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PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 18 (4): 173-178, 2015
ISSN 1028-8880


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RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/pjbs.2015.173.178

# Effects of Artificial Shelter and Chitosan on Growth Performance of Freshwater Prawn (*Macrobrachium lanchesteri* de Man, 1911)

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ARTICLE INFO
*Article History:*
Received: June 10, 2015
Accepted: July 08, 2015

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ABSTRACT

Two experiments were carried out at the Department of Fisheries, Khon Kaen University, Khon Kaen, Thailand during September 2013 to February 2014 aiming to pursue both the better growth performance and survival rate of the freshwater prawns (*Macrobrachium lanchesteri*) where a Completely Randomized Design (CRD) with three replications was used for each experiment. For experiment 1, different percentages of grass shelter were used, i.e. 0% (Control, T1), 25% (T2), 50% (T3) and 75% (T4). For experiment 2, different percentages of commercial chitosan were used, i.e. 0% (Control, T1), 25% (T2), 50% (T3) and 75% (T4). Both experiments were carried out at the same time and each of them was lasted for 75 days. The results showed that the added grass shelter gave significantly higher Total Weight (TW) and Survival Rate (SR) than the control treatment. However, there were no significant differences found on TW and SR among the added grass shelter treatments (T2 up to T4). Chitosan gave significantly higher TW, AW and Number of Molting (NM) than the control treatment. There were no significant differences on TW, NM and AW found amongst the chitosan treatments. The results of the experiment 1 showed that the best rate of grass shelter for the culturing of the *Macrobrachium lanchesteri* was at a rate of 25% (T 2) and the best rate of the chitosan was at a rate of 4% (Diet 3) for the Experiment 2.

**Key words:** Chitosan, grass shelter, *Macrobrachium lanchesteri*, shelter area

## INTRODUCTION

*Macrobrachium lanchesteri* is one of the most important species of freshwater prawns. They commonly inhabited in wetland areas of many countries, especially in the Southeast Asian countries such as Thailand, Malaysia, Singapore and others (Holthuis and Rosa, 1965). This type of prawns is commonly found in most rice-paddy fields, particularly in the rainy season. The prawn could rapidly multiply its population under any static freshwater (Johnson, 1968). This type of aquatic living creature is considered as an important source of protein for the local inhabitants of many Southeast Asian countries, especially in Thailand. Therefore, this freshwater prawn species has its significant role in supplying protein for human consumption hence, some further utmost development related to this respect is of a tangible value. In addition, *M. lanchesteri* plays an important role in terms of both local and international food trading supplies and also as live food for aquarium and cultured fish as well as the use in scientific research pertaining to knowledge upon biochemistry and physiology of prawn reproduction (Suckcharoen, 1980; Chong and Khoo, 1988).

Prawns are mainly benthic animals as a result their growth depended heavily on the availability of two-dimensional area rather than a three-dimensional volume in the aquaculture system (Tidwell *et al.*, 1998). This limitation on production is exacerbated by the territorial nature of the prawns (Cohen *et al.*, 1981). Therefore, an increase in the surface area in the aquatic system may result in an increase in the amount of prawn production (Tidwell *et al.*, 1998). Most of the territorial prawns have confined its populations under the benthic area in the ponds without any supplementation of substrates. This causes an increase in total weight of the prawns and the corresponding amount of available bottom area per unit of body weight decreased. As a result, growth rates of the prawns decrease and mean individual weights at any harvest can be relatively low, particularly in the ponds with comparatively higher densities of the prawns (Tuly *et al.*, 2014). If the provision on the substrates of a pond can be changed from a two-dimensional area to a three-dimensional area, hence it could provide a greater area for the prawns (D'Abramo *et al.*, 2006). Several studies have demonstrated the benefits derived from the adding amount of different artificial substrates to the production units as to increase its available surface area (Keshavanath *et al.*, 2001; Mamun *et al.*, 2010; Tidwell *et al.*, 1998). Cohen *et al.* (1983) reported that when substrate was provided into the ponds then it increased the prawn production by 14% with an increase in the average body size by 13%. Tidwell *et al.* (2000) showed that the prawns supplemented with substrate attained 33% larger than without and also with an increase of 24% of the total yield compared with those without.

Chitosan is a linear homopolymer of b-(1, 4)-2-amino-2-deoxy-D-glucose and is prepared by the alkaline deacetylation of chitin derived from shrimp and crab shells. Its contents included the natural biological properties such as immunological function and bacterio-static activity and these properties are valuable for both plant and animal applications (Rinaudo, 2006). Chitosan has a variety of applications in medicine, agriculture and aquaculture (Niu *et al.*, 2011). In aquaculture, it was used as an immunostimulant to protect salmonids against bacterial disease (Anderson and Siwicki, 1994; Siwicki *et al.*, 1994) and also in gilthead sea bream, *Sparus aurata* (Esteban *et al.*, 2000; Esteban *et al.*, 2001; Ortuno *et al.*, 2000; Cuesta *et al.*, 2003). However, the effects of dietary chitin and chitosan on Crustacea are not well justified (Niu *et al.*, 2011) and also along with the effects of chitosan on *Macrobrachium lanchesteri* have not been widely evaluated. Therefore, the objective of this investigation was to evaluate the effects of different levels of chitosan and grass shelter applications on growth performance of the *M. lanchesteri*. The acquired results may be of tangible value for growers of prawns, particularly for those growers in Thailand.

## MATERIALS AND METHODS

This study was carried out at the Department of Fisheries, Faculty of Agriculture, Khon Kaen University during September 2013 to February 2014. The work consisted of two experiments, i.e. experiment 1 concerned with different levels of artificial grass shelter diet, experiment 2 on the formulation and proximate composition of the experimental diets include the use of different ingredients such as fish meal, rice bran, rice flour, binder, fish oil, vegetable oil, lard oil, vitamins and etc. (Table 1 and 2). In general, basal diet normally contained fish meal, rice bran and flour as sources of proteins and carbohydrates. Fish oils, vegetable and lard are used as sources of lipids. The use of basal diets contained approximately 35% protein and 4% lipids have been proven to be optimal for a rapid growth of prawns of the *Macrobrachium lanchesteri* (Suriya, 2004-unpublished data). For experiment 1, different percentages of artificial grass shelter were added to the cultured system i.e. 0% (Control, T1), 25% (T2), 50% (T3) and 75% (T4). With experiment 2, a commercial chitosan was supplemented to the basal diets at different concentrations i.e. 0% (Control, Diet 1), 2% (Diet 2), 4% (Diet 3) and 6% (Diet 4). All ingredients were milled into powder to pass through a 320 mm mesh screen and were thoroughly mixed with oils and then added with a certain amount of water as to produce stiff dough. The dough was then used to pass through a mincer to form pellets. The pellets were finally dried in an oven at 50°C to attain a moisture content of approximately 10% and then the pellets were kept in a fridge at 4°C, hence the pellets were ready for experimental uses.

Table 1: Formulation and proximate composition of the ingredients with its required amount for use in experiment 1 (% on dry matter basis)

| Ingredients | Amount used (%) |
|---|---|
| Fish meal | 58.0 |
| Rice bran | 16.7 |
| Rice flour | 9.0 |
| Binder (α-starch) | 0.0 |
| Fish oil | 0.8 |
| Vegetable oil | 1.0 |
| Lard oil | 0.2 |
| Vitamin and trace minerals[1] | 4.0 |
| Vitamin C | 0.1 |
| Choline chloride | 0.2 |
| **Proximate composition** | |
| Protein | 35.59 |
| Fat | 4.44 |
| Fiber | 13.86 |
| Ash | 13.69 |
| Total | 100.00 |

[1]Vitamin and trace minerals (mg/1,000 g of diet): Vitamin comprises of vitamin A: 4,000 IU, Vitamin $D_3$: 2,000 IU, Vitamin E: 50 mg, Vitamin K: 10 mg, Thiamine: 20 mg, Riboflavin: 20 mg, Pyridoxine: 20 mg, Calcium pantothenate 200 mg, Niacin: 150 mg, Biotin: 2.0 mg, Folic acid: 5 mg, vitamin $B_{12}$ 0.2 mg, Inositol: 400 mg and Ethoxyquin: 200 mg, Trace minerals comprise of iron: 30 mg, Zinc :20 mg, Manganese: 25 mg, Copper: 5 mg, Cobalt: 0.05 mg, Iodine: 5 mg and Selenium: 0.2 mg

**Experimental preparations:** The prawns of *Macrobrachium lanchesteri* of a similar size being used in this study were those being bred under natural breeding conditions. Both matured male and female prawns were allowed to grow for three months in a finishing-cultured earth pond with an area of 400 $m^2$. Prior to the experimental work, 100 female prawns those reached a stage 5 of matured eggs were chosen and distributed into a plastic cage attached to the concrete tanks. The tank has a dimension of 260×220×50 $cm^3$. The tank was regularly aerated and it was fixed with natural sub-sanded filter water system. Within 2-5 days of culturing, the prawn's larvae were hatched and cultured in the concrete tank for

Table 2: Formulation and the proximate composition of the ingredients for the diets 1-4 being used in experiment 2 (% dry matter basis)

| | Amount used (%) | | | |
|---|---|---|---|---|
| Ingredients | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
| Chitosan | 0 | 2 | 4 | 6 |
| Fish meal | 28 | 28 | 28 | 28 |
| Soybean meal | 12 | 12 | 12 | 12 |
| Squid meal | 5 | 5 | 5 | 5 |
| Shrimp head meal | 5 | 5 | 5 | 5 |
| Broken rice | 18 | 18 | 18 | 18 |
| Wheat flour | 12 | 12 | 12 | 12 |
| Wheat gluten | 6 | 6 | 6 | 6 |
| Yeast (*Saccharomyces cerevisiae*) | 2 | 2 | 2 | 2 |
| Tuna oil | 1 | 1 | 1 | 1 |
| Soybean oil | 1 | 1 | 1 | 1 |
| Lecithin | 1 | 1 | 1 | 1 |
| Premix | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace minerals[2] | 0.05 | 0.05 | 0.05 | 0.05 |
| Spirulina | 0.1 | 0.1 | 0.1 | 0.1 |
| Rice husk | 8.6 | 6.6 | 4.6 | 2.6 |
| **Proximate composition** | | | | |
| Protein | 36.72 | 36.96 | 37.29 | 38.97 |
| Fat | 4.07 | 4.15 | 4.44 | 4.6 |
| Fiber | 6.02 | 5.49 | 5.08 | 2.31 |
| Ash | 6.78 | 7.41 | 7.87 | 9.91 |

[2]Vitamin and trace minerals (mg/1,000 g of diet): Vitamin comprises of vitamin A: 4,000 IU, Vitamin $D_3$ :2,000 IU, Vitamin E: 50 mg, Vitamin K: 10 mg, Thiamine : 20 mg, Riboflavin : 20 mg, Pyridoxine: 20 mg, Calcium pantothenate: 200 mg, Niacin: 150 mg, Biotin: 2.0 mg, Folic acid: 5 mg, Vitamin $B_{12}$: 0.2 mg, inositol 400 mg and Ethoxyquin: 200 mg, Trace minerals comprise, Iron: 30 mg, Zinc: 20 mg, Manganese: 25 mg, Copper: 5 mg, Cobalt: 0.05 mg, Iodine: 5 mg and Selenium: 0.2 mg

23 days. The larvae were fed with different kinds of food where appropriate i.e., for the first ten days, they were fed with *Chlorella* algae at a concentration of a $2\times10^6$ cell $mL^{-1}$ and red yolk boiled- egg then followed with the use of red yolk boiled-egg plus a small size of *Moina* up to 23 days. From day 24-26, the shrimps were fed under control diet where the diet contains 35% protein.

**Experimental design:** The post-larvae of the *Macrobrachium lanchesteri* from the prepared tank were used for this study. For both experiment 1 and experiment 2, 2,400 individual prawns of a similar size with average weights ranged from 1.7-2.2 g were allocated into the 24 plastic tanks (150 individual prawns for each replicated tank, 12 tanks for the first experiment and another 12 tanks for the second experiment) and each tank had a capacity of 10 L. The experimental design used for both experiments was a Completely Randomized Design (CRD) with three replications. Throughout the experimental period, the diets were hand-fed to the prawns *ad libitum* twice a day i.e., at 8:00 am and 4 pm. The water temperature was maintained at a range between 24-27°C and pH values were within a range from 6.9-7.5. The dissolved oxygen was maintained no less than 6.7 mg $L^{-1}$. Both experiments were carried out for 75 days. Water in each tank was discarded away approximately 30% and immediately replaced with the same volume of cleaned water once a week.

**Measurements on growth:** At the end of the feeding trial, the prawns from each tank were bulk weighed for live weights. The growth performance and survival rate of the *Macrobrachium lanchesteri* were calculated with the use of the following measurement parameters and calculations, i.e.:

Total Weight (TW) = final prawn weight per tank

Average Weight (AW) = TW/SN (SN = survival number)
Survival number (SN) = Final prawn number per tank

Percentage of survival rate (SR%) = SN/150×100

**Statistical analysis:** The attained data was statistically analyzed where appropriate using a computer program (SAS, 1998). The Duncan's Multiple Range Test (DMRT) was used for least significant differences among the treatments used.

## RESULTS

For the first experiment, the results showed that mean values of total live weights of the prawns increased with an increase in the percentages of artificial grass shelter with mean values ranged from 12.55-18.67 g for T1 and T4, respectively (Table 3). All artificial grass shelter treatments gave significantly higher total live weights than the control treatment (T1). There was no significant difference found among the treated treatments (T2-T4). With the results on the average weights (individual), the average live weights were similar in all treatments with a range from 0.21-0.22 g for T2 and T4, respectively. For survival numbers, there was no statistical difference found among the treated prawns of T2 up

Table 3: Total weights AW, average weights, survival numbers and survival rate of the *Macrobrachium lanchesteri* as influenced by different levels of artificial grass shelter added to the cultured tanks

| Parameters | T1 (control) | T2 | T3 | T4 |
|---|---|---|---|---|
| TW (g/tank) | 12.55[b] | 17.73[a] | 17.99[a] | 18.67[a] |
| AW (g/individual) | 0.22[a] | 0.21[a] | 0.21[a] | 0.22[a] |
| SN (individuals/tank) | 56.25[b] | 81.00[a] | 82.25[a] | 83.25[a] |
| SR (%) | 37.50[b] | 54.00[a] | 54.83[a] | 55.50[a] |

Letters within rows indicated least significant differences of means of Duncan's Multiple Range Test (DMRT) at probability (p) of 0.05, TW: Total weight, AW: Average: weight, SN: Survival number, SR: Survival rate

Table 4: Total weights, average weights, survival numbers, survival rate and numbers of molts (numbers of molts or shell crusts) of the *Macrobrachium lanchesteri* as influenced by different levels of chitosan% added to the Diets 1 up to 4

| Diets growth | Diet 1(0%) | Diet 2 (2%) | Diet 3 (4%) | Diet 4 (6%) |
|---|---|---|---|---|
| TW (g/tank) | 17.79[c] | 18.68[bc] | 20.73[ab] | 22.11[a] |
| AW (g/individual) | 0.22[b] | 0.23[a] | 0.23[a] | 0.23[a] |
| SN (individual/tank) | 83[a] | 81.75[a] | 92.00[a] | 95.25[a] |
| SR (%) | 55.33[a] | 54.50[a] | 61.33[a] | 63.50[a] |
| NM | 29.00[b] | 38.00[a] | 40.00[a] | 37.00[a] |

Letters within rows indicated least significant differences of means of Duncan's Multiple Range Test (DMRT) at probability (p) of 0.05, TW:Total weight, AW: Average: weight, SN: Survival number, SR: Survival rate

to T4 but significantly higher than the control treatment (T1) with mean values range from 56.25-83.25 individual prawns. A similar trend as that of the survival numbers was found, i.e., there was no statistical difference found among the treated treatments (T2-T4) but the treated treatments gave significantly higher survival rates than the control treatment (T1) with mean values ranged from 37.50-55.50% for T1 and T4, respectively.

With the second experiment, the results showed that Total Weight (TW) of individual prawn increased with an increase in the chitosan concentrations in the diets with mean values ranged from 17.79-22.11 g for Diet 1 and 4, respectively (Table 4). For average weight (AW, g prawn$^{-1}$), an increase in the chitosan concentration did not increase average weight of the prawns but those treatments with the added chitosan (Diet 2 to 4) gave significantly higher average weights than the control treatment (Diet 1) with mean values ranged from 0.22-0.23 g for Diet 1 and 4, respectively. With Survival Number (SN), the results showed that there were no significant differences found among the four treatments used where they gave mean values ranged from 81.75-95.25 for Diet 2 and 4, respectively. A similar result as that of the SN was found with the survival rate (SR%), i.e. an increase in the chitosan concentration did not increase SR% of the prawns with mean values ranged from 54.50-63.50% for Diet 2 and 4, respectively. The results on number of molts (shell crust) showed that there were significant differences found among the added chitosan treatments of Diet 2 up to 4 but all of the added chitosan diets treatments gave significantly higher shell crusts than the control treatment (Diet 1).

## DISCUSSION

Several studies have demonstrated that juvenile prawns cultured under isolated social have grown more evenly than those being raised in groups (Ra'anan and Cohen, 1984). The studies also indicated that natural materials such as small dried tree branches, aquatic plants, gravels and shells can be introduced in to juvenile rearing tanks to reduce any aggressive interactions and the possible refuges for molting prawns (Ling, 1969; Tidwell *et al*., 2000; Tidwell and Coyle, 2008). In the present study, grass was used as artificial shelter added into the tanks for the rapid growth of the *Macrobrachium lanchesteri* prawns. The result indicated that total weight and survival rate were significant higher for those prawns added with a 25% of the artificial grass. The results were similar to the results attained from the previous studies on freshwater prawns of the *Macrobrachium rosenbergii* as reported by Tidwell *et al.* (1998), Tidwell and Coyle (2008) and Tuly *et al.* (2014). However, the results were not similar to the results derived from the previous studies on the *M. rosenbergii* of Tidwell *et al.* (2000). The difference may be attributable to the differences in species, sizes, experimental conditions and diets. The significant improvement on growth performance of the prawns may be due to the reduction in antagonistic interactions amongst the prawns where they have been shown to reduce stress, improved growth and thereby improved feed conversion efficiency (Karplus *et al*., 1992). In aquaculture systems without substrates, the territorial prawns may have mostly confined themselves at the bottom area of the pond, hence as the growing season progresses, the total weight of the prawns in the pond increases and the corresponding amount of the available bottom area per unit of body weight decreases. As a result, the growth rate decreases resulting in the low production of the prawns (Tuly *et al*., 2014). From these data, it appears that the primary benefit of the substrate is to provide the prawns an ability to physically separate themselves from each other, thus reducing prawn-prawn interactions and stresses.

The effects due to chitin and chitosan on growth performance of any aquatic animals are somewhat controversial (Niu *et al*., 2013). Several studies have proven some benefitted effects of the dietary chitosan on growth performance and immunity of the aquatic (Niu *et al*., 2011; Niu *et al*., 2013; Wang and Chen, 2005). Niu *et al.* (2011) reported that medium chitosan level gave benefitted effects on the growth and the survival of the *Litopenaeus vannamei* and the optimum supplement dietary of the chitosan level should be in a range between 2.13 and 2.67 g kg$^{-1}$ diet. However, Fox (1993) showed that chitin was not directly utilized by the *P. monodon*. In the present study, dietary supplementation of the chitosan had significantly higher Total Weight (TW), Average Weight (AW) and number of prawn crust than the control. No significant difference found on the TW and AW between dietary administrations of the chitosan. The highest values of the TW, AW and number of molting prawns were observed in prawns fed with 4% chitosan (Diet 3). Generally, chitosan is an active compound that was extracted from shell of crustacean, it is known as the vital component for the growth of any aquatic animals (Niu *et al.*, 2011). Therefore, the administration with the use of chitosan in the diet at a medium concentration could have been taken place as a part in the biosynthesis of the organism at a rapid speed (Niu *et al.*, 2011). This may positively improve the shrimp growth by increasing its frequency in molting and enhancing both the digestion and absorption of nutrition at a moderate level, in returns, this benefit may have increased the growth performance of the shrimps (Wang and Chen, 2005). Nevertheless, it was found that deficiency in the amount of chitosan in the diet did not show any effect on growth. This may imply that chitosan is essential for shrimp development, yet the administration level used in the diets was limited. With the present work, the results indicated that shrimps fed with the chitosan significantly improved growth performance. The results confirm the previous studies on the *Litopenaeus vannamei* (Niu *et al.*, 2011) and *Penaeus monodon* (Niu *et al.*, 2013). However, the results did not confirm the results of the previous work on *P. monodon* (Shiau and Yu, 1998). The reason for the differences in this context is not clear. It may be attributable to the differences in the development stages, species, diets and experimental conditions. Chitosan supplemented diet was also reported to enhance the growth of fish such as olive flounder, *Paralichthys olivaceus* (Cha *et al.*, 2008) and common carp, *Cyprinus carpio* (Gopalakannan and Arul, 2006). These studies were similar to the results of the present study although the two different species were used (fish versus shrimp). It has been demonstrated that fish can consume the chitosan in the diet due to the presence of a high chitinase activity in the stomachs (Cha *et al.*, 2008). The chitosan with its properties could combine strongly with metallic ionsor proteins, so its supplementation in the diet could consequence impede the digestion or absorption in some fishes those had no chitosanase activity (Niu *et al.*, 2011). The improvement on growth performance of the shrimps in the present study may be due to the presence of the chitosanase activities in stomachs of the *Macrobrachium lanchesteri*. However, some further investigations are needed in order to clarify this assumption.

## CONCLUSION

The use of grass as substrates significantly increased both total live weights and survival rates of the cultured prawns compared with the control treatment. Grass substrates could possibly have helped in reducing stress and improved feed conversion efficiency amongst the cultured prawns. The use of chitosan significantly improved growth performance of the shrimps but not the prawns.

## ACKNOWLEDGMENTS

The authors wish to express their thankful gratitude to the Faculty of Graduate Schools of Khon Kaen University for financial assistance, the Khon Kaen Inland Fisheries Research and Development Center, Dr. Pissamai Somsueb, Fish Nutrition Institute, Inland Fisheries Research Development Division, Department of Fisheries, Ministry of Agriculture and Cooperatives for help in using laboratory facilities and finally staff members of the Department of Fisheries, Faculty of Agriculture, Khon Kaen University for their kind assistance.

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