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Effects of dietary protein levels on growth performance and feed utilization of juvenile rice-field crab (*Esanthelphusa dugasti*)

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To evaluate the effects of dietary protein on growth performance and feed utilization of juvenile rice-field crab, *Esanthelphusa dugasti*. Quadruplicate groups of rice-field crab (initial wet body weight = 0.0063±0.0004 g crab^{-1}; initial carapace width and length = 2.34±0.004 and 2.14±0.006 mm crab^{-1}, 25 crabs per tank) were fed with 30%, 35%, 40% and 45% of crude protein level diets. After 30 days, body weight gain and specific growth rate of the crab fed diet containing 40% of protein were significantly higher than the other groups ($P<0.05$). The group of dietaries containing 35% and 40% crude protein had the best feed conversion ratio (FCR). Significantly highest protein efficiency ratio (PER) were observed in rice-field crab fed diets containing 35% crude protein ($P<0.05$). Whereas survival rate and proximate composition in carcass of crab were not significantly influenced by different dietary protein levels ($P>0.05$). Therefore, the dietaries containing 35 to 40% crude protein are optimal for survival rate, growth performance and feed utilization of juvenile rice-field crab.

Keywords: Rice-field crab, protein level, growth performance, feed utilization

INTRODUCTION

The rice-field crab (*Esanthelphusa dugasti*), which is one of the freshwater crab, is an ecologically important species in the inland waters of Myanmar, Cambodia, Lao PDR, Vietnam and Thailand (Naiyanetr, 1978; Naiyanetr, 1982; Ng and Naiyanetr, 1993; Naiyanetr, 1994; Ng and Kosuge, 1995; Yeo and Quynh, 1999; Yeo, 2004; Cumberlidge et al., 2009; Cumberlidge et al., 2011). The crab, *E. dugasti*, belongs to the Parathelphusidae family that endemically distributes in northeast Thailand and also is an economically important species, which is considered as a source of cheap protein for Thai people (Yeo, 2004; Wangkahart and Wisespongpand, 2010). However, the rice-field crab is normally collected from the wild that are regarded as a potentially intermediate host to *Paragonimus* genus causing the paragonimiasis in human, as a final host (Yaemput et al., 1994; Blair et al., 1999; Odermatt et al., 2007; Uruburu et al., 2008). Moreover, the rice-field crab naturally captured from rice fields are contaminated with herbicides and pesticides used in agricultural activities; thus, consumption of the wild-caught crab has a risk to exposure to toxins in human (Cumberlidge et al., 2009).

Some evidences indicate that decreasing amounts of the freshwater crabs are mainly associated with habitat deterioration and pollution resulting from industrial and agricultural developments (Cumberlidge et al., 2009; Cumberlidge et al., 2011). In Thailand, the rice-field crab as the pests of rice seedling are eliminated by farmers using pesticides. Notably, some of them are reported as the threatened species of freshwater crabs by Cumberlidge et al*.* (2009)*.* Evidently, the threatened species of freshwater crab are accounted for 32*.*2% of total 101 species of freshwater crabs in Thailand (Cumberlidge et al*.*, 2009; Cumberlidge et al*.*, 2011)*.*

In the present, there is no any rice-field crab aquaculture; thus, the productivity of rice-field crab is completely obtained from the wild*.* Nevertheless, the survival rate of this wild-caught crab during nursing stage is very low that is due to a lack of the knowledge and information on nutrient requirement of the rice-field crab*.* Several published studies have investigated for the optimum protein requirements of some crabs, namely Chinese mitten crab (*Eriocheir sinesis*), mud crab (*Scylla serrata*) and swimming crab (*Portunus trituberculatus*) (Mu et al*.*, 1998; Catacutan, 2002; Lin et al*.*, 2010; Unnikrishnan and Paulraj, 2010; Jin et al*.*, 2013; Huo et al*.*, 2014; Nguyen et al*.*, 2014), except for rice-field crab*.* These studies indicate that the dietary protein is a key of success in the crab aquaculture*.*

The initial study, Mu et al*.* (1998) determined the effects of dietary protein levels, ranging from 29*.*8 to 54*.*8% crude protein, on growth performance of the juvenile Chinese mitten crab (initial body weight = 1*.*08 g crab^{-1})*.* At the end of 35-day trial, the crabs fed the diet containing 39*.*0% crude protein had the best growth rate, feed conversion ratio (FCR) and protein efficiency ratio (PER); however, the carcass protein content was not observed in this study*.* Based on the results of this study, the authors demonstrated that the optimal protein levels for growth of juvenile Chinese mitten crab were in the range of 39*.*0 to 42*.*5% crude protein*.* Recently, Chinese mitten crab (initial body weight = 3.56 g crab^{-1}) was investigated for the effects of graded levels of dietary protein on growth performance and feed utilization (Lin et al., 2010). The growth rate, PER and carcass protein content of Chinese mitten crab significantly increased with increasing levels of dietary protein up to 34*.*9% crude protein; besides, the lowest FCR value was significantly observed in the crab fed 34% crude protein (Lin et al*.*, 2010)*.* The other studies have reported the optimal levels of protein requirement of each species of crabs for the maximal growth, feed utilization and survival rate*.* For example, the optimal levels of crude protein in diets of mud crab were approximately 32 to 47%, recommended by Catacutan (2002), Unnikrishnan and Paulraj (2010) and Nguyen et al*.* (2014)*.* In case of juvenile swimming crab, the optimal levels of crude protein in diets of swimming crab were higher than those of mud crab that were approximately 50 to 51*.*5%, recommended by Jin et al*.* (2013) and Huo et al*.* (2014)*.* It is in accordance with the reports of Guillaume (1997) and NRC (2011), the optimal levels of dietary protein ranged from 20 to 60% of crude protein are required for growth of shrimps and other crustaceans*.*

The development of rice-field crab aquaculture is necessary to maintain the long-term sustainability of aquaculture and also produce safety food, in terms of non-toxic and non-parasitic crab*.* The aims of this present study were to determine the effects of graded levels of dietary protein on survival rate, growth performance and body composition of *E. dugasti* juvenile in order to assess the protein requirement for development of a feed formulation.

MATERIALS AND METHODS

Experimental diets

The experimental diets were formulated to meet the protein requirement for crustacean based on Guillaume (1997) and NRC (2011). Shrimp meal, chicken egg, soybean meal, corn gluten meal and soybean meal were used as protein sources. Four experimental diets formulated to contain graded levels of protein by 30%, 35%, 40% and 45% crude protein (Table 1). Diets were prepared as described previously by New (2002). In brief, blending various ingredients in a blender before whole (yolk and white) chicken egg and cow's milk were added, then stir thoroughly in the blender and cooking the mixture in a steamer until it solidified into a custard which was then screened to size relating the age of the crab. Steamed diets could be fed directly or they might be kept under 4℃ for a few days for later used. All experimental diets analyzed for chemical compositions according to AOAC (1990).

Table 1: Formulation and proximate composition of the experimental diets.

Ingredient	Dietary protein levels (%)			
	30	**35**	**40**	**45**
Chicken egg (CP: 12.9%)	30	30	25	25
Cow milk (CP: 3.4%)	10	10	10	5
Shrimp meal (CP: 45%)	20	25	30	35
Soybean meal (CP: 42%)	30	20	10	5
Corn gluten (CP: 75%)	10	15	25	30
Total	100	100	100	100
Proximate composition (%)				
Moisture	35.65	35.75	33.55	32.75
Protein	31.65	34.80	39.75	44.65
Lipid	6.15	5.75	6.25	6.55
Ash	10.35	10.20	9.95	9.85

Experimental animal and conditions

Juvenile *E. dugasti* (hatching form the egg) were obtained from the hatchery of Department of Fisheries, Faculty of Agriculture, Khon Kaen University in Thailand. The crabs were acclimatized for three days in a 500-L fiberglass tank filled with 5 cm of de-chlorinated tap water and equipped with aeration. During the acclimation period, the crabs were fed with the dietary egg custard. Prior to the experiment, newly molted crab were measured carapace width and length, and weighed. The experiment consisted of four treatments with four replicate tanks per treatment as follows protein levels. Four hundred juvenile crab (initial wet body weight = 0.0063±0.0004 g crab^{-1}; initial carapace width and length = 2.34±0.004 mm crab^{-1} and 2.14±0.006 mm crab^{-1}) was randomly distributed into fiberglass tanks (30x40x25 cm and water depth 5 cm) at a density of 25 crabs per tank. Each fiberglass tank was provided with twelve PVC pipe (3.5 cm diameter and 5 cm length) as substrate and hide-out for the juvenile crab, and was covered over by a black plastic mesh to reduce the light intensity. The fiberglass tanks were provided with continuous aeration throughout the experimental period to sufficiently maintain the dissolved oxygen level and hand-fed with the experimental diets loaded on the feeding trays to near satiety three times daily at 9:00 a.m., 1:00 p.m. and 5:00 p.m. for 30 days. Feeding rate at the start of experiment was 15% of total body weight. The amount of diets was adjusted according to body weight and appetite of the crab by checking feed residue in the tray remaining 4 h after the feeding.

The 30% the water was drained and replaced with de-chlorinated tap water every three days to maintain water quality. Water quality parameters were measure according to a standard protocol (APHA, 2012) during the experimental period. The water temperature, pH and dissolved oxygen (DO) were measured daily: temperature was 28±2℃, pH was 8.0±0.5 and DO was 4.5±0.5 mg L^{-1}. Ammonia nitrogen and nitrite nitrogen were measured weekly and monitored throughout the experiment to ensure that the concentrations did not exceed 0.1 mg L^{-1}.

Sample collection and chemical analysis

Uneaten feed was collected after feeding for 4 h, washed, and oven-dried at 60℃ to determine the weight of feed intake. Feces were siphoned off daily before the feeding. The deaths and feed intake were recorded daily.

The carapace width and length, and weight of the crab were measured and sixteen crabs were collected and stored at -20℃ until analyzed for initially chemical composition in carcass prior to start the experiment. At the end of 30-day feeding trial, all the crab were weighed and measured for the carapace width and length. Sixteen crabs from each treatment were collected and stored at -20℃ until analyzed for a finally chemical composition in carcass, such as moisture, protein, lipid and ash, according to AOAC (1990).

Calculation and statistical analysis

All data were calculated as follows: survival rate (SR, %) = Nf x 100/Ni, weight gain (WG, g) = (Wf - Wi), specific growth rate (SGR, %/day) = (ln Wf - ln Wi) x 100/T, feed conversion ratio (FCR) = Df/WG, protein efficiency ratio (PER) = WG/Dp, where Ni is the number of initial crabs, Nf is the number of final crabs, Wi is the initial body weight (g), Wf is the final body weight (g), T is the feeding trial period (day), FI is feed intake (g), Df is the dry feed intake (g), Dp is the dry protein intake (g). All the data were subjected to one-way analysis of variance followed by Duncan's multiple range tests. Significant differences were clarified at P<0.05.

RESULTS

Growth performance, survival rate and feed utilization

The result of feeding of graded levels of dietary protein ranging from 30 to 45% crude protein showed that growth rate of rice-field crab from all treatment groups continuously increased through the experiment period (Table 2). The weight gain and SGR of rice-field crab significantly increased with increasing levels of dietary protein at 40% crude protein (P<0.05); however, the weight gain and SGR decreased when the dietary protein levels increased up to 45% crude protein (Table 2). A group of rice-field crab fed the diet containing 40% had the highest body weight gain (0.81±0.07 g $crab^{-1}$) and SGR (16.23±0.43%). Survival rate of all treatments was not significantly different (P>0.05), ranging from 89 to 90% in all groups.

The FCR and PER of rice-field crab fed diets for 30 days in the present study were significantly different among treatments (Table 2). Nonetheless, two groups of rice-field crab fed diets containing 35% and 40% showed the lowest FCR as 0.78±0.09 and 0.79±0.06, respectively. PER of rice-field crab (3.74±0.40) fed the diet containing 35% was higher than the other groups.

Chemical composition in carcass

Chemical compositions (Table 3), such as moisture, protein, lipid and ash, in carcass of rice-field crab were not affected by feeding of different levels of dietary protein for 30 days.

Table 2: Growth performance and feed utilization of rice-field crab in four groups.

Parameters	Dietary protein levels (%)			
	30	35	40	45
Final body weight (g $crab^{-1}$)	$0.64±0.04^{b}$	$0.73±0.10^{ab}$	$0.82±0.06^{a}$	$0.68±0.03^{b}$
Final carapace width (mm $crab^{-1}$)	12.07±0.30	12.07±0.69	11.96±1.20	11.79±0.50
Final carapace length (mm $crab^{-1}$)	10.57±0.11	10.78±0.60	10.78±0.95	10.33±0.36
Weight gain (g $crab^{-1}$)	$0.63±0.04^{b}$	$0.72±0.10^{ab}$	$0.81±0.07^{a}$	$0.67±0.03^{b}$
Feed intake (g $crab^{-1}$ day^{-1})	0.61±0.03	0.61±0.06	0.68±0.04	0.64±0.05
SGR (%)	$15.29±0.22^{b}$	$15.81±0.40^{ab}$	$16.23±0.43^{a}$	$15.42±0.29^{b}$
FCR	$0.91±0.05^{c}$	$0.78±0.09^{a}$	$0.79±0.06^{ab}$	$0.90±0.05^{bc}$
PER	$2.95±0.18^{b}$	$3.74±0.40^{a}$	$3.01±0.25^{b}$	$2.35±0.15^{c}$
Survival rate (%)	89±3.8	89±3.8	89±3.8	90±2.3

Note: Values are presented as means ± SD of four replicates. SGR: specific growth rate, FCR: feed conversion ratio, PER: protein efficiency ratio. Values in the same row with different letters are significantly different (P< 0.05)

Table 3: Carcass proximate composition (%) of rice-field crab at the end of the experiment

Proximate composition (%)	Dietary protein levels (%)			
	30	35	40	45
Moisture	68.90±1.69	69.05±3.04	69.20±1.55	68.15±4.03
Protein*	41.00±1.83	39.90±1.83	41.65±2.05	41.20±1.97
Lipid*	9.05±1.06	9.20±1.13	8.85±0.21	7.90±1.13
Ash*	39.45±2.33	38.20±2.12	39.20±2.26	39.65±2.33

Note: Values are presented as means ± SD of four replicates. Significant (P<0.05) differences between groups are indicated by different letters. *Expressed as percent of dry weight basis.

DISCUSSION

Based on the increasing trends of weight gain and SGR in the rice-field crab with increasing levels of dietary protein up to 40%, the results in the present study indicates that rice-field crab could be nursed with diets containing 40% of crude protein for 30 days. This level of dietary protein for rice-field crab observed in the present study is similar to the optimal levels of dietary protein for mud crab and Chinese mitted crab studied by Mu et al. (1998), Catacutan (2002) and Nguyen et al. (2014). Nguyen et al. (2014) revealed that the mud crab (initial weight = 20.43±9.81 g crab^{-1}) fed diet containing 40% crude protein (soy protein concentrate as a main source of protein) for 80 days displayed the highest weight gain and the most carapace width. Catacutan (2002) reported although the mud crab (initial weight = 11.18±0.66 g crab^{-1}) could be fed dietary protein (fish meal as a main source of protein) ranging from 32-40%, feeding 40% crude protein in the diet with either 6% or 12% lipid promoted the highest growth rate and the most carapace width of the mud crab in the 30-day-nursing trial. Similarly, protein requirement of juvenile Chinese mitted crab (initial weight = 1.06±0.17 g crab^{-1}) was reported at 39.0% crude protein (Mu et al., 1998). Conversely, Unnikrishnan and Paulraj (2010) recommended that feeding the diet containing 45% crude protein (fish meal, squid meal and shrimp meal as sources of protein) for the best growth of the mud crab (initial weight = 0.25±0.051 g crab^{-1}) in the 63-day-nursing trial. According to findings of Jin et al. (2013) and Huo et al. (2014), swimming crab required higher levels of dietary protein (approximately 50-51% crude protein) than those of rice-field crab and mud crab for the best growth performance. Contrast to the findings of the present study and Mu et al. (1998), the diet containing 35.1% crude protein (fish meal, soybean meal and rapeseed meal as sources of protein), which was lower than that of the present study, was suitable for the best growth of juvenile Chinese mitted crab recommended by Lin et al. (2010). This difference in optimum dietary protein levels for well growth of rice-field crab and the other crabs may be caused by many factors, including species, size, temperature, feed formulation, stock density, protein sources, quality and levels of non-protein energy in the diets (Guillaume, 1997; Lin et al., 2010; Jin et al., 2013; Huo et al., 2014). The optimum protein requirement level for juvenile rice-field crab in the present study was in the range of protein requirement levels for crustacean (Guillaume, 1997; NRC, 2011).

There was no a significant difference in survival rate of the rice-field crab fed graded levels of dietary protein, the survival rate ranged 89-90%. The results indicated that the protein levels were considered to be in the satisfied range for crustacean; therefore, this indicated that the experimental feed formulation in the present study was acceptable (Cuzon and Cahu, 1994). The mortality occurred in the present study results from cannibalism and incomplete molting of rice-field crab (Catacutan, 2002; Unnikrishnan and Paulraj, 2010).

Although the feed intake of rice-field crab were not affected by feeding different levels of dietary protein; feed utilization (FCR and PER) was statistically observed in the present study. FCR of rice-field crab fed diets graded dietary protein (30-45% crude protein) ranged from 0.78 and 0.91. The high FCR observed in the rice-field crab fed with diet contained 30% crude protein may be associated with intake of insufficient nutrient levels for raising the growth of crabs (Lin et al., 2010). This finding has been reported in Mu et al. (1998), Catacutan (2002), Unnikrishnan and Paulraj (2010) and Nguyen et al. (2014). The PER values of rice-field crab increased significantly with increasing levels of dietary protein up to 40% crude protein. However, feeding higher levels of dietary protein (45% crude protein) significantly reduced the PER value. This finding in the present study accorded with the studies of Mu et al. (1998), Lin et al. (2010), Unnikrishnan and Paulraj (2010) and Jin et al. (2013). The adverse impact on PER value related to feeding higher level of dietary protein than the optimal levels of dietary protein requirement for growth of the rice-field crab could be that the excess protein level was used as energy (Lin et al., 2010; Jin et al., 2013). Besides, Catacutan (2002) and Unnikrishnan and Paulraj (2010) explained that a negative relationship between intake of increasing protein levels above the optimal protein levels and declined PER led to catabolism of excess protein resulting in accumulation of ammonia nitrogen and free amino acids. This could become toxic which affected normal metabolism and growth of the crabs.

Previous studies have demonstrated that protein and lipid contents in the carcasses of several crab species increased significantly with increasing levels of dietary protein (Lin et al., 2010; Unnikrishnan and Paulraj, 2010; Jin et al., 2013). In contrast, chemical composition (e.g. protein, lipid, ash and moisture) in the carcass of the rice-field crab was not significantly influenced by feeding graded levels of dietary protein in the present study. In accord with chemical composition in the carcasses of Chinese hairy crab and mud crab studied by Mu et al. (1998) and Lin et al. (2010) did not significantly gain when feeding of increasing levels of dietary protein.

CONCLUSION

Feeding diets containing 35% of dietary protein resulted in the most efficient feed utilization of rice-field crab. Nevertheless, feeding diets containing 40% crude protein provided the highest growth rate of rice-field crab. The finding of the present study, effects of feeding diets containing graded levels of dietary protein for 30 days in nursing of the rice-field crab on growth performance and feed utilization indicate that dietary protein levels ranging from 35 to 40% are the most suitable for nursing of rice-field crab

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

The authors' responsibilities were as follows: SW conceptualized the study; PW, WP and SD designed and performed the experiments. PW, WP, KT and BY conducted research; PW, KT and BY provided the materials; PW, WP, KT and SD collected the data; PW, WP, BY and SW analyzed the data; PW, BY and SW wrote the manuscript. WP and SD provided comments and suggestions for improvements. All authors read and approved the final version.

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