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Effects of Chitosan Levels on Growth Performance, Feed Utilization and Survival Rate of Rice-field Crab (*Esanthelphusa dugasti*)

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Two experiments were conducted at Khon Kaen University, Nong Khai campus, Thailand during May to December 2015 to justify effects of chitosan of Experiment 1 on growth performance of rice-field crabs. The Experiment 1 had five chitosan treatments, i.e. 0, 1, 2, 3 and 4 % (on dry matter basis). Experiment 2 had two treatments, i.e. the best treatment of Experiment 1 (T3) was chosen in comparison with flesh fish meat treatment. A Completely Randomized Design (CRD) with four replications was used for Experiments 1 and 2. The results of the Experiment 1 showed that a chitosan level of 2 % of T3 gave significantly ($p < 0.05$) better growth performance on final weight gained, weight gained, average daily growth rate, specific growth rate and survival rate (%) of the rice-field crabs than the rest, except that of the feed conversion ratio where T1 (control) was the highest. Thus a chitosan level of 2 % (T3) in the feed diet is recommended for rice-field crab culture. With the Experiment 2, the feed diet of T3 of Experiment 1, i.e. with 2 % chitosan gave significantly ($p < 0.05$) higher survival rate (%) of rice-field crabs than the flesh fish meat of T1.

Keywords: Chitosan, *Esanthelphusa dugasti*, Rice-field crab, growth performance.

Rice-field crab (*Esanthelphusa dugasti*) is a freshwater crab of significant economic value since its fully developed body when matured is popularly used as an edible protein food, particularly for the people in northeastern region of Thailand[1]. This type of rice-field crabs is commonly distributed in most paddy fields in many Asian countries, e.g., India, Myanmar, Cambodia, Laos, Vietnam and Thailand[2-7].In Thailand, this type of crabs can be found in all regions of the country, particularly in paddy rice fields in northeastern region especially in the rainy season. This aquatic creature is widely accepted as an important source of protein for daily diets of the people in the region. Thus there is an urgent need to increase the production to some considerable extent in order to meet a high demand of domestic markets. Nowadays, the population of rice-field crabs tends to decrease more rapidly with time due to many reasons. One of them is an increase in modern agriculture. That is some huge amounts of herbicide and insecticide have been used in growing the crops, particularly in paddy rice fields. Therefore, it may be possible that this crab species may reach its extinction eventually. Even though some of them may have built up its high resistance to hazardous chemicals but still they are already contaminated with chemicals. This circumstance may create problem on health sanitation of the people. It is known that this crab species is recognized as a secondary intermediate host of *Paragonimus* genus (lung fluke) for mammals, including human beings

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as the final host. Thus consuming poorly cooked crabs containing metacercariae of *Paragonimus* spp. could undoubtedly harmful to health[8-10]. Therefore, culturing the crabs under aquaculture technique for human consumption could avoid contaminations of hazardous chemicals. In crab culture, a successful achievement may not only rely on good brood stock and suitable environment but also it may be depended on quality of feed diets. However, publication on this particular creature is relatively limited.

The use of chitosan chemical in feed diets in aquaculture has been reported to attain some certain achievements since this linear homopolymer of b-(1, 4)-2-amino-2deoxy-D-glucose was used by a number of workers, e.g. on plant and animal applications[11], on applications in medicine[12], agriculture and aquaculture. In aquaculture, it is utilized as an immunostimulant to protect salmonids against bacterial disease (Brook trout*, Salvelinus fontinalis*, against *Aeromonas salmonicida*[13], Rainbow trout, *Oncorhynchus mykiss*, against *A. salmonicida* and *Vibrio anguillarum*[14]), augmenting the respiratory burst and phagocytic activities in the gilthead sea bream (Esteban *et al*.[15,16], Ortuno *et al*.[17], 2000; Cuesta *et al*.[18]), immersion and dietary supplements (yellowtail, *Seriola quinqueradiata,* against *Pasteurella piscicida*[19]). Nevertheless, Niu *et al.*[12] stated that the effects of dietary chitosan are not well justified and the use of chitosan in feed diet of crustacean is relatively limited. Therefore, there is an urgent need to carry out more experiments on rice-field crabs with the use of different chitosan levels (%) in feed diets in order to justify the best diet formula and the best formula can be used for further work as to compare the results with the use of fish meat in place of ration diet. This is to provide adequate information for growers of rice-field crabs for the utmost economic benefit. The objectives of this investigation include an evaluation on the effects of different levels of chitosan in combination with feed diets on growth performance of the rice-field crabs (*Esanthelphusa dugasti*). The best feeding formula will be used in comparison with fish meat for feeding alone. The fish flesh meat is to be used as a feed diet. The attained results may of important value to growers of the rice-field crabs overseas, and particularly for the Thai growers.

MATERIALS AND METHODS

The various dried feed ingredients were collected, i.e. fish meal, broken rice, corn or maize, soybean meal, rice bran, wheat, crab meal, soybean oil, cellulose, limestone, and vitamins. Each of which was milled into powder to pass through a 320 mm mesh screen. The chitosan percentages of 0, 1, 2, 3 and 4 were added to form different diet formulae where appropriate (Table 1). The ingredients were thoroughly mixed for 5 min. by an electric mixer with an added amount of lipid. Deionized water (300 ml kg^{-1} dry ingredients mixture) was also added then placed in a mincer (soft-type pellets without steaming) to pass through a 2 mm diameter and the resulted strands were steamed for 5 min. before drying in an electric oven at 60 °C for 12 hrs then cut into a 2-3 mm in length and later kept in the fridge for further uses[20].

Experimental Procedure

Two experiments were carried out at the Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand during May to December 2015. For Experiment 1, a feed diet was formulated with the use of various ingredients to form a complete diet formula and then the same formulated feed diet was duplicated five times and each was added with chitosan levels of 0 (control), 1, 2, 3, and 4 % as to form five treatments altogether, i.e. 0 % (control, diet 1, T1), 1 % (diet 2, T2), 2 % (diet 3, T3), 3 % (diet 4, T4), and 4 % (diet 5, T5). The treatments are shown in Table 1. This experiment aimed to justify the growth performance of the rice-field crabs in relation to chitosan levels. The experiment was laid in a Completely Randomized Design (CRD) with four replications and it was carried out for 60 days. With Experiment 2, the experiment was conducted right after the Experiment 1 was terminated. Two treatments were used, i.e. T1 was conducted with use of the diet formula of T3 of the Experiment 1 was used to compare with a treatment with the use of flesh fish meat of common silver barb (*Barbodes gonionotus*). The experiment was carried out for 90 days, i.e. it reached 150 days of age at the end of the experimental period. Again, a Completely Randomized Design (CRD) with four replications was used. Twenty rice-field crabs were used for each replication and this was done for both experiments.

Juvenile rice-field crabs at an age of first molting period were taken from female brood stock cultured at the Department of Fisheries, Faculty of Agriculture, Khon Kaen University. For each replication, twenty crabs were randomly allocated into each acrylic container with a dimension of 30 x 42 x 8 cm in width, length and height, respectively. Twenty slots were established within each acrylic container and each has a dimension of 7.5 x 8.4 x 8 cm in width, length and height, respectively. Five holes for water drainage were made for each slot and each slot was occupied by a single crab. Each acrylic container was finally placed into a plastic container with a dimension of 34 x 46 x 12 cm in width, length, and height, respectively. None chlorine tap water of 3 litres was filled up in each container. These were carried out for Experiment 1. For Experiment 2, again twenty crabs derived from Experiment 1 were randomly allocated into each cement tank. Each tank has a dimension of 2 x 2 x 1 m in width, length and height, respectively. Each cement tank contained twenty plastic baskets and each plastic basket has a single crab in it. Each plastic basket has a dimension of 18 x 26 x 10 cm in width, length and height, respectively. The twenty plastic baskets were placed in rows (5 x 4) within a cement tank. This was done for each replication. Each tank was filled up with tap water up to 80 cm in height, i.e. with a volume of water of 3.2 m^3.

Culture Media and Feeding Managements

Juvenile crabs of each replication were fed 3 times daily by hand (9.00 am, 1.00 and 5.00 pm). The feed diet was placed into plastic trays and the given amount was adjusted according to body weight and its appetite by examining the remaining residues in trays at 3 hrs intervals after its first given period yet only three times daily. The remaining feed diet was dried off and weighed out as to calculate the consumed amount by the crabs. Feces were siphoned out daily before feeding was taken place and 50 % of exchanged water was filled in daily as to maintain cleaned water for the crabs. Water temperature was maintained at a range between 24.5 to 26.7 ^{0}C, and pH values were within a range between from 7.84 to 7.97. Dissolved oxygen was maintained no less than 3.5 mg L^{-1}. This was done for Experiment 1. For Experiment 2, the same procedure as that of Experiment 1 was carried out for Experiment 2 except an amount of 50 % of water in each replicated tank was siphoned away and replaced with new cleaned water within every fortnight. Temperature was at a range between 26.1 to 27.1 ^{0}C and pH values were within a range of 7.84 to 7.96. Other contributed items were similar to that of the Experiment 1.

Growth Measurements

The measurements in millimeters carried out with rice-field crab samples were initial carapace width, and length and final carapace width, and length along with the counting on numbers of time or frequency in molting. At the end of the experimental period, the growth performance and survival rate of the crabs were recorded and calculated with the use of the following formulae, i.e. survival rate (SR%) = Nf x 100/Ni; weight gained (WG) = Wf – Wi; average daily growth rate (ADG, g/crab) = Wg/T, specific growth rate (SGR, %/day) = (In Wf x In Wi) x 100/T; feed conversion ratio (FCR) = Df/WG, where Ni = number of initial crabs, Nf = number of the final crabs, Wi = initial live weight (g), Wf = final weight (g), T = time interval in days, Df = dry feed intake.

Analytical and Statistical Calculations

The established feed diets were analyzed for proximate composition followed that of the method described by Association of official analytical chemists (AOAC)[21]. The attained data on growth performance were calculated where appropriate for least significant differences of Duncan's Multiple Range Test (DMRT) with the use of a computer programme[22].

RESULTS

Initial and Final Values of Carapace of Rice-field Crabs of Experiment 1

With the results of Experiment 1, it showed that initial values of carapace width were similar in all five treatments with a mean value of 2.68 mm (Table 2). Initial mean values of carapace length were similar in all five treatments with a mean value of 2.46 mm. Final mean values of carapace width were highest with T3 and lowest with T1 (control treatment) with values of 13.63 and 12.73 mm, respectively. The difference was large and statistical significant ($p < 0.05$). For final carapace length, T3 attained the highest and lowest with T1 with mean values of 13.46 and 12.12 mm, respectively. The difference was large and statistical significant ($p < 0.05$). With molting

frequency, it showed that the highest number of molting was found with T3 and lowest with T1 with mean values of 6 and 5.25 times, respectively. The difference was large and statistically significant ($p < 0.05$).

With initial weights (Wi), the rice-field crabs initially attained a similar mean value of 0.0045 g in all five treatments (Table 3). At the end of the experimental period (60 days), final weights of the rice-field crabs ranged from 0.7828 to 1.0437 g for T1 and T3, respectively. The difference was large and statistically significant ($p = 0.05$). For weight gained (WG), mean values of weight

Table 1. Formulation and proximate composition of the ingredients of five treatments of five different feed diets used in Experiment 1 (% on dry matter basis).

Ingredients (%)	Amount used (%)				
	Diet1(T1)	Diet2 (T2)	Diet3 (T3)	Diet4 (T4)	Diet5 (T5)
Chitosan	0	1	2	3	4
Fish meal	10.5	10.5	10.5	10.5	10.5
Broken rice	1.35	1.35	1.35	1.35	1.35
Corn	4.50	4.50	4.50	4.50	4.50
Soybean meal	50.0	50.0	50.0	50.0	50.0
Rice bran	20.24	20.24	20.24	20.24	20.24
Wheat	3.00	3.00	3.00	3.00	3.00
Crab meal	0.5	0.5	0.5	0.5	0.5
Soybean oil	1.0	1.0	1.0	1.0	0.6363
cellulose	3.6363	2.6363	1.6363	0.6363	0
Limestone	4.21	4.21	4.21	4.21	4.21
Mineral [1]	0.7637	0.7637	0.7637	0.7637	0.7637
vitamin[2]	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100
Chemical composition by proximate analysis (g kg^{-1} dry weight on dry matter basis)					
Moisture	8.65	8.34	8.75	8.25	8.92
Protein	33.24	33.56	33.50	33.41	33.35
Lipid	6.02	6.15	6.10	6.21	6.75
Fibre	7.64	7.49	7.52	6.89	7.31
Ash	11.05	10.95	11.34	11.82	11.91

[1] Mineral kg^{-1}: Calcium carbonate ($CaCO_3$) 6 g., Magnesium sulfate ($MgSO_4.7\ H_2O$) 1 g., Zinc sulfate ($ZnSO_4.7\ H_2O$) 0.6 g., Ferrous sulfate ($FeSO_4.7\ H_2O$) 0.03 g., Copper sulfate ($CuSO_4.5H_2O$) 0.007 g., Potassium iodide (KI) 0.001g.
[2] Vitamin kg^{-1}: Vitamin A 30,000IU, Vitamin D 6,000IU, Vitamin E 9.000 mg., Vitamin K 5.250 mg., thiamine 3.750 mg., Riboflavin 6.000 mg., Pyridoxine 10.500 mg., Pantothenic acid 45.000 mg., Folic acid 0.750 mg., Ascorbic acid 45.000 mg., Cobalamin 0.045 mg.

Table 2. Initial carapace width and length, final carapace width and length and frequency in molting of the Esanthelphusa dugasti as influenced by five different levels of chitosan (T1-T5).

Treatments/parameters	Dietary chitosan levels				
	T1 (0%)	T2 (1%)	T3 (2%)	T4 (3%)	T5 (4%)
Initial carapace width, (mm)	2.68	2.68	2.68	2.68	2.68
Initial carapace length, (mm)	2.46	2.46	2.46	2.46	2.46
final carapace width, (mm)	12.73^{b}	13.52^{a}	13.63^{a}	13.24^{ab}	13.14^{ab}
final carapace length, (mm)	12.12^{b}	13.34^{a}	13.46^{a}	13.06^{a}	12.97^{a}
Frequency in molting (times)	5.25^{c}	5.75^{ab}	6^{a}	5.5^{bc}	5.5^{bc}

Letter(s) within rows indicated least significant differences (LSD) of means of Duncan's Multiple Range Test (DMRT) at probability ($p < 0.05$).

gained ranged from 0.7783 to 1.0392 g for T1 and T3, respectively. The difference was large and statistically significant (p = 0.05). With average daily growth rate (ADG), mean values of ADG of rice-field crabs ranged from 0.0130 to 0.0173 g for T1 and T3, respectively. The difference was large and statistically significant (p < 0.05). Specific growth rate (%) of rice-field crabs was highest

Table 3. Growth performance, feed utilization and survival rate of the *Esanthelphusa dugasti* as influenced by five different levels of supplementary chitosan (T1-T5).

Treatments/parameters	Dietary chitosan levels				
	T1 (0 %)	T2 (1 %)	T3 (2 %)	T4 (3%)	T5 (4%)
Initial weight (Wi)	0.0045	0.0045	0.0045	0.0045	0.0045
Final weight (Wf)	0.7828^{c}	0.9523^{ab}	1.0437^{a}	0.8928^{bc}	0.8238^{bc}
Weight gained (WG)	0.7783^{c}	0.9478^{ab}	1.0392^{a}	0.8883^{bc}	0.8192^{bc}
Average daily growth rate (ADG)	0.0130^{c}	0.0158^{ab}	0.0173^{a}	0.0148^{bc}	0.0137^{bc}
Specific growth rate (SGR, %)	8.92^{ab}	8.92^{ab}	9.07^{a}	8.81^{bc}	8.68^{c}
Feed conversion ratio (FCR)	1.55^{a}	1.29^{bc}	1.23^{c}	1.46^{ab}	1.53^{a}
Survival rate (SR, %)	75^{a}	77^{a}	77^{a}	76^{a}	72^{a}

Letter(s) within rows indicated least significant differences (LSD) of means of Duncan's Multiple Range Test (DMRT) at probability (p < 0.05).

Table 4. Initial carapace width, length, and final carapace width, length and frequency molting numbers of the *Esanthelphusa dugasti* as influenced by two types of feed diet, recorded at 90 days of age.

Treatments/Parameters	Type of feed diets	
	Fish flesh meat (T1)	Formulated feed diet (T2)
Initial carapace width, mm	11.90	11.90
Initial carapace length, mm	10.75	10.75
final carapace width, mm	32.01^{a}	31.80^{a}
final carapace length, mm	25.65^{a}	26.17^{a}
Frequency molting (time)	6.5^{a}	6.5^{a}

Letter(s) within rows indicated least significant differences (LSD) of means of Duncan's Multiple Range Test (DMRT) at probability (p < 0.05.).

Table 5. Initial and final weights (g), weight gained (g), average daily growth rate (g), specific growth rate (%) and survival rate (%) of the *Esanthelphusa dugasti* as influenced by fish flesh meat (T1) and formulated feed diet (T2), recorded at 90 days of age

Parameters	Type of feed diets	
	Fish flesh meat (T1)	Formulated feed diet (T2)
Initial weight (Wi)	0.77	0.77
Final weight (Wf)	11.59^{a}	11.12^{a}
Weight gained (WG)	10.82^{a}	10.35^{a}
Average daily growth rate (ADG)	0.120^{a}	0.115^{a}
Specific growth rate (SGR (%)	3.01^{a}	2.97^{a}
Survival rate SR (%)	67.50^{b}	78.75^{a}

Letter(s) within rows indicated least significant differences (LSD) of means of Duncan's Multiple Range Test (DMRT) at probability (p < 0.05.).

with T3 and lowest with T5 with mean values of 9.07 and 8.68 %, respectively. The difference was large and statistically significant ($p < 0.05$). For feed conversion ratio, T1 gave the highest and least with T3 with mean values of 1.55 and 1.23, respectively. The difference was large and statistically significant. With survival rate (SR), T2 and T3 attained the highest with a similar value of 77 % and lowest with T5 with a mean value of 72 %.

Growth Measurements on Rice-field Crabs of Experiment 2

For initial carapace width, mean value of carapace width was 11.90 mm for both treatments (T1 and T2) and initial carapace length of 10.75 also for both treatments (Table 4). At the end of the experimental period (90-day of age), mean values of final carapace width were 32.01 and 31.80 for T1 and T2, respectively. There was no statistical significant differences found, whilst mean value of final carapace length was 6.5 mm for both treatments and there was no statistical significant differences found. With mean values of initial weights, it was found that mean values of individual crab of both treatments were the same (0.77 g) whilst mean values of the final weights were 11.59 and 11.12 g for T1 and T2, respectively (Table 5). For weight gained, it was found that mean values were 10.82 and 10.35 g for T1 and T2, respectively. There was no statistical difference found. With average daily growth rate, T1 and T2 gave values of 0.120 and 0.115, respectively. There was no difference found between the two treatments. Similarly, there was no statistical difference found between T1 and T2 on specific growth rate (%). Both treatments gave values of 3.01 and 2.97 % for T1 and T2, respectively. For survival rate, T1 was lesser than T2 with values of 67.50 and 78.75 %, respectively. The difference was large and statistically significant ($p < 0.05$).

DISCUSSION

At the beginning of the experimental period, it was found that initial values of carapace width of the rice-field crabs were similar in all five treatments. The results indicated that individual rice-field crab attained a similar size thus the crabs should have had a similar form of growth and development. At the end of the experimental period of 60 days, the results showed that carapace width of T3 (2 % chitosan) was the highest and significantly ($p < 0.05$) higher than T1 (control). Higher levels of chitosan higher than T3 gave a similar carapace width as that of T3. The results indicated that chitosan had its significant effect on growth of the rice-field crabs at only this level. This trend was also found with carapace length along with frequency in molting where T3 gave the highest. Therefore, an amount of 2 % of chitosan of T3 should be an utmost level for use in the feed diet. Chitosan hastened growth of aquatic creatures had been reported by a number of workers such as , Nui *et al.*[12], Esteban *et al.*[15,16], Ortuno *et al.*[17], Cuesta *et al.*[18], Wang and Chen[23] and Cha *et al.*[24]. A clearer effect of chitosan was evidently found on final weights of rice-field crabs. That is T3 gave the highest weight than the rest and the difference was large and statistically significant ($p < 0.05$). This trend was also found with weight gained, average daily growth rate, specific growth rate and survival rate. This must be attributable to a 2 % chitosan chemical added to the feed diet hastened growth of the rice-field crabs. A decline in final weight, weight gained, average daily growth rate, specific growth rate and survival rate was found with T4 and T5. The decline may be due to an excessive amount needed for growth of the rice-field crabs. Nui *et al.*[12] stated that medium chitosan level in the feed diet gave benefited effects on growth and survival rate of the *Litopenaeus vannamei* and recommended that the optimum supplemented level was at a range of 2.13 to 2.67 g kg^{-1} feed diet. Nui *et al.*[25] reported that the optimum supplement of dietary chitosan level for the culturing of *Penaeus monodon* was at a rate of 0.19-0.21%. Therefore, the best level of chitosan to be used for rice-field crab culture is 2 % (T3). This result was attained from an age of the rice-field crabs of 60 days. In the study of Attasart *et al.*[26], The chitosan feed additive dose at 100 ppm was found to positively affect the juvenile shrimp (about 2 g wet weight) growth and frequency of molting, the results reflect that chitosan can promote the shrimp growth, it was the same with ours. So the positive effect of chitosan maybe also relate to the rice-field crab culture. Rice-field crabs are susceptible to many pathogens that either lower their resistance or enhance the pathogenicity of the pathogens. Therefore, chitosan utilization should be immuneopotentiating and

antimicrobial functions in animals[27]. Furthermore, chitosan can play an important role in organic crab farming in aspects to improve feed quality, water quality management, the rice-field crab health and the culture system sanitation[26].

With the results of Experiment 2, it revealed that there was no statistical significant found between T1 (flesh fish meat) and T2 (formulated feed diet) on final carapace width and length of the rice-field crabs. Both of them were similar. A similar trend was also found with frequency numbers in molting. That is both gave a mean value in molting of 6.5 times. The results suggested that both feed diets possessed a similar effect in promoting growth of the rice-field crabs. A similar trend as that of width and length of the rice-field crabs was also found with final weights, weight gained, average daily growth rate and specific growth rate. However, a significant result was found with survival rate (%). That is T2 of the formulated feed diet was statistically higher than T1 ($p < 0.05$). This may be attributable to perhaps chitosan contents in the formulated feed diet encouraged a rapid growth of the rice-field crabs of T3 apart from other nutrient contents in the feed diet. Thus this result confirms a significant effect of chitosan of the former experiment (Experiment 1). In general, natural food available for aquatic creature like fish should perhaps be able to facilitate growth of the rice-field crabs as that of the formulated feed diet. But it turned out that survival rate (%) of rice-field crabs was significantly higher for formulated feed diet than fish flesh meat alone. This suggested adequate nutritive elements in the formulated feed diet for a better survival of the rice-field crabs. Therefore, this formulated feed diet should have reached its ultimate standing for use in culturing rice-field crabs for some considerable production.

CONCLUSIONS

The results of this study revealed that chitosan chemical has its significant effect on growth and development of the rice-crabs. The most appropriate level of chitosan to be added to the feed diet is 2 % (on dry matter basis). Other higher levels may not necessary for use in the feed diet. Chitosan of 2 % in the feed diet of T3 of the Experiment 1 when used in the Experiment 2 significantly ($p < 0.05$) encouraged survival rate (%) of the rice-field crabs better than the use of fish flesh meat alone (T1 of Experiment 2).

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