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Growth performance, feed efficiency and whole-body chemical composition of the oriental river prawn, *Macrobrachium nipponense*, fed different dietary protein to lipid ratio

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Abstract

A 56 days grow-out experiment with three protein levels (35, 40 and 45%) and three lipid levels (5, 10 and 15%) was conducted to evaluate the optimum protein to lipid ratio for oriental river prawn (*Macrobrachium nipponense*). Four hundred and five prawns, with mean (± SD) weight of 1.40 ± 0.04 g were distributed in 27 glass aquaria and were fed four times daily. Results showed that interaction between protein and lipid was significantly affected all of the growth indices (p<0.05). Prawns fed the diet containing 45% protein and 5% lipid showed the highest growth rate, protein and lipid efficiency ratios and protein and lipid productive values with the lowest feed conversion ratio. Increasing dietary protein levels reduced body moisture and protein content increased but had no significant effect on lipid content (p>0.05). The results of this study showed that oriental river prawn represents the best performance at level of 45% protein and 5% lipid.

Keywords: Nutrients requirements, Feed conversion ratio, Body composition, *Macrobrachium nipponense*

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Introduction

The oriental river prawn (*Macrobrachium nipponense*) is naturally distributed in China, Japan, Korea, Vietnam, Myanmar and Taiwan (Cai and Ng, 2002; De Grave and Ghane, 2006; New, 2009), its culture started in Southeast Asian countries at the 1990s, and at present China is the largest producer in the world with 203033 metric tons (Mt) in 2014 (FAO, 2014). The annual world production of freshwater prawns (without crayfish and crabs) has significantly increased from 50000 Mt in 1995 to > 496202 Mt in 2014 (FAO, 2014). Despite its small size, it has great capability to be cultured (Sun *et al.,* 2016). It can tolerate cold winter weather, then has greater survival and growth rates compared to the *M. rosenbergii* in the larval stage (De Barros and Valenti, 2003), it is a rival for the *M. rosenbergii* in the prawn culture industry (Freeman, 1990; Maclean and Brown, 1991); and suitable species for culturing where there is brackish, low salinity or freshwater (Ma *et al.,* 2016).

Feeding costs are one of the expensive items in prawn culture and constitute 40-50% of the total operational costs (Cui *et al.,* 2016). At present, the main challenge in commercial aquaculture is improvement of feed formulations for optimizing growth and increasing prawn health (Ding *et al.,* 2015). The achievement of commercial production of freshwater prawns depends on the affective use of available food resources (Koshio *et al*., 1993; Du and Niu, 2003). Various factors may influence the efficiency of prawn culture, but reducing mortality rates or reducing pathogenic agents, is one of the important points (De Araujo and Valenti, 2007). Raising productivity in prawn culture depends on feed formulations and nutrients that provide energy (Keysami *et al*., 2007).

It is necessary to determine the desirable level of proteins in formulated diet of the prawns in order to make optimal use of protein and reduce costs (Jin *et al.,* 2013). Overuse of protein reduces the efficiency, leads to unreasonable rises in diet costs (Abdel-Tawwab and Ahmad, 2009), and increases ammonia in the environment (Jacinto *et al*., 2004), which increases the pollution and reduces water quality (Kim *et al.,* 2012). While, insufficient amount of dietary protein prevents production of new tissues and retards growth (Kuhn *et al.,* 2016). Therefore, it is emphasized that levels of the 10 essential amino acids for prawns should never be less than the minimum required levels (Yoshikawa *et al.,* 2016). Despite terrestrial animals, aquatic animals including crustaceans need high dietary protein levels around 24-57% (Bages and Sloane, 1981; Shu *et al.*, 2016), for instance, 27% protein requirements for *Portunus tritubunus* (Huo *et al.,* 2014), 29-30% for Red swamp crayfish *Procambarus clarkia* (He *et al*., 2009), 27% for black tiger shrimp *Penaeus monodon* (Hajra *et al.,* 1988), 24% for *Portunus pelagicus* (Serang *et al.,* 2007), 21% for *Litopenaeus vannamei* (Hu *et al.,* 2008) and 32% for juvenile Australian red claw crayfish, *Cherax*

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quadricarinatus (Cortés *et al.*, 2005). Most commercial prawn feeds are formulated for intensive culture systems with protein content of 35-50% (Alava and Lim, 1983; Xie *et al*., 2007). However, optimum dietary protein levels of freshwater prawns depend on numerous factors such as age, feeding level, protein quality, dietary lipid level and quality of ingredients (Shiau, 1998; Goda, 2008). Prawns cannot synthesize sterols and, therefore, must obtain them from external sources to survive (Yao *et al*., 2006). Energy intake through lipids reduces the need for breaking down protein to produce energy, and protein can be used for new cells and tissues to grow (Sheen and D'Abramo, 1991). Lipids are the main energy source for aquatic organisms that have less ability in utilizing carbohydrates as energy source (Goda, 2008). Moreover, excessive lipids, raise the concern that it will accumulate in the carcass and viscera of the prawns, reduce the quality of prawns and production efficiency because of lacking the ability to emulsify excess lipids (Chou *et al*., 2001).

Determining protein and lipid requirements helps to formulate a diet for rapid growth of aquatic organisms (Brauge *et al*., 1995). Goda (2008) studied the effects levels of proteins and lipids in diet on the post larval stages of freshwater prawns (*M. rosenbergii*), in which a diet containing 30% proteins and 10% lipids recommended for this species. Noveirian *et al*. (2012) used five treatments of 0, 3, 6, 9, and 12% lipids and a constant level (40%) of protein in the diet of oriental river prawn. The results indicated the best growth was achieved in diet containing 9% lipids, and increasing the level of lipids in the diet reduced body moisture and increased carcass lipid. Moreover, body protein increased in the treatments with diets containing 9 and 12% lipid.

The aim of the present study was to formulate a diet for the oriental river prawn with a suitable protein: lipid ratio according to culture condition considering features of economical and physiological point of views. To achieve this, the effects of three different levels of protein and lipid were investigated on growth and production of *M. nipponense*.

Materials and methods

Prawn culture conditions

This study was conducted at the aquaculture laboratory of the Faculty of Matural Resources, University of Guilan in Sowmeh Sara (37°20′N, 49°20′E, Guilan, Iran) for 8 weeks from July to September 2015. The studied prawns with the mean weight of 1.40 ± 0.04 g and length of 5 ± 0.15 cm were caught in the Siah Darvishan River (37°25′N, 49°30′E, Guilan, Iran) and transferred to the experimental site.

The prawns were kept in a 300-liter fiberglass tank for 2 weeks to become acclimatized to the experimental conditions. During this period, they were fed *ad libitum* with a commercial trout diet (50% protein, 13% lipids, 12% ash, 10-12% moisture, 1549 KJg^{-1}, 0.5-0.9-mm crumble; Chineh Company, Hashtgerd, Alborz, Iran). After the acclimatization period, 15 prawns were put in each of 27 glass aquaria with

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length, width and height of 70, 40, and 45 cm, respectively. Each aquarium contained 60 L of tap water that had been continuously aerated for 24 hours for dechlorination. Aeration of the aquaria continued through the experiment using air-stone connected to a central aerator (Alberta, Washington DC, USA). Each day, one third of the water in each aquarium was replaced with fresh dechlorinated water before feeding.

A 12:12 h light:dark schedule was used during the culture period, and the light source was a fluorescence lamp turned on during the day and off at night. Water quality that included dissolved oxygen (mg L^{-1}), temperature (°C), and chlorine were measured daily, and other parameters such as total ammonia, water hardness, and pH during each bioassay test using laboratory kits and digital instruments. A heater (Sonpar, Guangzhou, China) was used to keep the temperature of the water in the aquariums. A digital WTW pH meter (340I SET^{-1}, Oberbayern, Germany) was used to measure pH. Dissolved oxygen in water was measured with a digital WTW instrument (OXi 340 SET^{-1}, Oberbayern, Germany) and chlorine with a Pooltester chlorine laboratory kit (AF 10 HR 6, Tehran, Iran). A BTM laboratory kit (5229, Tehran, Iran) was employed to measure water hardness and a BTM instrument (5548, Tehran, Iran) to measure total ammonia, according to APHA (1989). During the experiment, temperature was kept at 24.5±0.05 °C, dissolved oxygen at 7.00±0.03 mg L^{-1}, pH at 6.25±0.03, chlorine at less than 0.2 mg L^{-1}, hardness at 124.6 mg L^{-1}, and total ammonia at 0.043±0.021 mg L^{-1}.

Diet preparation and experimental design

The prawns were divided into 9 treatments (each with 3 replications) that included 9 diets with different levels of protein (35, 40, and 45%) and lipids (5, 10, and 15%) and were fed for 8 weeks. They received feed five times a day (06:00, 10:00, 14:00, 18:00 and 22:00 hours) (Etefaghdost *et al.*, 2015). Total daily feed was initially set at 2% of body weight in each experimental unit (Ettefaghdoost, 2015).

Table 1 lists the compositions and the approximate analyses of the experimental diets. The ingredients of each diet, except for the fish oil, were mixed for 10-15 minutes, the fish oil was then added, and the mixture was stirred again for 10-15 minutes. Ultrapure water Merck Millipore® (Sigma-Aldrich, St. Louis, USA) equaling 30% of the dry matter weight was also added to the diet. The mixture was turned into strands with the diameter of 2-2.5 mm using a meat grinder. The pellets were distributed uniformly on trays and put in an oven at 50 °C for 12 hours to dry completely. While drying, the trays were taken out of the oven systematically, the strands were stirred to become uniformly warm, and the trays were put back in the oven. The dried strands were grounded and kept at -16 °C. The daily feed was put in a refrigerator at 4 °C.

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Table 1: Composition and proximate analysis of experimental diets for the oriental river prawns (*Macrobrachium nipponense*).

	Experimental diets								
Protein (%)		**35**			**40**			**45**	
Lipids (%)	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**
Ingredients (%)									
Fish meal[1]	30	30	30	30	30	30	30	30	30
Soy meal	30	30	30	30	30	30	30	30	30
Wheat meal	10	10	10	10	8	8	7	7	6
Corn meal	10	10	10	10	8	8	7	7	6
Fish oil[1]	0	5	10	0	5	10	0	5	10
Casein[2]	0	0	0	9	9	9	16	16	16
Vitamin premix [3]	1	2	2	2	2	2	2	2	2
Mineral premix [4]	2	2	2	2	2	2	2	2	2
Filler (CMC) premix[5]	16.4	10.4	5.4	6.4	5.4	0.4	5.4	0.4	0.4
Vitamin C[6]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Dicalcium phosphate[7]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (%)									
Moisture	8.03 ± 0.90	7.74 ± 0.60	8.25 ± 0.70	9.12 ± 0.50	9.07 ± 0.23	9.00 ± 0.84	9.42 ± 0.50	8.72 ± 0.12	9.06 ± 0.71
Crude protein	34.73 ± 0.41	34.56 ± 0.63	34.68 ± 0.71	39.81 ± 0.54	39.69 ± 0.78	40.00 ± 0.32	44.65 ± 0.23	44.37 ± 0.45	44.64 ± 0.12
Crude lipid	4.83 ± 0.23	9.71 ± 0.59	14.33 ± 0.58	4.01 ± 0.61	9.15 ± 0.83	15.27 ± 0.15	4.69 ± 0.96	10.73 ± 0.90	15.43 ± 0.87
Ash	13.26 ± 1.67	14.88 ± 1.43	15.84 ± 1.55	14.79 ± 1.69	14.94 ± 1.86	15.79 ± 1.66	13.99 ± 1.75	16.23 ± 1.88	15.86 ± 1.49
Gross energy (KJ g^{-1})	1693 ± 0.49	1736 ± 0.62	1887 ± 2.27	1826 ± 0.19	1906 ± 0.41	1895 ± 2.14	1852 ± 0.66	1912 ± 0.71	2088 ± 1.46

[1] The Iranian Fisheries Research Organization (Bandar Anzali, Iran)
[2] The Milatech Company (Mashhad, Iran)
[3] The Science Laboratories (Qazvin, Iran). Each 1000 g premix contained 160000 IU vitamin A, 400000 IU vitamin D_3, 40 g vitamin E, 2 g vitamin K_3, 6g vitamin B_1, 8 g vitamin B_2, 12g vitamin B_3, 40 g vitamin B_5, 4 g vitamin B_6, 2g vitamin B_9, 60 g vitamin C, 20 g vitamin B_7, 20 g BHT
[4] The Science Laboratories (Qazvin, Iran).Each 1000 g mineral complement contained 20 g Fe, 60 g Zn, 4000 mg Se, 2000 mg Co, 5000 mg Cu, 4000 mg Mn, 80 mg I, 80000 mg Choline Chloride
[5] The Kimia Tehran Acid Company (Tehran, Iran)
[6] The Science Laboratories (Qazvin, Iran). Each 1000 g vitamin C contained 500 g Stay-C
[7] The Arastaban Company (Tehran, Iran)

Growth indices

Biometrics tests for all of the prawns were conducted at the culture period and every two weeks interval. To perform the tests, feeding was stopped for 24 hours and the prawns were then individually weighed using an electronic balance (Sartorius, Göttingen, Germany) with accuracy of 0.01g. Growth and feed efficiency indices including average weight gain (WG), percentage body weight increase (BWI), survival rate (SR), feed conversion ratio (FCR), specific growth rate (SGR), average daily growth (ADG), protein efficiency ratio (PER), lipid efficiency ratio (LER), protein productive value (PPV), and lipid productive value (LPV) were calculated as follows:

WG (g)=final body weight (g)-initial body weight (g)

BWI (%)=100×(WG(g)/initial weight (g))

ADG (g day^{-1})= ((WG (g)/initial weight (g)) × culture period (days))

SGR (% day^{-1}) = 100 × [Ln (final body weight)-Ln(initial body weight)]/ culture period (days)

FCR = feed intake (g)/weight gain (g)

PER(g)=weight gain (g)/protein intake (g)

LER (g)=weight gain (g)/lipid intake (g)

PPV (%)=100×(protein gain (g)/protein intake (g))

LPV (%)=100×(lipid gain (g)/lipid intake (g))

SR (%)= 100×(final number of prawns/ initial number of prawns)

Analytical methods

To determine the nutritional values of the experimental diets and proximate

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whole body composition, samples were taken from each treatment and determined according to procedures of AOAC (1995). Prawns were randomly sampled from each experimental treatment (four prawns in each replicate) and transferred to the Nutrition Laboratory. Crude protein was measured by Kjeldahl method (N × 6.25; Bakhshi, Tehran, Iran) and crude lipid by Soxhlet method using the diethyl ether solvent (40-60 °C) and moisture by drying the samples in an oven at 102±2 °C until they reached constant weights. The ash content was measured by putting the samples in an electric furnace at 550 °C for 8 h, and gross energy contents of the diets were calculated using the ADCP (1983) standard.

Statistical analysis

The Kolmogorov-Smirnov test was first used to check the normality of the data, and the Levene test for the homogeneity of the variances. Two-way ANOVA with interaction and Tukey's test were then employed to compare the significance of differences between the means. All tests were performed using SPSS Version 22 (IBM Co., New York, USA) at the confidence level of 95%. Data in the text were expressed as mean ± standard deviation (SD).

Results

Fig. 1 shows the effects of proteins and lipids in the diet on the growth of oriental river prawns. The protein and lipid levels, and also their effects, significantly influenced growth (Table 2, $p<0.05$).

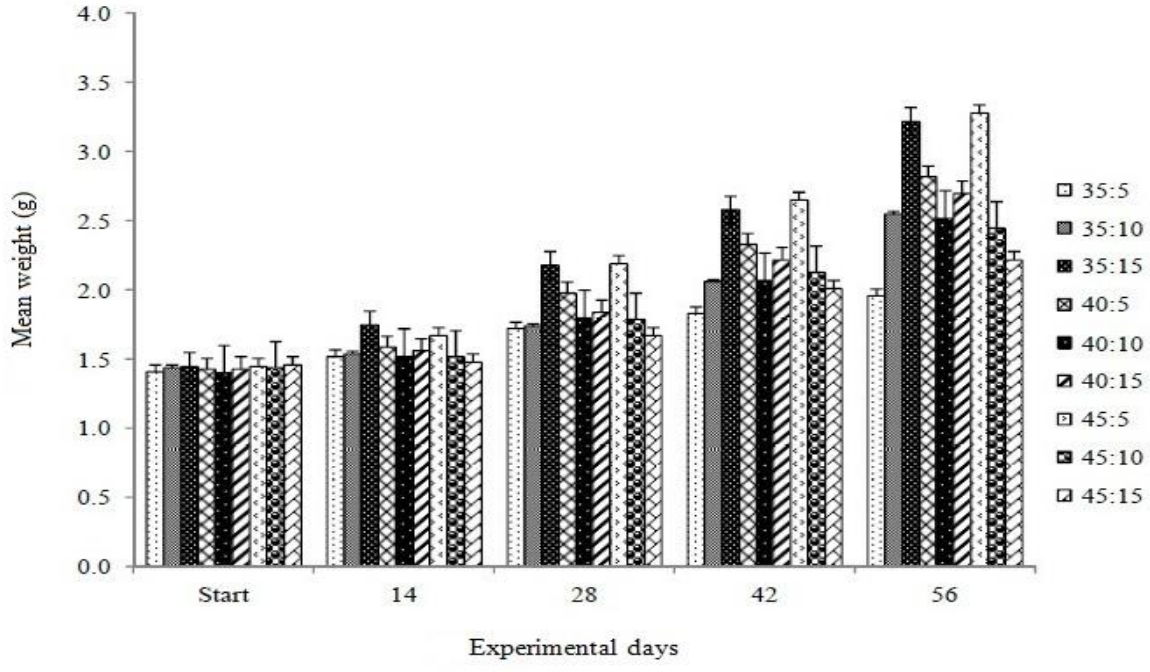


Figure 1: Mean individual weight of oriental river prawn, *Macrobrachium nipponense* fed different experimental diets during the culture period (every 2-week measurements) (mean± standard deviation).

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Table 2: Growth performance of oriental river prawn, *Macrobrachium nipponense* fed different experimental diets for 56 days (mean± standard deviation, n=3).

Proteins: lipids ratio	FW (g)	WG (g)	BWI (%)	ADG (%)	SGR (%/day)	FCR	SR (%)
35:5	1.96 ± 0.05 ^{f}	0.56 ± 0.01 ^{f}	40.00 ± 0.72 ^{f}	0.71 ± 0.01 ^{f}	0.26 ± 0.01 ^{e}	2.94 ± 0.39 ^{ab}	73.33 ± 1.83 ^{d}
35:10	2.55 ± 0.04 ^{d}	1.15 ± 0.00 ^{c}	82.15 ± 0.47 ^{cd}	1.48 ± 0.02 ^{cd}	0.46 ± 0.02 ^{c}	2.23 ± 0.35 ^{c}	66.67 ± 1.12 ^{de}
35:15	3.22 ± 0.06 ^{a}	1.82 ± 0.02 ^{a}	130.00 ± 0.6 ^{a}	2.32 ± 0.01 ^{a}	0.65 ± 0.01 ^{a}	1.39 ± 0.02 ^{d}	88.90 ± 2.22 ^{c}
40:5	2.82 ± 0.05 ^{b}	1.42 ± 0.01^{b}	101.43 ± 0.6 ^{b}	1.81 ± 0.02 ^{b}	0.54 ± 0.01 ^{b}	1.79 ± 0.03 ^{cd}	91.11 ± 1.10 ^{b}
40:10	2.52 ± 0.04 ^{cd}	1.12 ± 0.00 ^{c}	80.00 ± 0.32 ^{cd}	1.42 ± 0.02 ^{cd}	0.46 ± 0.01 ^{c}	2.34 ± 0.06 ^{bc}	73.33 ± 1.50 ^{d}
40:15	2.70 ± 0.05 ^{bc}	1.30 ± 0.01^{ab}	92.86 ± 0.55 ^{c}	1.67 ± 0.05 ^{c}	0.51 ± 0.02 ^{b}	1.99 ± 0.04 ^{cd}	75.56 ± 1.61 ^{d}
45:5	3.28 ± 0.04 ^{a}	1.88 ± 0.00 ^{a}	134.29 ± 0.55 ^{a}	2.39 ± 0.03 ^{a}	0.66 ± 0.02 ^{a}	1.32 ± 0.04 ^{d}	97.78 ± 1.00 ^{a}
45:10	2.45 ± 0.04 ^{d}	1.05 ± 0.00 ^{d}	75.00 ± 0.54 ^{d}	1.34 ± 0.01 ^{d}	0.43 ± 0.03 ^{c}	2.39 ± 0.03 ^{abc}	60.00 ± 2.53 ^{e}
45:15	2.22 ± 0.04 ^{e}	0.82 ± 0.00 ^{e}	58.57 ± 0.75 ^{e}	1.05 ± 0.05 ^{e}	0.36 ± 0.01 ^{d}	3.04 ± 0.03 ^{a}	64.50 ± 1.80 ^{de}
Two-way ANOVA							
Proteins	0.000	0.000	0.000	0.000	0.000	0.000	0.421
Lipids	0.000	0.000	0.000	0.000	0.000	0.058	0.001
Proteins × lipids	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Values with different letters show significant differences between various columns (p<0.05)

FW (F=320.906, df=3, p<0.001), WG (F=381.500 df=3, p<0.001), BWI (F=276.736, df=3, p<0.001), ADG (F=26.482, df=3, p<0.001), and SGR (F=17.077, df=3, p<0.001) were significantly influenced by protein levels. The treatment with 45% protein showed the greatest difference with the other treatments. Moreover, final weight (F=302.246, df=3, p<0.001), WG (F=597.500, df=3, p<0.001), BWI (F=897.11, df=3, p<0.001), ADG (F=147.511, df=3, p<0.001), SGR (F=321.000, df=3, p<0.001) were significantly affected by protein and lipid levels. Treatments with 35% protein and 15% lipid, and with 45% protein and 5% lipid, were significantly different from the other treatments.

Dietary protein and lipid levels separately and in combination, significantly influenced SR of oriental river prawns (Fig. 2, p<0.05). The highest SR was observed in 45% protein and 5% lipid, and the lowest in 45% protein and 10% lipid, and these two rates were significantly different from those of the other treatments (p<0.05).

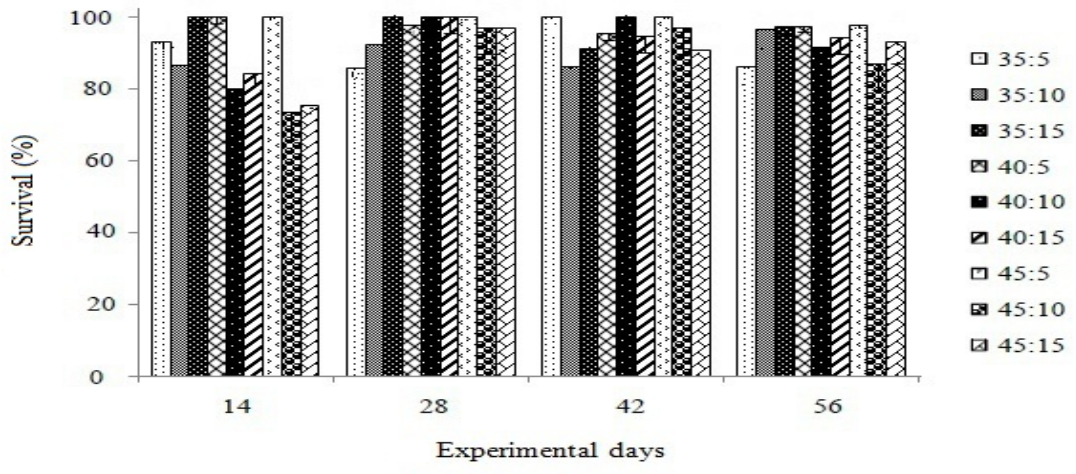


Figure 2: Survival rate of oriental river prawn, *Macrobrachium nipponense* fed different experimental diets during the culture period (every 2-week measurements) (mean± standard deviation).

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Results in Table 3 indicated that PER was significantly affected by protein and lipid levels in the diet (F=53.032, df=3, p<0.001). Increases in protein level of the diet significantly increased PER, and the maximum was belonged to the treatment with 45% protein and 5% lipid (F=6.435, df=3, p=0.008). However, decreases in the lipid level of the diet significantly reduced PER, and the lowest was observed in the treatment with 35% protein and 5% lipid (F=6.145, df=3, p=0.009).

Table 3: Feed efficiency indices of oriental river prawn, *Macrobrachium nipponense* fed different experimental diets for 56 days (mean± standard deviation, n=3).

Protein: lipid ratio	PER (%)	LER (%)	PPV (%)	LPV (%)
35:5	0.98 ± 0.03 ^{f}	1.67 ± 0.01 ^{cd}	5.68 ± 0.80 ^{e}	5.66 ± 2.97 ^{c}
35:10	1.11 ± 0.01 ^{bc}	1.71 ± 0.02 ^{cd}	8.70 ± 0.16 ^{abc}	13.95 ± 3.55 ^{bc}
35:15	1.17 ± 0.03 ^{ab}	1.74 ± 0.03 ^{c}	9.88 ± 0.50 ^{ab}	14.03 ± 1.93 ^{bc}
40:5	1.12 ± 0.03 ^{bc}	2.91 ± 0.04 ^{b}	7.96 ± 1.18 ^{bcd}	16.00 ± 1.65 ^{b}
40:10	1.07 ± 0.02 ^{cde}	1.68 ± 0.03 ^{cd}	10.18 ± 0.60 ^{a}	7.93 ± 0.14 ^{bc}
40:15	1.10 ± 0.04 ^{bcd}	1.49 ± 0.03 ^{e}	9.36 ± 0.40 ^{abc}	10.82 ± 1.49 ^{bc}
45:5	1.24 ± 0.02 ^{a}	3.53 ± 0.01 ^{a}	10.09 ± 0.60 ^{ab}	29.00 ± 3.23 ^{a}
45:10	1.03 ± 0.01 ^{def}	1.62 ± 0.01 ^{c}	7.41 ± 1.30 ^{cde}	6.38 ± 2.77 ^{c}
45:15	1.01 ± 0.03 ^{ef}	1.26 ± 0.06 ^{f}	6.21 ± 0.45 ^{de}	9.73 ± 2.72 ^{bc}
Two-way ANOVA				
Protein	0.008	0.000	0.004	0.000
Lipid	0.009	0.000	0.073	0.000
Proteins×lipids	0.000	0.000	0.000	0.000

Values with different letters show significant differences between various columns (p<0.05)

LER was influenced by protein and lipid levels in the diet (F=1287.297, df=3, p<0.001). With increasing the protein content of the diet, LER significantly increased (F=499.116, df=3, p<0.001), while decreasing lipid content of the diet significantly increased LER. The maximum LER was observed in treatment with 45% protein and 5% lipid, which was significantly different from those of the other treatments (F=4083.523, df=3, p<0.001).

PPV was also significantly affected by protein and lipid levels in the diet (F=24.864, df=3, p<0.001). With increasing protein content of the diet, PPV significantly increased and reached maximum in the treatments with 40% protein and 10% lipid and 45% protein and 5% lipid, which were significantly different from those of the other treatments (F=7.483, df=3, p=0.04). PPV increased with increases in lipid, but there were no significant differences between the treatments (F=3.042, df=3, p=0.073).

LPV was influenced by protein and lipid levels in the diet (F=49.491, df =3, p<0.001). Increases in the protein content of the diet significantly increased LPV (F=20.462, df=3, p<0.001), but decreases in lipid content of the diet significantly increased LPV and reached maximum in the treatment with 45% protein and 5% lipid (F=38.201, df =3, p<0.001).

The mutual effects of protein and lipid levels influenced FCR (F=43.132, df=3, p<0.001); with increases in protein

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content FCR significantly declined and reached its minimum values of 1.39 and 1.32 in the treatments with 35% protein and 15% lipid and 45% protein and 5% lipid, respectively. These minimum values were significantly different from those of the other treatments (F=19.944, df=3, p<0.001). FCR did not significantly change at different lipid contents of the diet (F=1.723, df=3, p=0.058).

Table 4 shows the effects of various diets on the biochemical body compositions in oriental river prawns. Body moisture content was influenced by the mutual effects of protein and lipid contents of the diet (F=7.091, df=3, p=0.001). With increasing protein level in the diet, body moisture significantly declined and reached minimum in the treatment with 45% protein and 5% lipid (F=8.893, df=3, p=0.002). However, body moisture significantly increased with increasing lipid level of the diet and reached maximum in the treatment of 35% protein and 10% lipid (F=9.414, df=3, p=0.001). Body protein content was affected by the mutual effects of dietary protein and lipid levels (F=25.009, df=3, p<0.001), but body lipid was not influenced by the various protein levels (F=1.527, df=3, p=0.241). Mutual effects of protein and lipid contents were significant on body lipid content (F=22.562, df=3, p<0.001), which increased significantly with increasing lipid content of the diet (F=22.80, df =3, p<0.001), but dietary protein level did not significantly affect the body lipid (F=3.417, df=3, p=0.53). The mutual effects of dietary protein and lipid levels significantly influenced body ash content (F=26.184, df=3, p<0.001), which rose with deceasing dietary protein and lipid levels and reached maximum in the prawns fed by 35% protein and 5% lipid (F=27.957, df=3, p<0.001; F=108.219, df=3, p<0.001).

Table 4: Proximate whole carcass composition of oriental river prawn, *Macrobrachium nipponense* fed different experimental diets for 56 days (mean± standard deviation, n=3).

Protein: lipid ratio	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Start of the culture period	75.68 ± 0.22	10.76 ± 0.27	2.71 ± 0.40	7.40 ± 0.23
35:5	74.44 ± 0.47 ^{ab}	13.54 ± 0.39 ^{e}	3.11 ± 0.21 ^{c}	7.85 ± 0.26 ^{a}
35:10	75.10 ± 0.24 ^{a}	15.02 ± 0.08 ^{cd}	4.66 ± 0.50 ^{ab}	6.50 ± 0.42 ^{bc}
35:15	72.37 ± 0.20 ^{bcd}	15.60 ± 0.25 ^{bcd}	5.65 ± 0.41^{a}	5.86 ± 0.32 ^{cd}
40:5	72.10 ± 0.49 ^{cd}	15.22 ± 0.66 ^{bcd}	3.83 ± 0.12 ^{bc}	7.11 ± 0.10 ^{ab}
40:10	73.40 ± 0.38 ^{abcd}	16.46 ± 0.34 ^{ab}	3.82 ± 0.02 ^{bc}	5.77 ± 0.33 ^{cd}
40:15	72.22 ± 0.55 ^{cd}	16.00 ± 0.23 ^{abc}	4.98 ± 0.31 ^{a}	5.49 ± 0.33 ^{de}
45:5	71.65 ± 0.27 ^{d}	17.11 ± 0.38 ^{a}	5.65 ± 0.44 ^{a}	4.44 ± 0.09 ^{f}
45:10	73.62 ± 1.85 ^{abcd}	15.43 ± 0.82 ^{bcd}	3.60 ± 0.39 ^{c}	4.76 ± 0.25 ^{ef}
45:15	73.86 ± 0.76 ^{abc}	14.67 ± 0.29^{de}	4.75 ± 0.57 ^{ab}	5.41 ± 0.26 ^{de}
		Two-way ANOVA		
Protein	0.002	0.000	0.053	0.000
Lipid	0.001	0.241	0.000	0.000
Proteins × lipids	0.001	0.000	0.000	0.000

Values with different letters show significant differences between various columns (p< 0.05)

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Discussion

During the period of this study, there was no significant difference in the water quality of experimental treatments. The water qualities were pointed out within the acceptable range for the species of prawn. Diets were designed with using extremely digestible ingredients resembling to those used in experimental diets for prawns and other decapods crustaceans (Zhang *et al.,* 2013). The results of the various studies show that increasing of dietary protein with the optimum lipid levels, increase and improve growth factors. If the dietary protein levels were insufficient, it will affect on culture conditions and decline the growth parameters of prawns. This indicated the desirability of diets with high protein content for feeding of this species. These results confirm the statement reported by Brauge *et al*. (1995) that reducing the protein content in the diet of *M. rosenbergii* decreased the growth rate. In the present study, results confirm the principle that using a diet with optimum protein level, increases energy levels attended with increasing of growth indices and the desired level of protein was observed in the treatment with 45% dietary protein. Koshio *et al.* (1993) studied the effects of various quantities of protein at constant energy levels on growth, digestibility, and excretory nitrogen in 4 g Japanese shrimp (*P. japonicus*) that used five different diets with constant energy level and different protein contents (from 21 to 61%) and found no significant differences in SGR, WG, and FCR, which those results were different with the present study. Moreover, Vijayagopal *et al*. (2009) conducted experiments on Indian white shrimps (*P. indicus*) in which juvenile shrimps (12-15 mm length) were fed various levels of protein (from zero to 60%) for 30 days. Results showed that the optimum level of protein requirement was 30%, which results agree with those we obtained in our study. Xie *et al*. (2007) fed oriental river prawns (mean weight 0.28±0.07g) with different levels of protein (28-44%). Prawns that received a diet with 40% protein had the maximum weight and the minimum FCR, and that protein levels of 37.8 to 40.3% reached the maximum WG, the minimum FCR, and the highest digestive enzyme activity and immunity. In another experiment, Zhang *et al*. (2008) investigated the effects of various dietary levels of protein (30.17 to 45.45%) on growth of oriental river prawns with the mean weight of 0.067 g. Diets with 39.32 to 41.67% crude protein had higher growth rates compared to the other treatments. Jacinto *et al*. (2004) studied the effects of 7 diets with different protein contents (20, 25, 31 ,37, 43, 49, and 55%) on the growth of juvenile *Cherax quadricarinatus* and they observed the highest growth indices in lobsters fed diets with 20 and 31% protein. Comparison that, with those obtained in our study on oriental river prawns indicates that the best growth indices are not achieved by excessive use of protein together with lipid, that are optimal levels of protein and lipid for each species, and that exceeding these optimal levels lead to reduction in

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growth indices (Cortés and Hernandez, 2005). The treatment with 45% protein and 15% lipid reduced growth indices significantly because the mutual effects of the protein and lipid levels increased protein and lipid contents of the prawns, accumulation amino acids in the body which was accompanied by reduced growth indices (McGoogan and Gatlin Iii, 1999). Catacutan (2002) reported that when three levels of protein (32, 40, and 48%) were used at the same energy level in feeding of crab *Scylla serrata*, the best growth rate and FCR were obtained at protein level of 32%. Our results also indicated that increasing protein content of the diet significantly reduced FCR.

In our study, increased levels of protein and lipid in the diet significantly raised protein efficiency, which contradicts the results of Xu *et al*. (2013) on feeding juvenile red swamp crayfish (*P. clarkii*) with three diets having protein levels varying from 24 to 30% for 8 weeks and found that protein efficiency in carcass declined with increases in protein content of the diet, and the minimum protein efficiency and PPV were achieved in the treatments with low levels of protein and lipid. The previous studies showed low protein levels in the diet led to protein being used in energy production instead of being utilized for growth (Wouters *et al*., 2001; Alaf and Vijaya, 2005).

Determining the optimal lipid content is of great economical, nutritional, and environmental importance (Karalazos *et al.*, 2011). Recently conducted research provides basic information for designing diets. Zhao *et al.* (2015) reported the juvenile mud crab (*S. paramamosain*) could accept diets containing up to 11.63% lipids, while Alaf and Vijaya (2005) found on Indian white shrimp that juvenile shrimps tolerate a maximum of 10% lipid in diet. In another research, Izquierdo *et al*. (2006) reported juvenile Pacific white shrimps use a maximum 10% lipid in diet.

Results of our research indicate the desirable level of lipid that enables mutual effects with protein on growth, survival and the lowest FCR in oriental river prawns is 5%. This optimal level was found in a treatment with the maximum protein content of 45%. However, with lower protein content in diet, the percentage of utilized lipids increased and the maximum lipid content of 15% was found in the treatment of 35% protein and 15% lipid, with growth and protein efficiency indices and PPV significantly different from the other treatments. These results are similar to the result of Hysmith *et al*. (1992) that they reported diets with low protein and high lipid contents, and with high protein and low lipid contents, led to better growth of *P. aztecus*. Therefore, since shrimps have limited ability to use high ranges of lipid levels as an energy source, only carbohydrates in large amounts can be a suitable and cheap substitute for providing energy to overlap with protein (Asaduzzaman *et al.*, 2008). Zhang *et al.* (2013) conducted a research regarding the effects of various levels of lipids on Indian white shrimp and found the minimum and maximum

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lipid levels for improving LPV were in the range of 6 to 14% agrees with the results we observed in our research.

Wang *et al*. (2010) studied on the fatty acids metabolism on oriental river prawns concluded that great increases in lipid levels reduced LPV and also decreased survival and growth rates, because prawns did not accept higher levels of lipid and could not emulsify it (Muralisankar *et al.*, 2016). These results confirm our study in which the reduction in survival rate, and the increase in feed intake, led to increases feed conversion ratio in treatments with high protein levels and 15% lipids. This indicates that lipid required by oriental river prawns are provided in the range of 5 to 10%, but protein content at the minimum level (35%), higher levels of lipid can be used to improve the growth index and the FCR and reduce costs.

It can be inferred from reports that carcass lipid content greatly depends on adjusting protein-energy ratio in commercial diets for marine shrimps and freshwater prawns (Celada *et al*., 1993; Xu *et al*., 2013; Sabry-Neto *et al*., 2016). These conclusions agree with our research in which growth factors and survival of prawns fed on diets containing about 15% lipids were negatively affected at high protein content of the diet. This is due to the structure of the digestive system in crustaceans as they are omnivores (Cui *et al*., 2016). Moreover, contrary to various types of carnivores, prawns and most crustaceans can utilize high levels of carbohydrates and starches (Wang *et al*., 2016).

Body composition in cultured species is influenced by internal factors such as size, age, and external factors including feed composition (Snyder *et al*., 2016). In this study, carcass protein and lipid increased, and ash content decreased with increases in the levels of protein and lipid in the diet. Moreover, carcass moisture significantly increased with increases in lipid in the diets, while it significantly declined with increased levels of proteins in the diet. These results confirm by Goda (2008) which conducted on various levels of protein (30, 35, and 40%) and lipid (10 and 14%). Previous finding showed that in freshwater prawns (*M. rosenbergi*) carcass moisture significantly declined when protein content was raised up to 40%, while it significantly increased compared to other treatments when the dietary lipid level increased to 14%. Results of our study indicate that if protein level is low (35%) and remained so, and if lipid levels were raised up to 15%, components of the carcass improved compared to the start of the culture period. However, if high protein level is used (45%), the best improvements in protein and lipid contents, and reduced ash content in the carcass were observed in treatments that had a fixed 5% lipid level in the diet, and if higher levels of lipid is used, carcass moisture percentage rose and protein and lipid contents significantly declined. This indicates that lipid can serve as an energy source for the body and be used when protein level is low, and that with increases in the protein levels, the lipid content of the diet must be reduced. These results are

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comparable to Huo *et al*. (2014) that increases in protein and lipid percentages in the diet with the suitable ranges improved body components in juvenile swimming crab (*P. trituberculatus*) as the dietary lipid level increased from 5% to 13% at the constant protein level.

Our results showed that oriental river prawn is a species with high protein demand. This prawn could utilize high dietary protein content up to 45%, while the dietary lipid with more than 5% at high protein content would not increase growth and feed efficiency. Received the growth performance, survival rate and feed efficiency simultaneously, 45% dietary protein and 5% dietary lipid content could be set as an eligible dietary formulation for this species. While use of low-protein diets to reducing production costs, the diet containing 35% protein and 15% lipid is recommended.

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