

## Evaluation of different dietary protein and energy levels on growth performance and body composition of narrow clawed crayfish (*Astacus leptodactylus*)

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Received: January 2011

Accepted: June 2011

### Abstract

Nine practical diets containing increasing percentages of crude protein (CP) and energy (30%, 35%, 40% and 300kcal/100g, 370kcal/100g and 450kcal/100g) were fed to narrow clawed crayfish (mean individual weight=17±2.3 g) during an 8 week feeding trial. Weight Gain, Feed conversion ratio (FCR), Protein Efficiency Ratio (PER), Net Protein Utilization (NPU), Daily Food Consumption (DFC) and body composition of tail-muscle meat of narrow clawed crayfish were determined. At the conclusion of the experiment, comparing the growth parameters in response to interaction between protein and energy levels demonstrated that dietary number 2 (30/370) resulted in higher WG, NPU, PER, and the lowest FCR whereas dietary number 3 (30:450) showed higher SGR and DFC. Comparison of the body composition results indicates the greatest amount of protein absorption in diet number 2 (30/370). Results from this study indicate that narrow clawed crayfish can be fed a practical diet containing 30% protein and 370 Kcal/100gr. Reducing CP levels in narrow clawed crayfish diets may help reduce operating costs and thereby increase producers' profits.

**Keywords:** Narrow clawed crayfish, *Astacus leptodactylus*, Protein level, Energy level diet

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## Introduction

Freshwater crayfish (*Astacus leptodactylus*) belonging to the Astacidae family are widely distributed over all eastern European countries, with a high distribution in Poland, Russia and Turkmenistan and found naturally in western parts of Europe including Germany and France (Koksal, 1998).

*Astacus leptodactylus* is the most important crayfish species, which is distributed naturally in Iran with high density in western rivers of the Caspian Sea and Anzali Lagoon. To make new stocks of this valuable species, some were released in Ghorigol Lake as well as Aras Reservoir, Voshmgir and Sheikh Ali Kelaye Lagoon (Naviri, 1994). Production and export of this aquatic animal reached around 200 tons which was mainly harvested from Aras Reservoir (Naviri, 1994). In last years, two research studies in the field of parasitology and also growth, molting and survival response of juvenile narrow clawed crayfish, *Astacus leptodactylus*, which Fed on Two Sources of Dietary Oils, were conducted (Nekuie Fard et al., 2011; Valipour et al., 2011)

In Iran, considerable interest was aroused for diversification of aquatic animals. As narrow clawed crayfish had particularly good potential for rearing, knowledge of culture requirements and performance are needed. Formulation of a good and proper dietary with acceptable protein and energy content is obligatory in this way as very little information is available on successful compounded feeds for crayfish culture.

In fish and crustaceans farming, food and feeding can represent up to 50-60 % of the total operating costs of an aquaculture

enterprise (Naviri, 1994). As nutrient requirements of *Astacus leptodactylus* is not known completely, thus for boosting the culture process of this species, knowing its dietary requirements is vital (Tacon, 1990).

Protein is generally the most important component in a prepared diet which is necessary for animals. They provide essential amino acids that animals cannot synthesize, or cannot synthesize in sufficient quantities to enable the maintenance of good rates of growth (Thoman et al., 1999). Despite terrestrial animals, aquatic ones including crustaceans need high protein levels around 24-57 % of dietary (Thoman et al., 1999). Protein levels influence diet costs directly and reducing this level influences growth rate of farming species, so balancing this level is very important in both physiological and economical views (Thoman et al., 1999).

The assessment of the energy value of dietary is central to the study of animal feeding. Energy is considered as primary requirement for aquatic animals. As for maintenance as well as body growth, energy is required; this part of the dietary is very important and is considered as a basal component of food but practically protein is considered as the most important part of the food as the main cost belongs to this part. If the energy content of a dietary is high, deposition of lipid will increase and also food consumption will decrease which results in nutrient imbalance in the body. Energy of food is consumed first for body maintenance, locomotion, and it is then considered for body growth. So if

energy-protein ration is low in a dietary, protein is consumed as the energy source (New, 1987).

In another study (Hysmith et al., 1972) it is reported that application of dietaries with low protein-high energy level as well as high protein-low energy level resulted in good growth in *Penaeus aztecus*. In the case of protein efficiency ratio along with increasing protein levels in food, Colvin (1976) obtained the same results on *Penaeus indicus*. In this study, four different dietaries with different protein levels from 21% to 53% were administrated for feeding larvae during 3 weeks. Results demonstrated that PER decreases along an increasing dietary protein level.

In another study (Alva and Lim, 1983), it was reported that increasing dietary protein up to a defined limit will increase growth rate and beyond this limit, it doesn't have any meaningful effects. In this study, 8 different dietaries containing 25% to 60% protein level were used on black tiger shrimp larvae. Administration of a 40% protein level dietary resulted in the best growth, PER and FCR.

Investigation of the effects of different dietaries on body composition should be carried on equipped nutritional laboratories. Results indicated a reverse relation between dietary protein level and body lipid content in a way that with increasing protein contents in the dietary, body lipid content decreased. According to the results obtained from an investigation on *Eriocheir sinensis* (Mu et al., 1998), protein levels in juvenile crabs were not affected by protein levels in food. But in

another study (Chen et al., 1994), it was reported that *Eriocheir sinensis* which was fed with diets containing higher protein levels, it represented higher body protein levels than those fed with a lower amount. The objective of the present study was formulating a diet for the narrow clawed crayfish with an acceptable protein-energy ratio according to Iran culture situation considering aspects of economical and physiological views. To achieve this aim, effects of three different levels of protein and energy were investigated on growth and production of *Astacus leptodactylus*.

## Materials and methods

### *Experimental animals and system*

To start this investigation, 135 narrow clawed crayfish with an initial body weight of 15 to 20 gr were captured from Aras Reservoir (39°06'23.18" N, 44°22'16.84" E) located in Western Azerbaijan province, Iran. Animals were transferred to the Research Center and stocked in round 110 lit capacity polyethylene tanks filled with 80 L of freshwater. Before the experiment commenced, the animals were adapted to experimental conditions for 2 weeks.

The experiment was conducted from June to October 2009 in Sefidrood (Astane Ashrafieh) Fisheries Research Center, which under authority of Iranian Fisheries Research Institute. After this time, the experimental units consisting of 27 tanks were filled with 80 lit of freshwater and equipped with an aerator. Then they were weighed and randomly divided into nine experimental units in three replicates, five animals in each tank.

### *Diet preparation and experimental design*

Casein and Gelatin as main protein sources contained 73.5% and 90% protein, respectively whereas fish meal and soybean meal as other protein sources contained 62.5% and 41.5 % protein respectively. Dextrin as a main carbohydrate source contained 90.3 % carbohydrate.

Casein, gelatin, dextrin and fish oil were considered as protein and energy sources to manipulate and balance dietaries whereas other components were fixed in all diets. So discrepancy in digestibility kept, at least. Nine dietary treatments (n = five crayfish per treatment) were formulated and prepared to contain three protein levels (30%, 35% and 40%), and three energy levels (300, 370 and 450 kcal/100 gr food) (Table 1 and 2). Diets were prepared by mixing the dry ingredients in an electric mixer (A-200 T; Hobart, Troy, OH) before the lipid source was added. The cooked bread flour was added to the mix in the end and the resulting dough was made to pass three times through a meat grinder fitted with a 2mm diameter die. The resulting strands were steamed for 5 min before drying in an oven for 5 h at 70 °C (Grieve, Round Lake, IL). Dried diets were cut into 3–5 mm lengths and stored in the refrigerator.

#### *Analysis*

The composition of each of the ingredients and body composition were analyzed to determine the moisture, protein, lipid, energy, and ash percentages. Analysis information of food ingredients are presented in table 3. Nutritional values of the experimental diets are presented in table 4. Protein levels were found around levels which were considered as

experimental diets. Moisture was determined by placement of a 2gr sample into a convection oven (135°C) for 2 hours until reaching a constant weight (Association of official analytical chemists, (AOAC) (2000). Protein was determined by combustion (A.O.A.C, 2000). Lipid was determined by the acid hydrolysis method (A.O.A.C, 2000) and ash was determined by placing a 2gr sample in a muffle furnace (600 °C) for 2 hours (A.O.A.C, 2000). Available energy (AE) was calculated from physiological fuel values of 4.0, 4.0, and 9.0 kcal/g for protein, carbohydrate, and lipid, respectively (Garling and Wilson, 1977; Webster et al., 1999).

#### *Feeding trials and system maintenance*

Total daily feed was initially set at 3-4 % total weight in each experimental unit. Through checking remained food in tanks, crayfish were fed to apparent satiation twice a day (10:00 and 17:00) over 60 days. Water management was done through siphoning out the residuals every day and water exchange at 80 -100 % of volume. All the tanks and equipments were washed thoroughly every 3 days.

To investigate the effects of different diets, biometry was done every 15 days and crayfish performance was measured by the calculation of the following parameters (Tacon, 1990):

Weight Gain (WG):  $WG = \{ [Final\ Weight - Initial\ Weight] / Initial\ Weight \} \times 100$

Specific growth rate (SGR), or average growth per day:  $SGR = [(\ln W_2 - \ln W_1) / days] \times 100$

Where  $\ln W_2$  is the natural logarithm of weight at time 2 and  $\ln W_1$  is the natural logarithm of the initial weight.

Feed conversion ratio (FCR):  $FCR = \text{feed intake (g)}/\text{Weight gain (g)}$

Protein Efficiency Ratio (PER):  $PER = \text{Weight gain (g)}/\text{Protein intake (g)}$

Net Protein Utilization (NPU):  $NPU = [\text{Protein gain (g)}/\text{Protein intake (g)}] \times 100$

Daily Food Consumption (DFC):  $DFC = [\text{consumed food (g)}/(\text{feeding days} \times \text{body weight})/2] \times 100$

#### Statistical analysis

Statistical analysis was done using the SPSS and Excel software. Checking data for normality was done by Kolmogorov-Smirnov test before any analysis. Data were presented as mean  $\pm$  SEM.

Differences between treatments were determined using one-way analysis of variance, followed by Duncan's multiple comparison test ( $p < 0.05$ ).

#### Results

Crayfish performance in response to different protein levels is presented in table 5. Protein level increasing from 30% to 40% results in increasing FCR value but decreases the rest of the values. Increasing the protein levels of the dietary, significantly decreases PER and NPU ( $p < 0.05$ ). Comparison between the rest of the values revealed no significant differences between experimental groups.

**Table 1 :dietary treatments**

Diets	Dietary Characteristics	
	Protein (%)	Gross energy (kcal/100 gr food)
1	30	300
2	30	370
3	30	450
4	35	300
5	35	370
6	35	450
7	40	300
8	40	370
9	40	450

**Table 2 :Composition of experimental diets (%)**

Ingredients (%)	Diets								
	1	2	3	4	5	6	7	8	9
Casein	4	4	4	4	3	11.7	3	3	15
Gelatin	6.6	6	6.1	12.7	12.8	7	20	18.8	10.2
Dextrin	1.5	18	35.7	1.5	15	29.3	1.5	13.8	23.9
Wheat meal	1.5	1.5	2.9	1.5	1.5	1	1.5	1.5	1
Fish meal	26	26	26	26	26	25	26.3	26	25
Corn meal	11.4	9	1.5	5.1	6.3	1.5	1.5	1.9	1.5
Soybean meal	9	9	8	9	9	8	7.3	9	7
Cod oil	6.5	6.7	7.7	6.7	6.6	8.5	6.3	6.7	8.4
Cellulose	26.4	12.7	1	26.4	12.8	1	25.7	12.4	1
Vitamin mix	2	2	2	2	2	2	2	2	2
Mineral mix	2	2	2	2	2	2	2	2	2
Choline Chloride	1	1	1	1	1	1	1	1	1
Vitamin C	1	1	1	1	1	1	1	1	1
Crayfish Meal	1	1	1	1	1	1	1	1	1

**Table 3: Proximate composition of experimental dietaries ingredients**

Ingredients	Protein %	Lipid %	Carbohydrate %	Moisture %	Fiber %	Ash %	Gross energy (Kcal/100 gr food)
Casein	73.5	0.5	16.8	2.2	0.05	6.99	445.0
Gelatin	90	2	3	4	0.03	0.3	396.0
Dextrin	4.4	0.8	90.3	4.5	0.16	0.6	525.0
Fish meal	62.5	8	7.79	6.2	1.57	14	388.0
Corn meal	8	4.98	73	10.3	2.19	1.48	415.0
Wheat meal	9.5	1	75.6	11.6	1.35	0.9	382.0
Soybean meal	41.5	1.5	32.5	11.9	5.56	7	346.0
Fish oil	-	100	-	-	-	-	963.9

**Table 4: Ingredients analysis of experimental dietaries**

Composition	Dietary								
	1	2	3	4	5	6	7	8	9
Protein %	30.85 ± 1.89	31.33 ± 2.57	32.3 ± 3.40	36.31 ± 3.04	34.9 ± 2.33	35.95 ± 3.12	41.34 ± 4.40	40.2 ± 2.55	40.05 ± 3.18
Lipid %	9.88 ± 0.92	9.29 ± 0.21	9.08 ± 0.12	9.88 ± 0.56	10.1 ± 0.65	10.3 ± 0.17	9.99 ± 0.54	10.06 ± 0.42	10.28 ± 0.78
Ash %	1.25 ± 0.12	1.55 ± 0.15	1.39 ± 0.58	1 ± 0.18	0.9 ± 0.34	1.57 ± 0.19	0.93 ± 0.43	1.58 ± 0.74	1.32 ± 0.66
Moisture %	7.85 ± 0.16	8.42 ± 0.22	6.82 ± 0.24	5.13 ± 0.76	8 ± 0.89	4.88 ± 0.65	5.36 ± 0.23	8 ± 0.76	4.77 ± 0.97
NFE %	50.17±2.35	49.41±2.02	50.41±1.55	47.68±3.21	46.1±2.98	47.3±3.06	42.38±3.89	40.16±2.45	43.58±1.99
Gross energy Kcal/100gr food	302.82 ± 10.78	371.64 ± 10.01	454.44 ± 8.09	314.93 ± 11.35	379.92 ± 15.13	461.74 ± 22.13	318.46 ± 9.37	373.35 ± 17.12	465.18 ± 25.17
Protein/Energy ratio(mg/Kcal)	108.48	84.30	71.07	118.47	91.86	77.85	129.81	107.67	86.09

**Table 5: Effects of different protein levels on growth parameters of narrow clawed crayfish (mean ± S.E.)**

Protein level (%)	Parameters					
	SGR	FCR	PER	NPU	DFC	WG
30	0.22 ± 0.01 <sup>a</sup>	8.62 ± 0.74 <sup>a</sup>	0.41 ± 0.02 <sup>b</sup>	27.14 ± 1.65 <sup>a</sup>	3.68 ± 0.07 <sup>a</sup>	16.07 ± 1.09 <sup>a</sup>
35	0.20 ± 0.01 <sup>a</sup>	8.95 ± 0.86 <sup>a</sup>	0.31 ± 0.02 <sup>a</sup>	19.55 ± 0.88 <sup>b</sup>	3.67 ± 0.07 <sup>a</sup>	14.01 ± 1.35 <sup>a</sup>
40	0.17 ± 0.01 <sup>a</sup>	9.34 ± 0.70 <sup>a</sup>	0.25 ± 0.01 <sup>a</sup>	13.80 ± 0.96 <sup>c</sup>	3.67 ± 0.04 <sup>a</sup>	14.39 ± 0.98 <sup>a</sup>

SGR = Specific growth rate, FCR = Feed conversion ratio, PER = Protein Efficiency Ratio,  
 NPU = Net Protein Utilization, DFC = Daily Food Consumption, WG = Weight Gain  
 Mean with different superscripts in the same column are significantly different at least at p<0.05

Effects of different energy levels on crayfish growth parameters are presented in table 6. Increasing food energy levels from 300 to 450 kcal/100 gr food results in

increasing FCR and DFC and decreasing PER. Evaluation of the parameters revealed significant differences except from NPU and SGR.

**Table 6: Effects of different energy levels on growth parameters of narrow clawed crayfish (mean  $\pm$  S.E.)**

Parameters						
Energy level (kcal/100gr)	SGR	FCR	PER	NPU	DFC	WG
300	0.20 $\pm$ 0.01 <sup>a</sup>	8.10 $\pm$ 0.81 <sup>a</sup>	0.36 $\pm$ 0.02 <sup>b</sup>	20.11 $\pm$ 1.56 <sup>a</sup>	3.42 $\pm$ 0.03 <sup>a</sup>	15.04 $\pm$ 1.18 <sup>ab</sup>
370	0.18 $\pm$ 0.01 <sup>a</sup>	7.83 $\pm$ 0.03 <sup>a</sup>	0.38 $\pm$ 0.02 <sup>b</sup>	22.84 $\pm$ 1.94 <sup>a</sup>	3.64 $\pm$ 0.3 <sup>b</sup>	17.44 $\pm$ 1.28 <sup>b</sup>
450	0.21 $\pm$ 0.01 <sup>a</sup>	11.98 $\pm$ 0.01 <sup>b</sup>	0.25 $\pm$ 0.01 <sup>a</sup>	17.55 $\pm$ 1.09 <sup>a</sup>	3.96 $\pm$ 0.1 <sup>c</sup>	11.99 $\pm$ 1.01 <sup>a</sup>

SGR = Specific growth rate, FCR = Feed conversion ratio, PER = Protein Efficiency Ratio,

NPU = Net Protein Utilization, DFC = Daily Food Consumption, WG = Weight Gain

Mean with different superscripts in the same column are significantly different at least at  $p < 0.05$

**Table 7: Effects of interaction between protein and energy levels on growth parameters of narrow clawed crayfish (mean  $\pm$  S.E.)**

Parameters						
Protein:Energy	SGR	FCR	PER	NPU	DFC	WG
30:300	0.16 $\pm$ 0.02 <sup>a</sup>	7.25 $\pm$ 1.6 <sup>a</sup>	0.46 $\pm$ 0.05 <sup>cd</sup>	25.53 $\pm$ 2.91 <sup>f</sup>	3.27 $\pm$ 0.06 <sup>a</sup>	16.02 $\pm$ 1.91 <sup>a</sup>
30:370	0.17 $\pm$ 0.03 <sup>a</sup>	7.27 $\pm$ 1.13 <sup>a</sup>	0.48 $\pm$ 0.05 <sup>d</sup>	31.53 $\pm$ 3.92 <sup>g</sup>	3.77 $\pm$ 0.02 <sup>cd</sup>	19.51 $\pm$ 2.42 <sup>a</sup>
30:450	0.32 $\pm$ 0.03 <sup>c</sup>	11.34 $\pm$ 1.06 <sup>ab</sup>	0.31 $\pm$ 0.02 <sup>abc</sup>	24.38 $\pm$ 1.64 <sup>f</sup>	3.99 $\pm$ 0.01 <sup>d</sup>	12.69 $\pm$ 1.21 <sup>a</sup>
35:300	0.27 $\pm$ 0.01 <sup>bc</sup>	8.46 $\pm$ 0.94 <sup>ab</sup>	0.34 $\pm$ 0.04 <sup>abcd</sup>	19.19 $\pm$ 1.70 <sup>d</sup>	3.50 $\pm$ 0.07 <sup>ab</sup>	14.41 $\pm$ 2.43 <sup>a</sup>
35:370	0.16 $\pm$ 0.04 <sup>a</sup>	7.88 $\pm$ 1.54 <sup>a</sup>	0.38 $\pm$ 0.05 <sup>bcd</sup>	22.72 $\pm$ 1.60 <sup>e</sup>	3.53 $\pm$ 0.04 <sup>b</sup>	16.83 $\pm$ 2.54 <sup>a</sup>
35:450	0.17 $\pm$ 0.02 <sup>a</sup>	13.52 $\pm$ 1.86 <sup>b</sup>	0.23 $\pm$ 0.04 <sup>ab</sup>	16.75 $\pm$ 0.70 <sup>c</sup>	3.98 $\pm$ 0.0 <sup>d</sup>	10.78 $\pm$ 2.41 <sup>a</sup>
40:300	0.16 $\pm$ 0.01 <sup>a</sup>	8.60 $\pm$ 1.61 <sup>ab</sup>	0.27 $\pm$ 0.03 <sup>ab</sup>	15.62 $\pm$ 2.39 <sup>bc</sup>	3.49 $\pm$ 0.02 <sup>ab</sup>	14.68 $\pm$ 1.78 <sup>a</sup>
40:370	0.21 $\pm$ 0.01 <sup>ab</sup>	8.35 $\pm$ 1.05 <sup>ab</sup>	0.28 $\pm$ 0.02 <sup>ab</sup>	14.27 $\pm$ 1.89 <sup>b</sup>	3.63 $\pm$ 0.03 <sup>bc</sup>	15.99 $\pm$ 1.91 <sup>a</sup>
40:450	0.14 $\pm$ 0.03 <sup>a</sup>	11.07 $\pm$ 1.11 <sup>ab</sup>	0.21 $\pm$ 0.02 <sup>a</sup>	11.51 $\pm$ 0.17 <sup>a</sup>	3.90 $\pm$ 0.04 <sup>d</sup>	12.51 $\pm$ 0.66 <sup>a</sup>

SGR = Specific growth rate, FCR = Feed conversion ratio, PER = Protein Efficiency Ratio,

NPU = Net Protein Utilization, DFC = Daily Food Consumption, WG = Weight Gain

Mean with different superscripts in the same column are significantly different at least at  $p < 0.05$



Comparing the growth parameters in response to interaction between protein and energy levels is presented in table 7. According to these it has been demonstrated that dietary number 2 (30:370) resulted in higher WG, NPU, PER, and the lowest FCR whereas dietary number 3 (30:450) showed higher SGR and DFC. There is a significant difference in all parameters except WG and FCR ( $p > 0.05$ ).

Effects of different protein levels on biochemical compositions of narrow

clawed crayfish body are presented in table 8. No significant difference was measured between the body composition parameters in different experimental groups except from body protein. The experimental group fed by the dietary containing 30 % protein showed the highest body protein value while those fed by 40 % protein demonstrated the lowest value of body protein ( $p < 0.05$ ). Increasing the dietary protein levels resulted in increasing the lipid levels of body composition.

**Table 8: Effects of different protein levels on narrow clawed crayfish body composition (mean  $\pm$  S.E.)**

<b>Body Composition</b>				
<b>Protein level (%)</b>	<b>Protein</b>	<b>Lipid</b>	<b>Ash</b>	<b>Digestible Energy (Kcal/100gr)</b>
30	$82.09 \pm 0.51^b$	$1.51 \pm 0.09^a$	$1.66 \pm 0.33^a$	$432.82 \pm 15.60^a$
35	$80.60 \pm 0.27^a$	$1.86 \pm 0.19^{ab}$	$2.61 \pm 0.36^a$	$437.02 \pm 30.15^a$
40	$79.91 \pm 0.46^a$	$1.94 \pm 0.11^b$	$2.06 \pm 0.26^a$	$437.24 \pm 11.97^a$

Mean with different superscripts in the same column are significantly different at least at  $p < 0.05$

In table 9, the effects of different energy levels on biochemical compositions of the body of narrow clawed crayfish are demonstrated. Comparison between means obtained from different experimental groups, reveals significant differences in protein, lipid, energy and ash of the body composition ( $p < 0.05$ ). The highest levels of protein, lipid and energy of body belonged to the group which were fed by the dietary

containing 370 kcal energy/100 gr and the lowest value was recorded in the group consuming the dietary containing 450 kcal energy/100gr ( $p < 0.05$ ). The group fed by the dietary containing 450 kcal energy/100 gr demonstrated the highest levels of ash in body composition whereas the lowest level was recorded in the group which used the 370 kcal energy/100 gr dietary ( $p < 0.05$ ).

**Table 9: Effects of different energy levels on narrow clawed crayfish body composition (mean  $\pm$  S.E.)**

Energy level (kcal/100gr)	Body Composition			
	Protein	Lipid	Ash	Digestible Energy (Kcal/100gr)
300	80.41 $\pm$ 0.45 <sup>a</sup>	1.90 $\pm$ 0.12 <sup>b</sup>	1.42 $\pm$ 0.06 <sup>a</sup>	435.99 $\pm$ 23.76 <sup>ab</sup>
370	81.81 $\pm$ 0.60 <sup>b</sup>	2.01 $\pm$ 0.12 <sup>b</sup>	1.52 $\pm$ 0.05 <sup>a</sup>	442.14 $\pm$ 19.50 <sup>b</sup>
450	80.39 $\pm$ 0.27 <sup>a</sup>	1.40 $\pm$ 0.06 <sup>a</sup>	3.40 $\pm$ 0.25 <sup>b</sup>	428.95 $\pm$ 16.89 <sup>a</sup>

Mean with different superscripts in the same column are significantly different at least at  $p < 0.05$

Effects of different interaction between protein and energy on body compositions are showed in table 10. Comparing the means of experimental groups revealed that the highest body lipid and energy belonged to the group which was fed by dietary number 4 whereas the lowest values belonged to the group consuming

dietary number 1 ( $p < 0.05$ ). The latter group, demonstrated the highest value of body ash while the lowest value of this parameter was recorded in the group fed by dietary number 7 ( $p < 0.05$ ). The highest value of body protein was achieved in the group which was fed by dietary number 2.

**Table 10: Effects of different interaction between protein and energy levels on narrow clawed crayfish body composition (mean  $\pm$  S.E.)**

Protein-Energy interaction	Body Composition			
	Protein	Lipid	Ash	Digestible Energy (Kcal/100gr)
30:300	86.52 $\pm$ 0.65 <sup>ab</sup>	1.35 $\pm$ 0.20 <sup>a</sup>	2.01 $\pm$ 0.04 <sup>a</sup>	481.06 $\pm$ 14.61 <sup>b</sup>
30:370	89.82 $\pm$ 1.28 <sup>b</sup>	2.32 $\pm$ 0.10 <sup>c</sup>	1.45 $\pm$ 0.01 <sup>a</sup>	518.92 $\pm$ 0.00 <sup>ef</sup>
30:450	89.48 $\pm$ 0.58 <sup>b</sup>	1.67 $\pm$ 0.03 <sup>b</sup>	2.30 $\pm$ 0.72 <sup>b</sup>	499.24 $\pm$ 27.92 <sup>b</sup>
35:300	84.96 $\pm$ 0.48 <sup>ab</sup>	3.01 $\pm$ 0.03 <sup>bc</sup>	1.56 $\pm$ 0.06 <sup>a</sup>	523.12 $\pm$ 0.00 <sup>f</sup>
35:370	87.50 $\pm$ 0.42 <sup>b</sup>	2.42 $\pm$ 0.27 <sup>c</sup>	2.10 $\pm$ 0.13 <sup>a</sup>	501.99 $\pm$ 15.25 <sup>cd</sup>
35:450	85.48 $\pm$ 0.29 <sup>ab</sup>	1.45 $\pm$ 0.02 <sup>b</sup>	5.67 $\pm$ 0.01 <sup>c</sup>	512.09 $\pm$ 43.62 <sup>a</sup>
40:300	86.38 $\pm$ 1.11 <sup>a</sup>	2.42 $\pm$ 0.14 <sup>bc</sup>	1.29 $\pm$ 0.00 <sup>a</sup>	495.94 $\pm$ 15.07 <sup>cd</sup>
40:370	85.98 $\pm$ 0.94 <sup>a</sup>	2.49 $\pm$ 0.17 <sup>bc</sup>	1.67 $\pm$ 0.04 <sup>a</sup>	500.17 $\pm$ 15.19 <sup>de</sup>
40:450	84.97 $\pm$ 0.17 <sup>ab</sup>	1.99 $\pm$ 0.00 <sup>b</sup>	4.08 $\pm$ 0.01 <sup>b</sup>	499.26 $\pm$ 15.17 <sup>c</sup>

Mean with different superscripts in the same column are significantly different at least at  $p < 0.05$

## Discussion

Effects of protein and energy levels of food are studied on many aquatic animals

so far. It's demonstrated that in the case of applying different diets with the same

energy levels, some growth parameters like WG and SGR have a positive relationship with increasing protein-energy ratio in food, while other parameters such as FCR, PER and NPU have a negative correlation and decrease with increasing food protein-energy ratio (Rodriguez et al., 2006).

In the present study, results confirm the fact that using a dietary with optimal protein level, increases energy levels accompanied with increasing growth parameters. Also in case of using dietaries with the same energy levels, growth parameters increased except from FCR, PER and NPU which all decreased (Davis and Arnold, 1997; Das et al., 1991; Hajra et al., 1988; Bautista, 1986).

Results of the present study indicate that dietary protein level can be reduced to 30% for narrow clawed crayfish grown in tanks at the size and stocking densities used here, thus reducing diet costs. Narrow clawed crayfish fed the 30% protein diet had similar growth and survival compared with narrow clawed crayfish fed diets containing 35% and 40% protein. Webster et al. (1994) reported that small (mean weight 0.022 g) juvenile red clawed required a 33% crude protein diet for rapid growth and survival when grown communally in an indoor recirculating aquarium system.

Manomaitis (2001) evaluated the crude protein requirements of juvenile red clawed crayfish utilizing two age classes and reported that small (0.02 g) red clawed required a 40% protein diet, whereas larger (3.03 g) red clawed could be fed a diet containing 30% protein when grown

indoors. In comparison to these results, Catacutan (2002) reported that in a series of dietaries with constant energy level, application of a 32% protein level compared to 40% and 48% in feeding *Scylla serata* resulted in higher growth and better FCR. In another study, effects of 7 dietaries with the same energy content but different protein levels including 20, 25, 31, 37, 43, 49 and 55% were investigated on juvenile and early mature *Cherax quadricarinatus* (Jacinto et al., 2004). The higher growth index in juvenile and early mature crayfishes were observed in groups fed by dietaries containing 31 and 20%, respectively. Effects of 6 different dietaries with protein levels ranging from 29.8 to 54.8% with 347 to 353 kcal energy/100 gr were studied on Chinese crab, *Eriocheir sinensis*, during a 35 day period (Shim et al., 2000). Results showed that the best SGR, FCR, PER and body weight increasing were recorded in the experimental group fed by a 39% protein level dietary. Based on this study, although increasing protein level beyond the optimal level of 39% resulted in increasing FCR, it had no effects on growth parameters. These results were also in accordance with our obtained results. In the research conducted by Colvin (1976), PER of diets with 21, 38.3, 42.3, and 53.1% protein were recorded 1.56, 1.10, 0.96 and 0.78, respectively. Another study which was done on Indian white shrimp Yazdani (1996) reported 0.78, 0.86, 1.10, and 1.33 as PER values for four different diets with 45, 40, 35, and 30% protein content, respectively. Results obtained from these two studies are in contrast to

each other. In analysis done by Colvin (1976), increasing the levels of protein in food resulted in reduction of PER while in the second research (Yazdani, 1996), PER increased when dietary protein increased. Results obtained from our study revealed that PER for diets containing 30, 35, and 40% protein were 0.36, 0.30, and 0.23 respectively. These results demonstrated that PER decreased when the levels of protein in food increased. In another study, effects of protein efficiency were investigated on two freshwater crayfish species (Jones et al., 1996). In this research, two different dietaries containing 15 and 30% protein were used. Protein efficiency ratio was low for the diets with lower protein content while the second diet with higher protein amount, showed higher protein efficiency ratio. According to these results, it is concluded that there is a direct relationship between diet protein content and protein efficiency ratio.

Considering these results, it is concluded that food conversion ratio will increase if diet protein content is increased, but this increasing is not statistically significant. In the research conducted by (Jones et al., 1996) the food conversion ratio in different experimental groups varied from 0.79 to 3.32. In this research, lower FCR was recorded in the group fed by the dietary containing 30% protein and 370 energy which is the same as our results. Comparing these results with those obtained from narrow clawed crayfish reveals that growth parameters didn't increase along with dietary protein level increase, but for each species an optimal protein level can be considered which beyond this level, growth

parameters decrease so the main cause for reducing growth parameter with increasing protein level of food can be described if the level of protein.

According to research conducted by Colvin (1976) it can be stated that the PER tended to decrease with the elevation of dietary protein content, in agreement with this study. According to research conducted by (Brauge et al., 1994) it can be stated that the reduce in growth rates of narrow clawed crayfish that were fed by diets containing 40% protein was caused to increase free amino acid in crayfish and these amino acids cannot be used for growth and should be diverted to NH<sub>3</sub> and then ejected.

In another study (Hajra et al., 1988) 6 different dietaries with the same protein level (46%) but a different total energy from 371.1 to 435.3 kcal/100 gr were administered on *Penaeus monodon* larvae. Results showed that increasing the energy levels up to an optimal level of 412.60 kcal/100 gr, increased body weight and FCR. Besides, other growth parameters such as PER, had a reverse relationship with food energy. Comparing these data with our findings reveals the same results in increasing dietary energy level with an optimal level of 370 kcal/100 gr on narrow clawed crayfish.

Increased energy caused to reduce growth factors and survival. Perhaps the increased energy, result in losing energy as heat and waste that is more justification on its growth, consequently (due to interfering enzymatic activities including digestive enzymes). Actual growth in crustaceans is followed through monitoring the increasing of body protein,

lipid, carbohydrate and ash and to achieve these data, analyzing the composition of animal body is necessary. In our study, levels of protein in diet inserted significant effects on body protein in narrow clawed crayfish. Also dietary energy content affected lipid, energy and ash contents of body in a direct manner. The highest body protein and energy was recorded when dietary energy increased up to 370 kcal/100 gr. Also the highest body ash was obtained when dietary energy increased to 450 kcal/100 gr. When dietary protein level increased, body protein level decreased. Comparing these data with Mu et al. (1998) findings reveals the same results. When dietary protein is more than physiological requirement, the excess amounting is deposited in the body in the form of lipid or carbohydrate (Cowey and Sarjent, 1979). In contrast, when the protein level is low and accompanied with low energy content, protein is used as an energy source instead of growth (Cowey and Sarjent, 1979). In the present study, different levels of protein in diets affected some growth parameters such as weight gain, mean body weight, FCR, PER, SGR and NPU. Considering the effects of dietary energy level on body lipid and energy content, similar results were obtained while the effects of dietary protein showed different results on body protein and lipid.

In shrimps, body growth also influenced by dietary protein, is under the effects of protein quality in food, too. Foods which contained two or more different protein sources are better consumed by shrimp than those consisting of only one source.

When the content of protein in crayfish food is increased, body protein increased, but when the level of protein in food increased more than the optimal, body protein decreased.

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