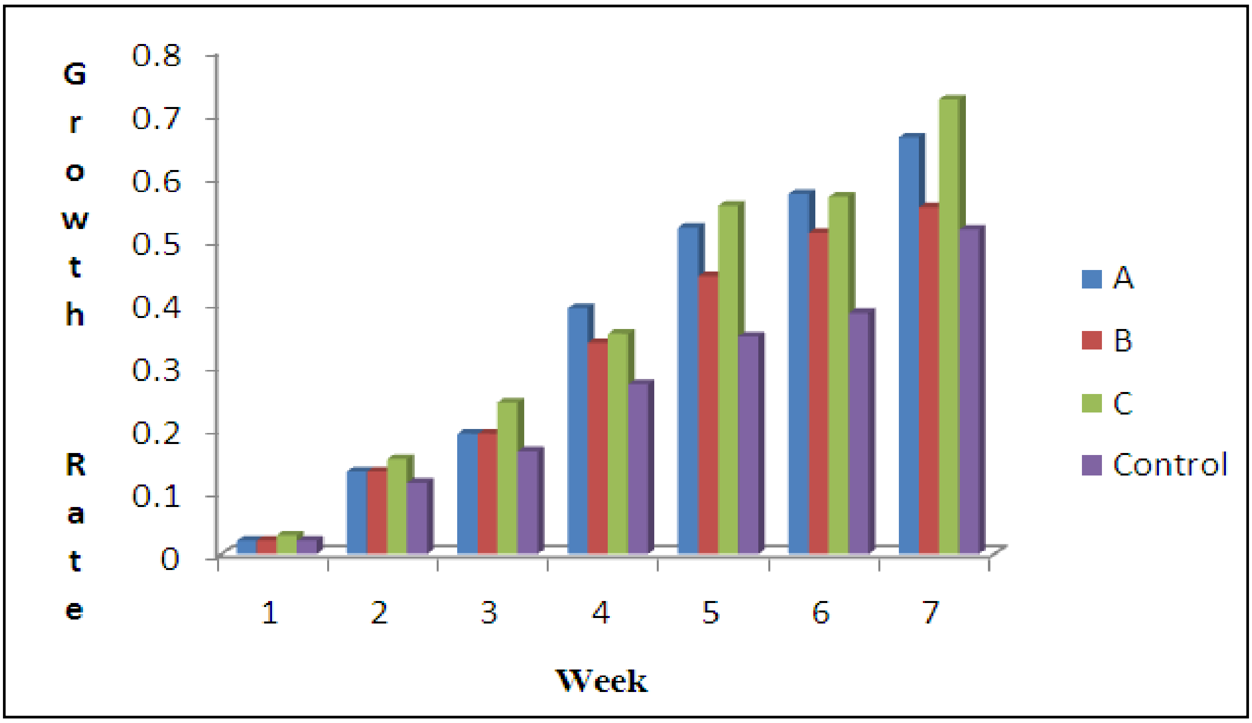

Figure 1. Weekly Accumulated Growth Rate of *Penaeus mondon* with treatments of K and Mg minerals in low salinity medium at BBAP Jepara

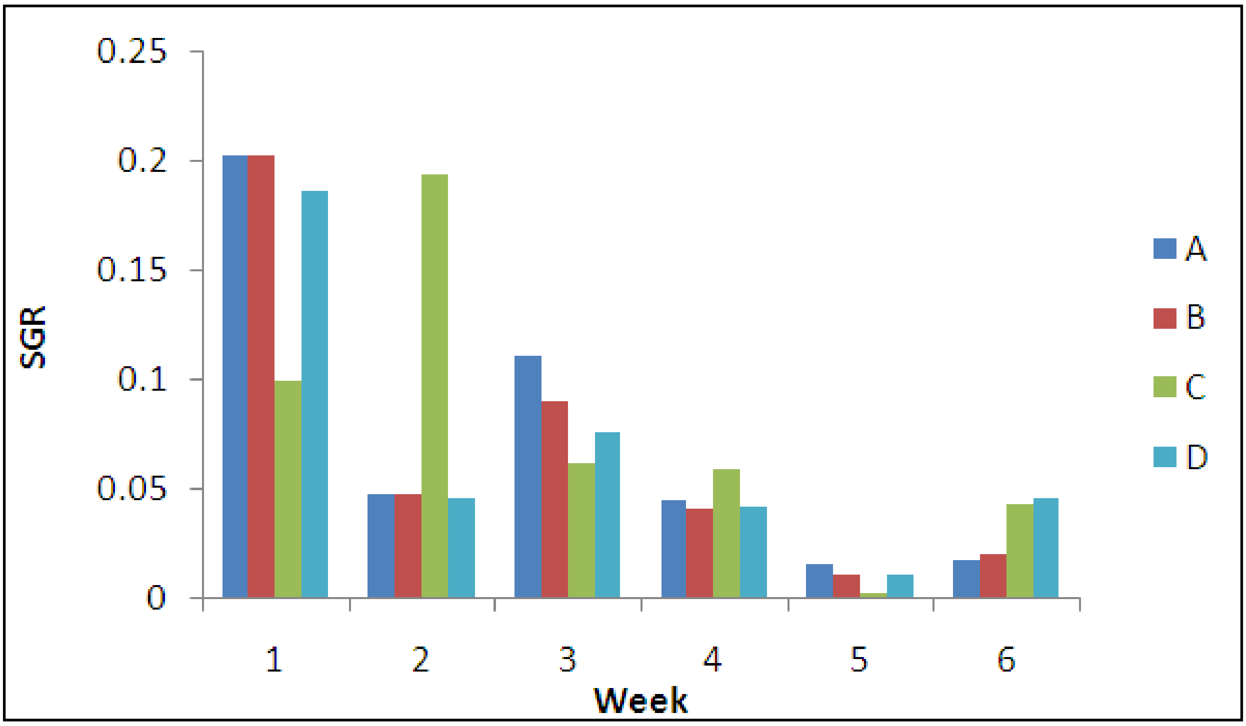

Figure 2. Weekly Specific Growth Rate of *Penaeus mondon* in low salinity medium at BBBAP Jepara

Table 2. Ionic Content (mg L^{-1} atau ppm) in the Brakish Water Ponds in Several Salinity Levels at Jepara *

Ion	4 ‰	8 ‰	12 ‰	16 ‰	20 ‰	25 ‰
Cl	2203	3299	6478	8917	10777	13676
SO4-	253.5	461.8	814.5	1143	1380	1878
PO4+	0.015	0.001	0.001	0.002	0.001	0.001
Ca	**26.16**	32.90	40.37	31.59	33.35	35.75
Mg	21.34	23.99	21.28	22.88	23.03	21.25
Na	**1749.8**	2365.3	2795.3	3119.8	2713.9	3104.0
K	204.5	308.2	345.9	339.5	291.6	455.9
Ratio :						
Na : K	**8.6 : l**	7.67 : 1	8.08 : 1	9.19 : 1	9.30 : 1	6.81 : 1
Mg : K	**0.10 : 1**	7.67 : 1	0.06 : 1	0.07 : 1	0.08 : 1	0.04 : 1
Ca : K	0.13 : 1	0.11 : 1	0.11 : 1	0.09 : 1	0.11 : 1	0.08 : 1
Mg : Ca	0.82 : 1	0.73 : 1	9.52 : 1	0.72 : 1	0.69 : 1	0.04 : 1

*) Analisis in the Lab of Waste Water BBTPPI, Semarang 2009.

Table 3. Ionic Content (mg L^{-1} or ppm) in 5, 15, 25, 35 ‰ seawater samples *

Ion	5 ‰	15 ‰	25 ‰	35 ‰**
Cl^-	2940	9010	15220	19353
Na^+	**9160**	11530	11530	10760
$SO4^{2-}$	---	---	---	2712
Mg^{2+}	240	520	930	1297
Ca^{2+}	**80**	320	860	412
K^+	50	160	400	399
P	0.014	0.014	0.28	--
Ratio :				
Na:K	**183.20 : 1**	72.06 : 1	28.82 : 1	26.97 : 1
Mg:K	**4.80 : 1**	3.25 : 1	2.32 : 1	3.25 : 1
Ca:K	1.60 : 1	2.00 : 1	2.15 : 1	1.03 : 1
Mg:Ca	3.00 : 1	1.62 : 1	2.32 : 1	3.15 : 1

*) Research analysis
**)Parnes *et al.*, (2004) seawater samples with salinity 35 ‰

The result of the mineralanalysis sugest that the important ions ratio of Na^+, K^+, Mg^{2+}, and Ca^{2+} in the sample of the brakish water ponds water in several salinity during the research was shown as in Table 2, which was found very different with the natural seawater. Ionic ratios of the seawater samples as in Table 3 had shown that ratio of Na:K = 8.6:1 in brakish-water sample with salinity of 4 ‰ was found much higher with other ions than those of higher salinity up to salinity of 25 ‰. The ratio of Na : K in the brakish-water ponds was 8.6 : 1, and Na : K in the seawater samples was 183.20 : 1. The difference of Na : K ratio in the brakish-water ponds and the natural seawater samples was then assumed as the limiting factor for the osmoregulatory capacity (OC) of the shrimp culture.

Based on the equilibrium state of seawaters (Castro and Huber, 2005) ratio of important ions of seawater should be always remaining the same even with different salinity. Ratio of Na : K with range from 25.9 : 1 to 27.0 : 1. Measurements of ionic ratios of brakish water ponds in different salinities Table 2 shows its difference with sample of seawater (Table 3). This was assumed as the indicator that water medium from brakish-water ponds was extreemly lower than the seawater sample and thus not as ideal for shrimp culture.

Mantel and Farmer (1983) and Pequeux (1995) had reviewed that osmoregulation is one of important physiological factor for aquatic organisms. Osmoregulation in aquaculture can be used as eraly indicators for water medium quality (Lignot *et al*., 2000 in Buckle *et al*., 2006). O*smoregulatory capacity* (OC) constitute as the difference / thresshold of hemolymph osmotic with the external medium (Charmenter *et al*., 1989 in Buckle *et al*., 2006). Some research eralier had reveale that with different of shrimp species had a different effect on moulting phase, where the isosmotic value of *Litopenaeus vannamei* was 717-823 mmol kg^{-1} (Chen *et al*., 1995). In the juvenile of *Fenneropenaeus chinensis* conclude that hemolymph osmolarity will increase due to the increase of the water and hemolymph osmolarity will decrease with the decrease of the water temperature, but this will made a different effect with *L. vannamei*. The culture of shrimp in low-salinity inland waters is increasing with considerable succes, but a problem of growth rate apprears to be associated with suboprimal ionic ratios of the water and the ability of the shrimp to osmoregulate at a given life stage (Davis and Saoud, 2004).

CONCLUSION

The study revealed that all mineral diet treatment had no significant effect (F cal < F tab; P > 0.05) to SGR and ΔG for *Penaeus monodon* cultured in low salinity. Although a combined mineral (K and Mg) treatment C had shows the highest of absolute growth (0.7023 gr) then followed by treatment A (0.6421 gr), B (0.5303 gr), and D (0.4951 gr). The best treatmnet of C with combination of 0.5% K and 0.5% Mg had gave the highest absolute growth. The wekly specific growth rate (SGR) had shown a steady decreasing pattern**,** with the lowest rate in week-5 and increase again in week-6. All mineral suplement in the diet had revealed a significant effect to the osmoregulatory capacity (OC) with (Fcal > F tab and P value < 0.05) with seawater medium variabel as already mentions earlier. All treatments had gave high survival rate (SR) of the *Penaeus monodon* post-larvae where traement A: 95.83%; B: 5.83%; C: 91.67% and control D: 85.50 %.

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REFERENCES

Buckle, F., B. Baron and M. Hermandez. 2006. Osmoregulatory Capacity of the Shrimp *Litopenaeus vannamei* at Defferent Temperature and Salinities, and Optimal Culture Environment. 54 (3): 745-753.

Castro, P., and M.E. Huber. 2005. Marine Biology. 5th ed. Pp. 4-144. McGraw-Hill International Edition. Singapore.

Chen, J.Ch., Nan, F.H.1995. Oxygen consumption and ammoniaN excretion of *Penaeus chinensis* (Osbeck,1765) juveniles at different salinity levels (Decapoda: Penaeidae). *Crustaceana* 68 (6), 712-719.

Cheng, K.M., Hu, C.Q., Liu, Y.N., Zheng, S.X.,Qi, J. 2005. Dietary magnesium requirement and physiological responses of marine shrimp *Litopenaeus vanamei* reared in low salinity water. *Aquacult. Num*. 11, 385-393.

Collins, A., B. Russell, A. Walls, and T. Hoang. 2005. Inland Prawn Farming: studies into the potensial for inland marine prawn farming in Queensland. Queensland Government. Dept. of. Primary Industries and Fisheries. 79 p.

Davis, D.A, I.P. Saoud, W.J. McGraw, and D.B. Rouse. 2002. Consideration for *Litopenaeus vannamei* Reared in Inland Low Salinity Waters. Pp. 73-90. *In*: Cruz-Suarez, L.E. Ricque-Marie, D. Tapia-Salazar, M. Gaxiola-Corte4s, and M.G. Simoes, N (Eds.). Avances en nutricion acuicola VI. Memorias del VI Simposium International de Nutricion Acuicola. 3 al 6 de Septiembre del 2002.Cancun, Quintana Roo, Mexico.

Davis, D.A., I.P. Saoud. 2004. Nutritional Considerations for Pacific White Shrimp Reared in Inland, Low-Salinity Waters. Global Aquaculture Advocate. 2 p.

Davis, D.A., C.E. Boyd, D.B. Rouse, I.P. Saoud. 2005. Effects of Potassium, Magnesium and Age on Growth and Survival of *Litopenaeus vannamei* Post-larvae Reared in Inland Low Salinity Well Waters in West Alabama. *In*

Daniels, H., P. Edwards, K. Brownlee and L. shewmon (Eds.) *Journal of the World Aquaculture Society*. 36(3); 417-424.

FAO. 2003. Health Management and Biosecurity Maintenance in white shrimp (*Penaeus vannamei*) Hatcheries in Latin America. FAO Fisheries Technical Paper no. 450. Rome. 66 p.

Gong H., Jiang D.H., Lightner, D.V.,Collins, C., Brock, D. 2004. A dictary modification apptoach to improve the osmoregulatory capacity of Litopenaeus vannamei cultured in the Arizona desert. *Aquacult Num*. 10, 227-236.

Kokarkin, C. 1999. Meningkatkan keberhasilan budidaya udang untuk masa mendatang. BPAP. Jepara. 15 p.

Lockwood, A.P.M. 1989. Aspect of the Physiology of Crustacea. W.H. Freeman and Company. San Francisco. 315 p.

Mantel, L.H., and L.L. Farmer. 1983. Osmotic and Ionic Regulation. Pp. 27-162. *In* Mantel, L.H. (Eds.). The Biology of Crustacea. Vol. 5 : Internal Anatomy and Physiological Regulation. Academicc Press, Inc. Tokyo.

Parnes, S., E. Mills, C. Segall, S. Raviv, C. Davis, A. Sagi. 2004. Reproductive Readiness of the Shrimp *Litopenaeus vannamei* Grown in Brackish Water System. Aquaculture xx(2004) xxx-xxx. Inpress. 14 p. Aquaculture. www.elsevier.com/locate/aqua-online. Available at 31 Maret 2008.

Pequeux. 1995. Osmotic regulation in crustaceans. J. Crustacean Biot 15, 1-60.

Roy, L.A. 2006. Physiological and Nutritional Requirements for the Culture of the Pacific White Shrimp *Litopenaeus vannamei* in Low Salinity Waters. Dissertation. The Graduate Faculty of Auburn University. Auburn. 126 p.

Roy, LA, DA Davis, IP Saoud, RP Henry. 2007. Supplementation of potassium, magnesium and sodium chloride in practical diets for the Pacific white shrimp *Litopenaeus vannamei* reared in low salinity waters. *Aquaculture Nutrition*. 13, 104-113.

Saoud, I.P., D.A. Davis, and D.B. Rouse. 2003. Suitability Studies of Inland Well Waters for *Litopenaeus vannamei* Culture. Aquaculture 217: 373-383. www.elsevier.com/locate/aqua-online. Available at Maret 2008.

Saoud, I.P. Davis, D.A. 2005. Effects of betaine supplementation to feeds of *Litopenaeus vannamei* reared et extreme salinities. *N. Am. J. Aquacult.* 67, 351-353.

Saoud IP, LA. Roy, DADavis. 2007. Chelated Potassium and Arginie Supplementation in Diets of Pacific White Shrimp Reared in Low-salinity Waters of West Alabama. North American *Journal of Aquaculture*. 69 : 265-270