Dietary Premix Levels, Calcium and Phosphorus Ratios on Growth Performance of Juvenile Rice-field Crabs (*Esanthelphusa dugasti*)

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ABSTRACT

Two experiments were conducted at the Department of Fisheries, Khon Kaen University, Nong Khai campus during June to November 2014 to investigate effects of dietary premix levels, calcium and phosphorus ratios on growth performance and survival rate (%) of juvenile rice-field crabs (*Esanthelphusa dugasti*). The objectives in carrying out this work include an evaluation on the effects of different levels of supplementary premix (Experiment1), calcium and phosphorus ratios on growth performance and survival rate of the rice-field crabs (Experiment 2). Experiment 1 consisted of five treatments of five levels of supplementary premix, i.e. 0, 1, 2, 3 and 4 g kg-1 of diet for T1 up to T5, respectively. With Experiment 2, five ratios between Ca and P were used, i.e. 1.1: 1.0 (T1 control), 2.1:1.0 (T2), 2.4:1.0 (T3), 3.1:1.0 (T4), and 3.5:1.0 (T5). A Completely Randomized Design (CRD) with four replications was used for both Experiments. The results of the Experiment 1 showed that the supplementary premix added to the feed diet was found to be a significant contributor in increasing both width and length of carapace and molting frequency of the rice-field crabs. An amount of 3g kg^{-1} of T4 added to the formulated diet gave the highest growth in width, and length of carapace and molting frequency of the rice-field crabs. An increase in dietary premix to the feed diet significantly increased weight gained (WG), average daily growth rate (ADG), specific growth rate (SGR %) and survival rate (SR %) of the rice-field crabs. For the Experiment 2, the highest mean values of carapace width, length and molting frequency of the rice-field crabs were found with a ratio between Ca and P of 2.4:1 (T3). An amount of 3g kg^{-1} of T4 added to the formulated diet gave the highest growth in width, and length of carapace and molting frequency of the rice-field crabs. An increase in dietary premix to the feed diet significantly increased weight gained (WG), average daily growth rate (ADG), specific growth rate (SGR %) and survival rate (SR %). The highest mean values of carapace width, length and molting frequency of the rice-field crabs were found with T3 with a ratio between Ca and P of 2.4:1.

Keywords: Feeding, rearing rice-field crab, *Esanthelphusa dugasti*, supplementary premix, calcium and phosphorus ratio

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INTRODUCTION

Rice-field crab (*Somanniathelphusa*) is a kind of rice paddy field animal in Thailand. It has an important economic value to the Thai people, particularly the people who are growing rice in the northeastern region of the country. This type of crabs is widely distributed in most Asian countries, particularly in Laos, Myanmar, Cambodia, Vietnam and Thailand (Naiyanetr, 1978, 1982, 1994; Ng and Naiyanetr, 1993; Yeo and Nguyen, 1999; Yeo, 2004; Yeo *et al*., 2008). Eleven species are well known to the Thai people they include *Esanthelphusa dugasti* or *Somanniathelphusa dugasti*, *S. germini* and, *S. neisi* (Rathbun, 1902), *S. sexpunctata* (Lanchester, 1906), *S. brandti* (Bott, 1968), *S. maehongsonensis* (Naiyanetr, 1978), *S. bangkokensis* (Naiyanetr, 1982), *S. denchaiiand S. nani* (Naiyanetr, 1984), *S. chiangmai* and *S. Phetchaburi* (Ng and Naiyanetr, 1993). The rice-field crab of *E. dugasti* species is the only species found in northeastern region of Thailand. It is considered to be an important species since its whole body is commonly used in making different food recipes for daily diets by the people in the region (Doolgindachbaporn *et al.,* 2011). Nowadays, it seems more likely that this rice-field crab species is almost approaching a stage of extinction since its population is rapidly decreasing with time due to an increase in number of the Thai populations and the expansion of many types of industries, particularly the modernization of rice producing industries where more of the pest insecticides and herbicides have been using in most paddy fields.

The Thai rice growers believe that all species of the crabs are of destructive organisms in their rice fields thus the crab species must be eliminated. Crabs normally make more damages to young rice plant seedlings. It is commonly known that the Thai people consume a large amount of edible rice field crabs annually. One important problem is rising now since the modernized agriculture used a huge amount of hazardous chemicals. This could possibly have had some significant effect to health of the Thai people. In addition, crabs are known to be a secondary intermediate host of *Paragonimus* genus (lung fluke) and humans are of the final host for this kind of disease since many Thai people consumed raw or poorly cooked crabs so its infection could derive from metacercariae of the *Paragonimus* spp. (Yaemput *et al*., 1994; Blair *et al*., 1999; Uruburu *et al*., 2008). Therefore, the culturing of rice-field crabs under both non-toxic and non-parasitic conditions may have some tangible contribution to health of mankind since there is always a high demand for fresh edible crabs of its kind in the local markets.

In aquaculture, vitamins and minerals have played its significant role in biochemical mechanisms especially in generating energy for cells of the body. It serves as an important component of the enzyme (Lovell, 1989). Thus vitamin supplements in the diets are greatly needed since aquatic bodies cannot synthesize vitamins on its own hence the diets must contain some considerable amounts of vitamin and minerals in order to hasten growth of the healthy aquatic organisms.

Calcium (Ca) and phosphorus (P) are the major minerals found in crabs, it has been advocated that crustacean including crab have a relatively high amount of ash up to 15.90 % in their bodies (Sze, 1973).Both calcium and phosphorus are the two major constituents of the inorganic portion in feed ration. They quantitatively function primarily as structural components of hard tissues, e.g. bond, exoskeleton, scales and teeth. Ca is essential for hard tissue structure, blood clothing, muscle contraction, nerve transmission, osmoregulation and as a co-factor for enzyme procession (Lall, 1989; Lovell, 1989) and most of the aquatic species can absorb Ca directly from its surrounding environments as to meet their Ca requirements (Deshimura and Yone, 1978; Lall, 1989; Coote *et al*., 1996; Davis and Gatlin, 1996). P possessed its crucial function in crabs since P is obviously limited under many rearing conditions such as a high degree of acid soils (Suksri, 1999). P is directly involved in all energy-yielding reactions and it has an integral role in cellular function, it is a key component of nucleic acids, phospholipids, phosphoproteins, adenosine triphosphate (ATP)

and several key enzymes (Lovell, 1989). P is mainly associated with Ca in the exoskeleton and also P is related to alkaline phosphatase enzyme, which responds to acclimation salinity and is associated with osmoregulation in crustaceans (Lovett *et al*., 1994). Crustaceans may need dietary sources of minerals for growth due to the repeated molting periods wherein minerals are lost, which is necessary to determine the required amount of Ca and P in rice-field crabs with a special emphasis on dietary as to ensure a rapid growth and a good health of the crabs. The objective in carrying out this work included an evaluation on the effects of different levels of premix, calcium and phosphorus ratios on growth performance of the rice-field crabs. The attained data may be of a tangible value for growers of the rice-field crabs in Thailand and elsewhere.

MATERIALS AND METHODS

This investigation was carried out at the Department of Fisheries, Faculty of Agriculture, Nongkhai Campus, Khon Kaen University, Khon Kaen, Thailand during June to November 2014 to investigate effects of dietary premix levels, calcium and phosphorus ratios on growth performance and survival rate (%) of juvenile rice-field crabs (E. dugosti). The work consisted of two experiments. For Experiment 1, the work focused on effects of dietary premix levels where five treatments with four replications were used, i.e. T1 (control) without supplementary premix but with the use of formulated feed diet alone. For T2 up to T5, formulated feed diet plus 4 supplementary premix levels were used, i.e. 1, 2, 3 and 4 g kg^{-1} for T2, T3, T4 and T5, respectively (Tables 1 and 2). With Experiment 2, the work aimed to find out how different ratios between calcium (Ca) and phosphorus (P) added to the formulated diet could affect growth of the rice-field crabs. Five ratios between Ca and P were 1.1:1.0 (T1, control), 2.1:1.0 (T2), 2.4:1.0 (T3), 3.1:1.0 (T4) and 3.5:1.0 (T5).The different sources of ingredients were used to produce a formulated diet for the five treatments used (Table 3). A Completely Randomized Design (CRD) with four replications was used for both experiments.

Table 1 Formulation of the ingredients of the feed diets of the five treatments (T1-T5) plus supplementary vitamin premix (% on dry matter basis)

Ingredients (%)/ Treatments (T)	Dietary premix levels				
	T1 (0)	T2 (1)	T3 (2)	T4 (3)	T5 (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	4.5	4.5	4.5	4.5	4.5
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	3.0	3.0	3.0	3.0	3.0
Crab meal	0.5	0.5	0.5	0.5	0.5
Soybean oil	1.0	1.0	1.0	1.0	1.0
cellulose	3.9363	3.8363	3.7363	3.6363	3.5363
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	0.1	0.2	0.3	0.4
Total	100.0	100.0	100.0	100.0	100.0

[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) 0.001 g

[2] Composition of vitamin premix is given in Table 2

Table 2 Components of vitamin premix added infeed diets of the five treatments (T1-T5) for rice-field crab culture of Experiment 1

Vitamins/Treatments	Dietary premix levels				
	T1 (0)	T2 (1)	T3 (2)	T4 (3)	T5 (4)
vitamin A (IU)	0	10,000	20,000	30,000	40,000
vitamin D (IU)	0	2,000	4,000	6,000	8,000
vitamin E (mg)	0	3.000	6.000	9.000	12.000
vitamin K (mg)	0	1.750	3.500	5.250	7.000
thiamine (mg)	0	1.250	2.500	3.750	5.000
riboflavin (mg)	0	2.000	4.000	6.000	8.000
pyridoxine (mg)	0	2.000	4.000	6.000	8.000
pantothenic acid (mg)	0	3.500	7.000	10.500	14.000
niacin (mg)	0	15.000	30.000	45.000	60.000
folic Acid (mg)	0	0.250	0.500	0.750	1.000
ascorbic acid (mg)	0	15.000	30.000	45.000	60.000
cobalamin (mg)	0	0.015	0.030	0.045	0.060

Table 3 Formulation and the proximate composition of the ingredients for feed diets of T1-T5 for rice-field crabs culture of Experiment 2 (% on dry matter basis)

Ingredients (%)/ Treatments	Ratios between dietary calcium (Ca) and phosphorus (P)				
	T1 (1.1:1.0)	T2 (2.1:1.0)	T3 (2.4:1.0)	T4 (3.1:1.0)	T5 (3.5:1.0)
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	4.5	4.5	4.5	4.5	4.5
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	3.0	3.0	3.0	3.0	3.0
Crab meal	0.5	0.5	0.5	0.5	0.5
Soybean oil	1.0	1.0	1.0	1.0	1.0
cellulose	7.8463	5.1163	3.6363	2.1763	0.69
Limestone	0	2.73	4.21	5.6700	7.1563
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} on dry weight basis)					
Moisture	9.57	8.79	8.74	9.03	8.60
Protein	30.58	31.91	33.25	33.50	33.93
Lipid	5.87	6.33	6.07	5.95	6.08
Fiber	7.02	6.35	7.72	6.85	7.30
Ash	7.53	10.51	10.94	12.81	14.98
Ca	1.07	2.17	2.43	3.20	3.79
P	0.96	1.02	1.02	1.04	1.07
Ca:P ratio	1.1:1	2.1:1	2.4:1	3.1:1	3.5:1

[1] Mineral kg^{-1}: Calcium carbonate ($CaCO_3$) 6 g, Magnesium sulfate ($MgSO_4.7\ H_2O$) 1 g, Zinc sulfate ($ZnSO_4.7\ H_2O$) 0.6g., Ferrous sulfate ($FeSO_4.7\ H_2O$) 0.03 g, Copper sulfate ($CuSO_4.5H_2O$) 0.007 g, Potassium iodide (KI) 0.001 g

[2] Vitamin kg^{-1}: Vitamin A30,000 IU, Vitamin D 6,000 IU, 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

The ration diets were prepared in the laboratory where all dry sources of ingredients were milled into powder to pass through a 320 mm mesh screen. They were weighed out accordingly then thoroughly mixed together with the use of an electric mixer then added with lipid source and thoroughly mixed for 5 min. Deionized water (300 ml kg^{-1} dry ingredients mixture) was added and mixed again for 5 min. The dough was allowed to pass through a 2 mm diameter electric mincer in order to form pellets (soft-type pellets without steaming). The sample strands were steamed for

5 min before drying in an electric oven at 60 °C for 12 hrs (Doolgindachbaporn *et al.*, 2005). Later on the dried diets were cut into 2−3 mm in length and finally stored in the refrigerator till used.

After the first molting stage, 100 juvenile rice-field crabs were randomly chosen from 400 rice-field crabs for use in the Experiment 1 and the same method was used for the rice-field crabs of the Experiment 2. All of the crabs were cultured at the Department of Fisheries, Khon Kaen University, Khon Kaen, Thailand. For the Experiment 1, initial mean values of carapace live weight, width and length were 0.0036 g, 2.65 mm and 2.65 mm, respectively whilst that of the Experiment 2 were 0.0035 g, 2.60 mm and 2.60 mm, respectively. The rice-field crabs of both experiments were cultured with the use of 20 acrylic containers. Each has a dimension of 30 x 42 x 8 cm in width, length and height, respectively. Each container was divided into 20 slots and each slot has a dimension of 7.5 x 8.4 x 8 cm in width, length and height, respectively. In each slot, five small holes were made for water to drain in and out and each slot was used to occupy by one rice-field crab. The crabs were fed with the prepared diets by hands for three times daily (9.00 am, 1.00 and 5.00 pm). The given amount of the diet was adjusted according to body live weight and their appetite by examining the remained diet residues in the tray at 3 hrs intervals after each given feeding period. The remaining feed stuffs were collected and oven dried and weighed out for dry weights as to attain the amount of the diet consumed by the crabs. Before feeding was taken place, feces were siphoned out daily and approximately 50% of cleaned water was replaced as to assure optimum water quality. The water temperature was maintained within a range from 24.5 to 27.5 °C and pH values were within a range from 7.1 to 7.9 with the dissolved oxygen no lesser than 3.5 mg L^{-1}. The rice-field crabs were allowed to grow for 60 days.

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 = the number of initial crabs, Nf = the number of the final crabs, Wi = the initial live weight (g), Wf = the final weight (g), T = time interval in days, Df = dry feed intake. The feed diets were analysed for proximate composition (AOAC, 1990). The attained data were calculated where appropriate with the use of a computer programme (SAS, 1998).

RESULTS

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

For Experiment 1, the resultsshowed that mean values of initial carapace width and length were similar in all levels of dietary premix with the value of 2.65 mm for both width and length of the crab carapace, respectively (Table 4). With final values of the carapace width, it showed that T1 (control) was the lowest and highest with T4 with mean values of 9.09 and 11.20 mm, respectively. The difference was large and statistical significant yet there was no significant difference found among T2 up to T5. For the final length, again it showed that mean value of T1 (control) was the lowest and highest with T4 with mean values of 7.99 and 10.97 mm, respectively. The difference was large and statistically significant. With the mean values of molting frequency, the results showed that the lowest number of molting was found with T1 (control) and highest with T4 with mean values of 4.5 and 5.25 times, respectively. The difference was large and statistically significant.

Table 4 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 premix (T1-T5) added to the feed diets being cultured for 60 days

Parameters/Treatments	Dietary premix levels (g kg^{-1})				
	T1 (0)	T2 (1)	T3 (2)	T4 (3)	T5 (4)
Initial carapace width, mm	2.65	2.65	2.65	2.65	2.65
Initial carapace length, mm	2.65	2.65	2.65	2.65	2.65
Final carapace width, mm	9.09^{b}	10.66^{a}	10.91^{a}	11.20^{a}	10.91^{a}
Final carapace length, mm	7.99^{d}	10.03^{b}	8.88^{c}	10.97^{a}	10.55^{ab}
Frequency molting (time)	4.50^{b}	5.00^{ab}	5.00^{ab}	5.25^{a}	5.00^{a}

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

Initial and Final Live Weights of Rice-field Crabs of Experiment 1

The results on growth performance revealed that initial mean values of live weights (Wi) of all treated crabs were similar with a mean value of individual crab of 0.0036 g (Table 5). With final live weights (Wf), it showed that the lowest was with T1 (control) and the highest was with T4 with mean values of 0.31 and 0.53 g, respectively. The difference was large and statistically significant. For weight gained, it revealed that T1 (control) was the lowest and the highest was with T4 with mean values of 0.31 and 0.52 g, respectively. For average daily growth rate (ADG), the results showed that the lowest was found with T1 (control) and the highest was with T4 with mean values of 0.0051 and 0.0087 g, respectively. With specific growth rate (SGR % day^{-1}), it revealed that the lowest was with T1 (control) and the highest was with T4 with mean values of 7.43 and 8.29%, respectively. For feed conversion ratio (FCR), the lowest was with T4 and the highest was with T1 (control) with mean values of 1.58 and 2.62, respectively. With survival rate (SR %), it showed that T1 (control) was the lowest and the highest was found with T4 with mean values of 63 and 77%, respectively.

Table 5 Growth performance, feed utilization and survival rate of the *Esanthelphusa dugasti* as influenced by five different levels of supplementary premix (T1-T5) added to the diets being cultured for 60 days

Parameters/Treatments	Dietary premix levels (g kg^{-1})				
	T1 (0)	T2 (1)	T3 (2)	T4 (3)	T5 (4)
Wi	0.0036	0.0036	0.0036	0.0036	0.0036
Wf	0.31^{b}	0.48^{a}	0.34^{b}	0.53^{a}	0.51^{a}
WG	0.31^{b}	0.48^{a}	0.33^{b}	0.52^{a}	0.51^{a}
ADG	0.0051^{b}	0.0079^{a}	0.0056^{b}	0.0087^{a}	0.0085^{a}
SGR (%)	7.43^{b}	8.14^{a}	7.57^{b}	8.29^{a}	8.26^{a}
FCR	2.62^{a}	1.63^{b}	2.34^{a}	1.58^{b}	1.63^{b}
SR (%)	63^{c}	68^{bc}	71^{ab}	77^{a}	70^{abc}

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

Initial and Final Values of Carapace of Rice-field Crabs of Experiment 2

With Experiment 2, the results showed that initial mean values of all treatments on carapace width were similar, i.e. 2.60 mm and its lengths were also similar, i.e. 2.60 mm in all treated crabs (Table 6). At the end of the experimental period, the results showed that carapace width value was lowest with T1 (control) and highest with T3 with values of 9.37 and 11.01 mm, respectively. The difference was large and statistically significant ($P < 0.05$). For the final carapace length, it showed that T1 (control) was the lowest and the highest was found with T3 with values of 9.18 and 10.73 mm, respectively. The difference was large and statistically significant ($P < 0.05$). With the numbers of molting, it was found that the lowest value of molting number was found with T1 and highest with T3 with values of 4.5 and 5.5 times, respectively. The difference was large and statistically significant ($P < 0.05$). However, there were no significant differences found among T2 up to T5.

Table 6 Initial carapace width, Initial carapace length, final carapace width, final carapace length and frequency molting of the *Esanthelphusa dugasti* as influenced by different ratios between calcium and phosphorus added to the feed diets being cultured for 60 days

Parameters/Treatments	Ratios between dietary calcium and phosphorus				
	T1 (1.1:1)	T2 (2.1:1)	T3 (2.4:1)	T4 (3.1:1)	T5 (3.5:1)
Initial carapace width, mm	2.60	2.60	2.60	2.60	2.60
Initial carapace length, mm	2.60	2.60	2.60	2.60	2.60
final carapace width, mm	9.37^{b}	10.07^{ab}	11.01^{a}	10.93^{a}	10.58^{a}
final carapace length, mm	9.18^{b}	9.90^{ab}	10.73^{a}	10.71^{a}	10.19^{ab}
Frequency molting (time)	4.50^{b}	5.25^{a}	5.50^{a}	5.50^{a}	5.25^{a}

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

Initial and Final Values of Live Weights of Rice-field Crabs of Experiment 2

With the results on growth performance, it was found that initial live weights (Wi) were similar in all treated crabs with a value of 0.0035 g crab^{-1} (Table 7). For the final live weights (Wf), it was found that T1 ranked the lowest and highest with T3 with average values of 0.39 and 0.52 g crab^{-1}, respectively. The difference was large and statistically significant ($P < 0.05$). With weight gained (WG), T1 (control) was the lowest and the highest was found with T3 with mean values of 0.39 and 0.52 g crab^{-1}, respectively. The difference was large and statistically significant ($P < 0.05$).

Table 7 Growth performance, feed utilization and survival rate of the *Esanthelphusa dugasti*as influenced by different ratios between calcium and phosphorus added to the feed diets being cultured for 60 days

Parameters/Treatments	Ratios between dietary calcium and phosphorus				
	T1 (1.1:1)	T2 (2.1:1)	T3 (2.4:1)	T4 (3.1:1)	T5 (3.5:1)
Wi	0.0035	0.0035	0.0035	0.0035	0.0035
Wf	0.39^{b}	0.47^{ab}	0.52^{a}	0.51^{a}	0.49^{ab}
WG	0.39^{b}	0.47^{ab}	0.52^{a}	0.51^{a}	0.48^{ab}
ADG	0.0067^{b}	0.0078^{ab}	0.0087^{a}	0.0084^{ab}	0.0080^{ab}
SGR (%)	7.84^{b}	8.16^{ab}	8.34^{a}	8.30^{a}	8.20^{a}
FCR	2.92^{a}	2.32^{ab}	2.27^{b}	2.20^{b}	2.39^{ab}
SR (%)	76^{a}	77^{a}	80^{a}	73^{a}	65^{b}

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

Daily Growth rate, Specific Growth Rate, Feed Conversion Ratio and Survival Rate of Rice-field Crabs of Experiment 2

For mean values of average daily growth rate (ADG), the results showed that ADG mean value was lowest with T1 (control) and highest with T3 with values of 0.0067 and 0.0087 g crab^{-1}, respectively. The difference was large and statistically significant ($P < 0.05$). With mean values of specific growth rate (SGR %), it was found that T1 ranked the lowest and highest with T3 with values of 7.84 and 8.34 crab^{-1}, respectively. The difference was large and statistically significant ($P < 0.05$). For mean values of feed conversion ratio (FCR), the results showed that T1 was the highest and the lowest was found with T4 with mean values of 2.92 and 2.20, respectively. The difference was large and statistically significant ($P < 0.05$). With values of survival rate (SR %), the results indicated that T5 was the lowest and the highest was found with T3 with mean values of 65.00 and 80.00%, respectively. The difference was large and statistically significant ($P < 0.05$) yet there were no significant differences found among T1 up to T4.

DISCUSSION

Supplementary Premix on Growth Items of Rice-field Crabs

It seems more likely that published data on rice-field crabs is relatively limited even though many of the Thai people consumed a large amount of fresh edible rice field crabs annually. With the results of the Experiment 1, it revealed that the added amount of supplementary premix to the formulated diet significantly increased carapace width of the rice-field crabs up to T2 and higher rates gave a similar result as that of T2. It was evidently shown that significant result on the final carapace length and molting frequency was ultimately found with T4. Thus T4 is considered to be the most appropriate rate of dietary premix for growth of the rice-field crabs, i.e. 3g kg^{-1} of the formulated feed diet. The results indicated that the growth in length and molting frequency required some additional amount of the supplementary premix in the ration hence supplementary premix has its significant role in culturing paddy field crabs. With the growth performance, the attained results, in most cases, showed that an increase in dietary premix to the feed diet significantly increased weight gained (WG), average daily growth rate (ADG), specific growth rate (SGR %) and survival rate (SR %). The results confirmed the works reported by Barrow *et al.* (2008) with the fish of rainbow trout (*Oncorhynchus mykiss*). They stated that when cultured the fish without supplementary premix, the fish gave significantly poorer growth and lower feed utilization efficiency than those fed with the supplementary premix after a period of six weeks and the survival rate was lesser than 50%. Other workers had also reported the significant effect of supplementary premix added to their feed diets for their fish experiments such as Taechajanta and Sitasit (1981) with juvenile *Clarias microcephatus*, Suhenda and Djadiredja (1985) with common carp (*Cyprinus carpio*), Butthep *et al.* (1985) with *Clarias microcephatus*. Nevertheless, Monje *et al.* (1996) with the experiment on fish species of *Clarias gariepinus*, they did not find significant effect on growth of the fish due to the added amount of the supplementary premix to their feed diet. However, they stated that the juvenile fish with non vitamin premix supplemented had lower body weight than the one fed with the added amount of supplementary premix.

Growth of Rice-field Crabs in Relation to Calcium and Phosphorous Ratios

The results of the Experiment 2 showed that final carapace width, carapace length and molting frequency of the rice-field crabs were significantly affected by ratios between calcium (Ca) and phosphorous (P), i.e. an increase in calcium level significantly increased carapace width and length and numbers of molting frequency and the most important ratios was found with T3 where a ratio between Ca and P being used was a 2.4:1. The result indicated that Ca plays its significant role in

growth of the rice-field crabs since Ca is needed as a major proportion for exoskeleton development where it is needed in a form of calcium carbonate, and it is an essential mineral for use in a large quantity for each molting period of the growing crabs (Zanotto *et al.*, 2004).Thus an appropriate ratio between Ca and P of 2.4:1 was found to be the utmost ratio being used to encourage a rapid growth of the rice-field crabs. A higher Ca:P ratio of 3.5:1 gave the highest average live weight of crabs of 0.3179 g with a survival rate of 45% was reported by Nonpila (2007). This required higher amount of Ca higher than the present work may be attributable to the differences in feed diet used or perhaps the differences in environmental conditions. Nevertheless, some other aquaculture species may also need different ratios between Ca and P for a rapid growth of its bodies such as a ratio of 2.06:1 for *Farfantepenaeus californiensis* (Huner and Colvin, 1977), 1:1 for *Penaeus japonicus* (Kanazawa *et al.,* 1984*),* and *Penaeus monodon* (Bautista, 1986), 1:1.7 for *Fenneropenaeus chinensis* (Li *et al.*, 1986).

CONCLUSIONS

With the results of the Experiment 1, the supplementary premix added to the diet was found to be an important contributor in increasing carapace width, length and molting frequency of the rice-field crabs. An amount of 3g kg^{-1} of T4 added to the formulated diet gave the highest growth in width, and length of carapace and molting frequency of the rice-field crabs. An increase in dietary premix to the feed diet significantly increased weight gained (WG), average daily growth rate (ADG), specific growth rate (SGR %) and survival rate (SR %). Supplementary premix is always required for a rapid growth of the rice-field crabs. It was found in the Experiment 2 that the highest values of final carapace width, length and molting frequency of the rice-field crabs were found with a ratio between Ca and P of 2.4:1.

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