Effects of different levels of energy and protein sources on the growth performance, feeding, survival rate and the chemical body composition of juvenile pacific white shrimp *(Litopenaeus vannamei)*

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Abstract

The present study was carried out in order to establish an economical effective diet for the pacific white shrimp in the southern part conditions of Iran. Three dietary energy levels (E1=262, E2=312, E3=362 Kcal 100 g^{-1} diet) and 6 ratios of fish meal (FM): soybean meal (SBM) [(P1= 100% FM+ 0% SBM), (P2= 80% FM+ 20% SBM),(P3= 60% FM+ 40% SBM), (P4= 40% FM+ 60% SBM), (P5= 20% FM+ 80% SBM),(P6= 0% FM+ 100% SBM)], 18 experimental diets were prepared. Completely randomized design was used to assign 54 polyethylene 300 litre round tanks provided by aeration and was stocked by 19 juvenile shrimp as 3 replicates to each treatment. Shrimps average weight was about 0.77 g at the start. After 56 days culture period, maximum growth and nutritional performance were observed in the P6E1 and P5E1 treatments. In addition, the highest survival rate of the shrimps was observed in the P1E1, P1E2, P3E3 and P5E3 treatments. Results indicated that protein, fat, fiber and ash contents of carcase were significantly affected by the treatments ($P<0.05$). Results of the present study suggest the replacement possibility of at least 80% of dietary fishmeal by soybean meal in the diet of pacific white shrimp in the conditions of southern part of Iran.

Keywords: Pacific white shrimp, Fish meal, Soybean meal

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Introduction

The world production of farmed shrimp, in spite of problems such as viral disease and global price fluctuations, has been expanding during recent years and according to FAO (2008) shrimp production with considerable increase has reached from about 850000 mt (metric tons) in 1995 to about 3.4 million mt in 2008 and pacific white shrimp has had maximum production with about 2.3 million mt in 2008. This increased production has been accompanied by a decrease in shrimp price, either because of depressed markets or overproduction. As shrimp aquaculture is expected to continue to increase in coming years, shrimp prices are likely to continue to fall as production exceeds demand, therefore challenging the profitability of this industry (Amaya et al., 2007).

Increased demand and need for increasing shrimp production as well as essentiality of increasing efficiency and profitability made fisheries researchers think of using modern methods of propagation and culture of shrimp including identification and introduction of exotic species with suitable capabilities to this industry. Pacific white shrimp (*Litopenaeus vannamei*) with considerable biocapabilities, is very good species for domestication in most regions of the world including south Iran regions.

The growth rate of pacific white shrimp is better than other farmed shrimp species and can grow up to 3 gr / week and reach up to 20 gr under intensive culture conditions (FAO, 2004). The stocking density of this species is very high and it is possible stocking of up to 150 / m^2 in pond culture and even as high as 400 / m^2 in controlled recirculated tank culture (FAO, 2004). Pacific white shrimp tolerates a wide range of salinities (0.5-45 ppt). This species is comfortable at 7-34 ppt, but grows particularly well at low salinities of around 10-15 ppt. This ability makes it a good candidate for the newer inland farms (FAO, 2004). Although pacific white shrimp will tolerate a wide range of temperatures, it grows best between 23-30° like the majority of the other tropical and subtropical species (FAO, 2004). Pacific white shrimp requires a lower protein (and hence cheaper) diet (20-35 percent) during culture as compared with blue shrimp and giant tiger shrimp (36-42 percent).

The possibility of producing efficient brood stock from reared shrimp and producing SPF (specific pathogen free) and SPR (specific pathogen resistance) brood stock from them and the high larval survival rates during hatchery rearing compared with other species, are other advantages of this species (FAO, 2004).

One important factor considered to reduce shrimp production costs and increase producers profitability, is the use of feeds with low levels of fish meal and high levels of less expensive, high quality plant protein sources. Fish meal is preferred among protein sources because it is an excellent source of proteins and indispensable amino acids, essential fatty acids, vitamins, minerals and attractants but limited availability and high demand make fish meal a costly ingredient (Amaya et al., 2007). Because of their low price and consistent nutrient composition and supply, plant protein sources such as oilseeds are often economically and nutritionally valuable alternatives to fish meal. Among plant protein sources, soybean meal has received considerable attention because of its balanced amino acid profile, consistent composition, worldwide availability and lower price (Akiyama, 1988). In consideration of importance of this species in the world shrimp industry, several studies have been conducted to replacement of fish meal with other plant and protein sources (Lim and Dominy, 1990; Swick et al., 1995; Davis and Arnold, 2000; Mendoza et al., 2001; Forster et al., 2003; Samocha et al., 2004; Goytortua-bores et al., 2006; Patnaik et al., 2006; Cruz-suarez et al., 2007; Amaya et al., 2007; Hernandez et al., 2008; Ju et al., 2009).

The objective of this study was to evaluate interaction of dietary energy and protein sources and to determine suitable ratio of soybean meal to fish meal in the diet of pacific white shrimp in order to attain the best growth performance and feeding rate of this species and finally increasing food efficiency via decreasing fish meal ratio as an expensive source in it and determining optimum level of digestible energy in the diet of this species in climatic conditions of southern part of Iran.

Materials and methods

shrimp and experimental units

Juvenile pacific white shrimp (*Litopenaeus vannamei*) (mean weight± SD., 0.77± 0.03 gr) were obtained from shrimp production ponds located in Choebde, Abadan and transported to Bandare Emam Khomeini Marine Fishes Research Station.

This study was conducted in indoor tanks with control of ambient temperature. Completely randomized design was used to assign 54 polyethylene 300 litre circular tanks provided by aeration (with one air stone in each tank) and was stocked by 19 juveniles as 3 replicates to each treatment. The water salinity used in this study was 15-17 ppt which is the best salinity for this species (Askary sary et al. 2008). The incoming seawater was filtered through a sand filter and then flowed through an UV irradiating unit and finally was mixed with filtered freshwater until optimum salinity was achieved.

During the experimental period, temperature, salinity and pH concentrations were daily measured in tanks. Photo period was set for 12h light : 12h dark cycle throughout the experiment by fluorescent lamps and indirect nature light from windows

Feeds and feed management

In this study, with the consideration of 3 digestible energy levels (E1=262, E2=312, E3=362 Kcal 100 g^{-1} diet) and 6 ratios of fish meal (FM) : soybean meal (SBM) [(P1= 100% FM+ 0% SBM), (P2= 80% FM+ 20% SBM), (P3= 60% FM+ 40% SBM), (P4= 40% FM+ 60% SBM), (P5= 20% FM+ 80% SBM), (P6= 0% FM+ 100% SBM)], 18 experimental diets were prepared(table 1). Diets were prepared at South Iran Aquaculture Research Center in Ahvaz. All major dry ingredients were mixed in a kitchen aid mixer. The plant oil and lecithin were blended then added to the mixture. Hot water (approximately 60°) was mixed into the mash to provide a consistency appropriate for pelleting and this is mixed for another 20 min. The resulting mash was passed through a meat grinder equipped with a 2 mm diameter die to produce pellets. The pellets were placed in the trays and dried in the oven for 12 h at 60° then allowed to cool overnight at room temperature and conserved in plastic bags at 4°. Crude protein in different diets was equal (36% diets). Two weeks before beginning feeding trial with experimental feeds, adaptation with new feeds and starter shrimp feed of havourash company (cp=38%) in different tanks was gradually conducted. Feeding was *ad libitum* and Shrimps were fed 3 times per day at 08:00, 14:00, 20:00 h. daily feed inputs were adjusted following observations of the quantity of feed residue present in each tank to determine whether rations were excessive, sufficient or insufficient. Left over feed and faeces were siphoned in morning and 10% of the water was exchanged daily before the first feeding. Biometry of all shrimp in each tank was conducted on a bi-weekly basis and by counting and weighing of all shrimp in each tank. Shrimp survival and mean weight was determined. In addition, in each biometry, at least one-third of carapace orbital length of shrimps in each tank was determined. At the conclusion of 8-week growth trial, all of shrimp were harvested, counted, measured and weighed and total biomass in each tank separately was dried by oven (at 115°). These samples, and samples provided before starting the experiment were transported to feeding laboratory of south Iran aquaculture research center in Ahvaz in order to carcass analysis. Crude protein was estimated using kjeldahl method (N× 6.25). Crude lipids were ether extracted by the soxhlet method. Crude fiber was obtained in a fat-free material sample by dilute acid and alkali treatment. Dry matter was determined by drying the sample in an oven at 105° for 16 h. Ash content was determined by incinerating samples in a muffle furnace at 550° for 12 h. Nitrogen-free extract (NFE) was calculated by the difference.

Statistical analysis

The physicochemical parameters record and biometries data were collected and calculations were conducted using excel software and the below parameters was calculated (tacon, 1987):

Weight gain (%) = 100 (mean final wet weight- mean initial wet weight/ mean initial wet weight)

Mean orbital carapace length increase (%) = 100 (mean final orbital carapace length- mean initial orbital carapace length / mean initial orbital carapace length)

SGR (specific growth rate, % day^{-1}) = 100 (ln average final weight – ln average initial weight) / number of days

FCR (food conversion ratio) = total dry feed intake (gr) / wet weight gain (gr)

PER (protein efficiency ratio) = wet weight gain (gr) / dry protein intake (gr)

ANPU (apparent net protein utilization, %) = 100 (final body protein – initial body protein) / total protein consumed (gr)

Yield = total final shrimp biomass in each tank;

Survival (%) = 100 (final number of shrimp / initial number of shrimp);

All statistical analysis were made by using the statistical analysis software program of SPSS 13. The data were subjected to two-way analysis of variance and then to the Duncan’s multiple range test to first determine whether significant differences existed among the dietary treatment means and then to identify where they occurred. Results were considered statistically significant at $P < 0.05$.

Table1: Ingredient composition (g/100 g diet) and proximate analysis (g/100 g dry weight) of experimental diets

Treatments	P1E1	P2E1	P3E1	P4E1	P5E1	P6E1	P1E2	P2E2	P3E2	P4E2	P5E2	P6E2	P1E3	P2E3	P3E3	P4E3	P5E3	P6E3
Ingredients																		
Fish meal	27	21.6	16.2	10.8	5.4	0	27	21.6	16.2	10.8	5.4	0	27	21.6	16.2	10.8	5.4	0
Soybean meal	0	6.9	13.8	20.7	27.6	34.5	0	6.9	13.8	20.7	27.6	34.5	0	6.9	13.8	20.7	27.6	34.5
Rice bran	17	17	17	17	16.95	15.06	17	17	17	17	16.95	14.50	17	17	15.8	13	12	10.50
Wheat bran	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.2	12.5	13.8	12.2	12.2
Casein	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Gelatin	5.77	5.77	5.77	5.77	5.65	5.70	5.77	5.77	5.77	5.77	5.65	5.73	5.77	5.77	5.72	5.60	5.90	5.90
Plant oil	0.37	0.38	0.38	0.38	0.45	0.79	6.62	6.63	6.63	6.63	6.70	7.12	12.87	12.88	13	13.04	13.74	14.02
Lecithin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Squid meal	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Shrimp meal	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Binder	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Preservative	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Zeolite	9	9	9	9	9	9	8.66	7.15	5.65	4.15	2.75	3.20	2.41	0.9	0.23	0.31	0.41	0.13
Filler	5.91	4.4	2.9	1.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proximate analysis																		
Crude protein	36	36.03	36.06	36.09	36	36	36	36.03	36.06	36.09	36	36	36	36.03	36.02	36	36.03	36
Crude fat	7.56	6.69	5.72	4.76	3.86	3.2	13.90	12.94	11.97	11.01	10.11	9.52	20.15	19.19	18.33	17.40	17.07	16.35
Crude fiber	10.77	11.23	11.69	12.15	12.59	12.22	10.77	11.23	11.69	12.15	12.59	11.98	10.77	11.23	11.21	10.65	10.43	*10.24*
Ash	13.04	13.10	13.15	13.21	13.26	12.94	13.01	12.91	12.82	12.73	12.63	12.25	12.38	12.29	12.05	11.63	11.42	11.15
NFE	14.20	16.10	18.01	19.91	21.79	23.14	14.20	16.10	18.01	19.91	21.79	22.87	14.20	16.10	17.83	19.69	20.34	21.80
Digestible energy	262	262.06	262.05	262.03	262.01	262.12	312	312.06	312.05	312.03	312.01	312.05	362	362.06	362.02	362	362.02	362.03
Fish meal (%)	100	80	60	40	20	0	100	80	60	40	20	0	100	80	60	40	20	0
Soybean meal (%)	0	20	40	60	80	100	0	20	40	60	80	100	0	20	40	60	80	100

Results

Interactive effects of protein ratios and energy levels as well as each of variables separately, without considering second variable, on growth performance and feeding indices of pacific white shrimp after 8 week using experimental diets are presented in tables from 2 to 10. Results from tables 2 and 5 showed that interactive effects of protein ratios and energy levels have significant effects on all of growth and feeding parameters ($P < 0.05$). The best growth performance and feeding parameters were observed in shrimp fed P6E1, P5E1 and P4E1 treatments (containing maximum soybean meal and minimum digestible energy) that for the most part had significant difference with shrimp fed diets containing highest fish meal and digestible energy ($P < 0.05$). The best survival rate was observed in shrimp fed P1E1, P1E2, P3E3 and P5E3 treatments (approximately 94%) that had significant difference only with shrimp fed P4E1 treatment (approximately 76%) ($P < 0.05$).

Results presented in tables 3 and 6 showed that all growth and feeding parameters with the exception of SGR, survival rate and final yield were significantly affected by different fish meal: soybean meal ratios ($P < 0.05$). The best growth performance and feeding indices were found in P5 and P6 protein ratios (containing highest soybean meal) that mostly had significant difference with P1and P2 protein ratios (containing maximum fish meal) ($P < 0.05$). The highest survival rate was found in P1 protein ratio although no significant difference was observed among the protein ratios ($P \geq 0.05$). Results presented in tables 4 and 7 indicated that all feeding and growth parameters with the exception of survival, yield, FCR, PER and ANPU were significantly affected by different digestible energy levels ($P < 0.05$). The best growth performances were observed in lowest digestible energy level (E1) that had significant differences with E2 and E3 energy levels ($P < 0.05$). The maximum survival rate was found in E3 energy level although had no significant difference with other energy levels ($P \geq 0.05$). Results presented in table 8 indicated that all chemical body composition parameters, with the exception of NFE and moisture content, were significantly affected by the interactive effects of protein ratios and energy levels ($P < 0.05$).

Maximum crude protein (62.28±1.51), crude lipid (6.10±1.14), crude fibre (5.69±0.31), ash content (12.15±0.54) were observed in shrimp fed P3E3, P1E3, P6E3 and P1E1 treatments respectively that had significant differences with (P1E2 and P5E3), (P4E1, P5E2, P6E1, P6E2 and P6E3), P5E3, (P1E3, P4E2 and P6E2) treatments respectively ($P < 0.05$). Maximum moisture content (13.86±0.89) and NFE (11.39±4.21) was observed in shrimp fed P2E3 and P5E3 treatments respectively that had no significant difference with other treatments ($P \geq 0.05$). Results presented in table 9 showed that some of chemical body composition parameters such as crude lipid and crude fibre were significantly affected by different fish meal : soybean meal ratios ($P < 0.05$).

Table 2: Interactive effect of different protein ratios and energy levels on some of growth indices of pacific white shrimp *(Litopenaeus vannamei)*

Treatment	Number of samples	Final weight (g)	Weight gain (g)	Weight gain (%)	SGR
P1E1	3	3.63 ± 0.51 ^{d}	2.88 ± 0.50 ^{de}	383.93 ± 63.05 ^{bcd}	2.81 ± 0.23 ^{cd}
P1E2	3	3.65 ± 0.17 ^{d}	2.90 ± 0.14 ^{de}	382.12 ± 11.87 ^{bcd}	2.81 ± 0.05 ^{cd}
P1E3	3	3.51 ± 0.33 ^{d}	2.78 ± 0.33 ^{de}	377.62 ± 45.74 ^{cd}	2.78 ± 0.17 ^{cd}
P2E1	3	3.75 ± 0.39 ^{cd}	2.98 ± 0.36 ^{cde}	385.46 ± 35.06 ^{bcd}	2.82 ± 0.13 ^{cd}
P2E2	3	3.91 ± 0.69 ^{cd}	3.17 ± 0.69 ^{cde}	422.88 ± 94.60 ^{bcd}	2.93 ± 0.34 ^{cd}
P2E3	3	3.30 ± 0.41 ^{d}	2.53 ± 0.40 ^{e}	329.85 ± 52.38 ^{d}	2.60 ± 0.22 ^{d}
P3E1	3	4.12 ± 0.64 ^{bcd}	3.34 ± 0.62 ^{bcde}	429.46 ± 71.31 ^{bcd}	2.96 ± 0.23 ^{bcd}
P3E2	3	4.27 ± 0.24 ^{bcd}	3.52 ± 0.22 ^{abcd}	470.64 ± 21.10 ^{abc}	3.11 ± 0.07 ^{abc}
P3E3	3	3.72 ± 0.43 ^{cd}	2.94 ± 0.41 ^{cde}	376.39 ± 44.45 ^{cd}	2.78 ± 0.17 ^{cd}
P4E1	3	4.65 ± 0.34 ^{abc}	3.88 ± 0.34 ^{abc}	500.78 ± 43.85 ^{ab}	3.20 ± 0.13 ^{abc}
P4E2	3	3.42 ± 0.22 ^{d}	2.64 ± 0.19 ^{de}	328.45 ± 14.32 ^{d}	2.64 ± 0.06 ^{d}
P4E3	3	3.59 ± 0.63 ^{d}	2.80 ± 0.62 ^{de}	352.02 ± 71.96 ^{cd}	2.68 ± 0.29 ^{d}
P5E1	3	4.98 ± 0.22 ^{ab}	4.22 ± 0.23 ^{ab}	554.40 ± 36.73 ^{a}	3.35 ± 0.10 ^{ab}
P5E2	3	3.68 ± 0.34 ^{d}	2.93 ± 0.36 ^{de}	390.93 ± 61.72 ^{bcd}	2.83 ± 0.21 ^{cd}
P5E3	3	4.20 ± 0.81 ^{bcd}	3.41 ± 0.81 ^{bcde}	428.37 ± 98.74 ^{bcd}	2.95 ± 0.33 ^{cd}
P6E1	3	5.17 ± 0.83 ^{a}	4.39 ± 0.83 ^{a}	561.23 ± 102.58 ^{a}	3.37 ± 0.28 ^{a}
P6E2	3	3.97 ± 0.39 ^{cd}	3.20 ± 0.40 ^{cde}	415.50 ± 62.31 ^{bcd}	2.92 ± 0.21 ^{cd}
P6E3	3	3.77 ± 0.30 ^{cd}	3.01 ± 0.29 ^{cde}	395.78 ± 40.80 ^{bcd}	2.85 ± 0.15 ^{cd}

SGR= specific growth rate

Table 3: effect of different protein ratios on some of growth indices of pacific white shrimp

Treatment cod	Treatment name	Number of samples	Final weight (g)	Weight gain (g)	Weight gain (%)	SGR
P1	100 A – 0 V	9	3.60 ± 0.32^{b}	2.85 ± 0.31^{b}	381.22 ± 39.50^{ab}	2.80 ± 0.15^{a}
P2	80 A – 20 V	9	3.65 ± 0.52^{b}	2.89 ± 0.52^{b}	379.39 ± 69.82^{b}	2.78 ± 0.26^{a}
P3	60 A – 40 V	9	4.04 ± 0.47^{ab}	3.27 ± 0.47^{ab}	425.50 ± 59.59^{ab}	2.95 ± 0.20^{a}
P4	40 A – 60 V	9	3.79 ± 0.66^{ab}	3.01 ± 0.66^{ab}	384.19 ± 83.84^{ab}	2.79 ± 030^{a}
P5	20 A – 80 V	9	4.20 ± 0.72^{a}	3.43 ± 0.72^{a}	445.84 ± 94.11^{a}	3.01 ± 0.30^{a}
P6	0 A – 100 V	9	4.19 ± 0.73^{a}	3.43 ± 0.73^{a}	444.54 ± 91.43^{a}	3.01 ± 0.28^{a}

SGR= specific growth rate

Table 4- effect of different energy levels on some of growth indices of pacific white shrimp

Treatment cod	Treatment Name (kcal/100g food)	Number of samples	Final weight (g)	Weight gain (g)	Weight gain (%)	SGR
E1	262	18	4.27 ± 0.73^{a}	3.51 ± 0.72^{a}	455.29 ± 89.65^{a}	3.04 ± 0.28^{a}
E2	312	18	3.82 ± 0.42^{b}	3.06 ± 0.43^{b}	403.45 ± 61.55^{b}	2.87 ± 0.22^{b}
E3	362	18	3.68 ± 0.52^{b}	2.91 ± 0.51^{b}	376.67 ± 61.51^{b}	2.77 ± 0.23^{b}

SGR= specific growth rate

Table 5: Interactive effect of different protein ratios and energy levels on some of growth and feeding indices of pacific white shrimp *(Litopenaeus vannamei*)

Treatment	Number of samples	Final Orbital Carapace Length (mm)	Orbital Carapace Length increase (mm)	Orbital Carapace Length increase (%)	Survival (%)	Yield (g)	FCR	PER
P1E1	3	17.30 ± 0.86 ^{de}	6.26 ± 0.93 ^{bc}	56.86 ± 9.32 ^{bcd}	94.74 ± 9.12 ^{a}	65.64 ± 13.27 ^{abc}	3.45 ± 0.50 ^{ab}	0.82 ± 0.11 ^{bc}
P1E2	3	17.44 ± 0.32 ^{cde}	6.25 ± 0.44 ^{bc}	55.99 ± 6.00 ^{bcd}	94.74 ± 5.26 ^{a}	65.67 ± 2.21 ^{abc}	3.24 ± 0.15 ^{ab}	0.68 ± 0.04 ^{bc}
P1E3	3	17.13 ± 0.60 ^{de}	6.38 ± 0.69 ^{bc}	59.50 ± 7.24 ^{abcd}	92.98 ± 3.04 ^{a}	61.61 ± 3.15 ^{abc}	3.30 ± 0.17 ^{ab}	0.84 ± 0.05 ^{bc}
P2E1	3	17.56 ± 0.64 ^{cde}	6.22 ± 0.25 ^{bc}	54.89 ± 1.31 ^{bcd}	87.72 ± 8.04 ^{ab}	62.27 ± 6.08 ^{bc}	3.62 ± 0.79 ^{ab}	0.79 ± 0.18 ^{bc}
P2E2	3	17.72 ± 1.16 ^{cde}	6.75 ± 1.43 ^{bc}	60.73 ± 14.34 ^{abcd}	92.98 ± 6.08 ^{a}	68.65 ± 8.18 ^{abc}	3.30 ± 0.37 ^{ab}	0.85 ± 0.09 ^{bc}
P2E3	3	16.83 ± 0.68 ^{e}	5.60 ± 0.85 ^{c}	50.07 ± 9.22 ^{cd}	87.72 ± 13.25 ^{ab}	55.57 ± 13.82 ^{c}	4.47 ± 1.88 ^{a}	0.69 ± 0.24 ^{c}
P3E1	3	18.20 ± 0.87 ^{bcde}	6.64 ± 0.71 ^{bc}	57.39 ± 5.29 ^{bcd}	89.47 ± 5.27 ^{ab}	70.02 ± 11.64 ^{abc}	3.21 ± 0.26 ^{ab}	0.87 ± 0.07 ^{bc}
P3E2	3	18.34 ± 0.36 ^{abcd}	7.36 ± 0.32 ^{ab}	67.08 ± 2.65 ^{ab}	92.98 ± 8.04 ^{a}	75.52 ± 8.80 ^{ab}	3.12 ± 0.40 ^{ab}	0.90 ± 0.11 ^{abc}
P3E3	3	17.51 ± 0.70 ^{cde}	6.05 ± 0.56 ^{bc}	52.84 ± 4.65 ^{bcd}	94.74 ± 5.27 ^{a}	66.74 ± 4.40 ^{abc}	3.26 ± 0.09 ^{ab}	0.85 ± 0.02 ^{bc}
P4E1	3	18.80 ± 0.43 ^{abc}	7.35 ± 0.36 ^{ab}	64.22 ± 3.54 ^{abc}	76.32 ± 3.73 ^{b}	67.53 ± 8.22 ^{abc}	3.80 ± 0.77 ^{ab}	0.75 ± 0.15 ^{bc}
P4E2	3	16.97 ± 0.38 ^{de}	5.53 ± 0.33 ^{c}	48.37 ± 4.09 ^{d}	87.72 ± 8.04 ^{ab}	57.23 ± 8.81 ^{bc}	3.21 ± 0.42 ^{ab}	0.87 ± 0.11 ^{bc}
P4E3	3	17.18 ± 1.05 ^{de}	5.54 ± 1.02 ^{c}	47.58 ± 8.62 ^{d}	92.98 ± 8.04 ^{a}	63.83 ± 14.43 ^{bc}	3.83 ± 1.24 ^{ab}	0.77 ± 0.22 ^{bc}
P5E1	3	19.42 ± 0.30 ^{ab}	8.24 ± 0.61 ^{a}	73.84 ± 7.45 ^{a}	89.47 ± 0.00 ^{ab}	84.57 ± 3.73 ^{a}	2.77 ± 0.23 ^{b}	1.01 ± 0.08 ^{ab}
P5E2	3	17.42 ± 0.49 ^{cde}	6.40 ± 0.95 ^{bc}	58.44 ± 11.32 ^{bcd}	84.21 ± 5.26 ^{ab}	58.72 ± 2.90 ^{bc}	3.05 ± 0.31 ^{b}	0.92 ± 0.09 ^{abc}
P5E3	3	18.26 ± 1.10 ^{abcde}	6.48 ± 0.95 ^{bc}	55.02 ± 7.37 ^{bcd}	94.74 ± 5.27 ^{a}	76.15 ± 18.91 ^{ab}	3.12 ± 0.71 ^{ab}	0.93 ± 0.24 ^{abc}
P6E1	3	19.62 ± 1.22 ^{a}	8.35 ± 1.17 ^{a}	74.07 ± 10.14 ^{a}	86.85 ± 11.17 ^{ab}	84.43 ± 2.76 ^{a}	2.43 ± 0.10 ^{b}	1.15 ± 0.05 ^{a}
P6E2	3	17.87 ± 0.61 ^{cde}	6.67 ± 0.81 ^{bc}	59.68 ± 8.57 ^{abcd}	82.46 ± 6.07 ^{ab}	61.96 ± 4.08 ^{bc}	3.26 ± 0.25 ^{ab}	0.85 ± 0.07 ^{bc}
P6E3	3	17.55 ± 0.51 ^{cde}	6.44 ± 0.69 ^{bc}	58.06 ± 7.22 ^{bcd}	92.98 ± 3.04 ^{a}	66.48 ± 3.64 ^{abc}	3.45 ± 0.30 ^{ab}	0.81 ± 0.07 ^{bc}

FCR= food conversion ratio

PER= protein efficiency ratio

Table 6: effect of different protein ratios on some of growth and feeding indices of pacific white shrimp *(Litopenaeus vannamei*)

Treatment	Treatment Name	Number of samples	Final Orbital Carapace Length (mm)	Orbital Carapace Length increase (mm)	Orbital Carapace Length increase (%)	Survival (%)	Yield (g)	FCR	PER
P1	100 A – 0 V	9	17.21 ± 0.56 ^{b}	6.30 ± 0.62 ^{ab}	57.45 ± 6.81 ^{ab}	94.15 ± 5.15 ^{a}	64.21 ± 7.24 ^{a}	3.33 ± 0.29 ^{ab}	0.84 ± 0.07 ^{ab}
P2	80 A – 20 V	9	17.37 ± 0.85 ^{b}	6.19 ± 0.98 ^{ab}	55.56 ± 9.94 ^{ab}	89.47 ± 8.73 ^{a}	62.16 ± 10.29 ^{a}	3.80 ± 1.16 ^{a}	0.78 ± 0.17 ^{b}
P3	60 A – 40 V	9	18.02 ± 0.70 ^{ab}	6.69 ± 0.74 ^{ab}	59.11 ± 7.33 ^{ab}	92.40 ± 5.95 ^{a}	70.76 ± 8.54 ^{a}	3.20 ± 0.25 ^{ab}	0.87 ± 0.07 ^{ab}
P4	40 A – 60 V	9	17.65 ± 1.06 ^{ab}	6.14 ± 1.07 ^{b}	53.39 ± 9.59 ^{b}	86.84 ± 9.33 ^{a}	62.28 ± 10.54 ^{a}	3.59 ± 0.82 ^{ab}	0.80 ± 0.15 ^{b}
P5	20 A – 80 V	9	18.23 ± 1.06 ^{a}	6.89 ± 1.12 ^{ab}	61.01 ± 11.19 ^{ab}	89.47 ± 6.29 ^{a}	71.72 ± 15.31 ^{a}	3.00 ± 0.45 ^{b}	0.95 ± 0.15 ^{a}
P6	0 A – 100 V	9	18.19 ± 1.09 ^{a}	7.00 ± 1.11 ^{a}	62.67 ± 10.03 ^{a}	87.50 ± 7.41 ^{a}	69.27 ± 10.08 ^{a}	3.13 ± 0.49 ^{ab}	0.91 ± 0.16 ^{ab}

FCR= food conversion ratio   PER= protein efficiency ratio

Table 7: effect of different energy levels on some of growth and feeding indices of pacific white shrimp (*Litopenaeus vannamei*)

Treatment	Treatment Name (kcal/100g food)	Number of samples	Final Orbital Carapace Length (mm)	Orbital Carapace Length increase (mm)	Orbital Carapace Length increase (%)	Survival (%)	Yield (g)	FCR	PER
E1	262	18	18.35 ± 1.07 ^{a}	7.04 ± 1.03 ^{a}	62.24 ± 9.30 ^{a}	88.07 ± 8.07 ^{a}	71.12 ± 11.52 ^{a}	3.25 ± 0.63 ^{a}	0.88 ± 0.16 ^{a}
E2	312	18	17.63 ± 0.68 ^{b}	6.49 ± 0.89 ^{b}	58.55 ± 9.45 ^{ab}	89.18 ± 7.32 ^{a}	64.63 ± 8.41 ^{a}	3.20 ± 0.29 ^{a}	0.88 ± 0.08 ^{a}
E3	362	18	17.41 ± 0.82 ^{b}	6.08 ± 0.79 ^{b}	53.85 ± 7.66 ^{b}	92.69 ± 6.55 ^{a}	65.01 ± 11.63 ^{a}	3.57 ± 0.95 ^{a}	0.81 ± 0.16 ^{a}

FCR= food conversion ratio   PER= protein efficiency ratio

Table 8: Interactive effect of different protein ratios and energy levels on chemical body composition of pacific white shrimp (*Litopenaeus vannamei*)

Treatment	Number of samples	Protein (%)	Lipid (%)	Fiber (%)	NFE	Ash (%)	Moisture (%)	ANPU
P1E1	3	60.75 ± 1.30 [abc]	4.09 ± 2.02 [abc]	4.57 ± 0.27 [ab]	7.69 ± 1.78 [a]	12.15 ± 0.54 [a]	11.42 ± 2.82 [a]	49.49±6.14 [bcd]
P1E2	3	56.41 ± 2.55 [c]	5.73 ± 1.26 [ab]	4.65 ± 0.41 [ab]	10.80 ± 4.67 [a]	10.26 ± 1.56 [ab]	12.83 ± 2.72 [a]	48.40±3.50 [bcd]
P1E3	3	58.88 ± 1.85 [abc]	6.10 ± 1.14 [a]	4.69 ± 1.22 [ab]	8.02 ± 1.90 [a]	9.55 ± 1.08 [b]	12.95 ± 2.28 [a]	49.66±2.70 [bcd]
P2E1	3	59.08 ± 0.83 [abc]	5.51 ± 0.94 [ab]	4.89 ± 0.75 [ab]	8.23 ± 1.60 [a]	9.78 ± 0.45 [ab]	12.53 ± 1.00 [a]	46.82±9.80 [bcd]
P2E2	3	60.60 ± 4.31 [abc]	5.29 ± 0.50 [abc]	5.07 ± 0.79 [ab]	7.09 ± 2.82 [a]	10.75 ± 1.78 [ab]	11.00 ± 2.30 [a]	51.27±5.03 [bcd]
P2E3	3	57.36 ± 2.84 [abc]	4.06 ± 2.59 [abc]	4.92 ± 1.00 [ab]	8.43 ± 2.12 [a]	11.42 ± 1.14 [ab]	13.86 ± 0.89 [a]	38.94±11.94 [d]
P3E1	3	60.26 ± 1.79 [abc]	4.61 ± 0.34 [abc]	5.23 ± 0.37 [ab]	7.43 ± 2.72 [a]	10.67 ± 0.41 [ab]	12.09 ± 1.26 [a]	52.34±3.35 [bcd]
P3E2	3	59.44 ± 0.70 [abc]	4.43 ± 0.86 [abc]	5.45 ± 0.63 [a]	8.02 ± 0.95 [a]	10.00 ± 0.51 [ab]	11.82 ± 2.05 [a]	53.45±6.37 [bcd]
P3E3	3	62.28 ± 1.51 [a]	5.74 ± 1.19 [ab]	4.98 ± 0.39 [ab]	7.07 ± 3.76 [a]	11.32 ± 0.75 [ab]	9.52 ± 0.16 [a]	53.17±2.62 [bcd]
P4E1	3	59.68 ± 3.30 [abc]	2.75 ± 0.99 [c]	4.91 ± 0.50 [ab]	9.72 ± 1.88 [a]	10.01 ± 1.97 [ab]	13.02 ± 1.54 [a]	43.43±6.15 [cd]
P4E2	3	61.92 ± 1.52 [ab]	4.46 ± 0.64 [abc]	5.08 ± 0.52 [ab]	8.41 ± 2.31 [a]	9.51 ± 0.63 [b]	9.69 ± 1.95 [a]	54.07±6.56 [bcd]
P4E3	3	58.16 ± 1.57 [abc]	4.09 ± 1.29 [abc]	4.46 ± 0.60 [ab]	10.95 ± 1.80 [a]	10.78 ± 0.39 [ab]	12.19 ± 1.26 [a]	44.93±13.22 [bcd]
P5E1	3	59.87 ± 0.65 [abc]	4.62 ± 0.05 [abc]	5.28 ± 0.27 [ab]	8.53 ± 2.19 [a]	10.44 ± 1.04 [ab]	11.29 ± 2.18 [a]	60.43±5.77 [ab]
P5E2	3	60.01 ± 2.69 [abc]	3.16 ± 0.89 [bc]	4.81 ± 0.44 [ab]	8.31 ± 4.83 [a]	10.97 ± 2.01 [ab]	13.14 ± 0.27 [a]	55.12±6.55 [bc]
P5E3	3	56.77 ± 2.94 [bc]	5.32 ± 2.55 [abc]	3.98 ± 0.24 [b]	11.39 ± 4.21 [a]	10.50 ± 2.55 [ab]	12.26 ± 4.18 [a]	52.81±14.46 [bcd]
P6E1	3	60.11 ± 2.04 [abc]	3.27 ± 0.33 [bc]	5.05 ± 0.38 [ab]	9.20 ± 0.84 [a]	10.77 ± 0.70 [ab]	12.73 ± 1.93 [a]	69.98±4.58 [a]
P6E2	3	59.04 ± 5.65 [abc]	2.79 ± 0.85 [c]	5.57 ± 1.37 [a]	8.90 ± 3.56 [a]	9.48 ± 0.56 [b]	12.32 ± 4.57 [a]	50.62±8.35 [bcd]
P6E3	3	59.79 ± 3.67 [abc]	3.31 ± 1.40 [bc]	5.69 ± 0.31 [a]	9.57 ± 2.66 [a]	10.27 ± 0.96 [ab]	11.29 ± 1.30 [a]	48.31±4.17 [bcd]

ANPU= apparent net protein utilization

NFE= nitrogen free extract

Table 9: Effect of different protein ratios on chemical body composition of pacific white shrimp (*Litopenaeus vannamei*)

Treatment	Treatment name	Number of samples	Protein (%)	Lipid (%)	Fiber (%)	NFE	Ash (%)	Moisture (%)	ANPU
P1	100A – 0V	9	58.68 ± 2.54 [a]	5.30 ± 1.61 [a]	4.64 ± 0.66 [b]	8.83 ± 3.06 [a]	10.65 ± 1.52 [a]	12.40 ± 2.38 [a]	49.18 ± 3.83 [ab]
P2	80A – 20V	9	59.01 ± 2.97 [a]	4.95 ± 1.55 [ab]	4.96 ± 0.74 [ab]	7.92 ± 2.03 [a]	10.65 ± 1.30 [a]	12.47 ± 1.82 [a]	45.67 ± 9.75 [b]
P3	60A – 40V	9	60.66 ± 1.76 [a]	4.93 ± 0.97 [ab]	5.22 ± 0.46 [ab]	7.50 ± 2.41 [a]	10.66 ± 0.76 [a]	11.14 ± 1.72 [a]	52.99 ± 3.86 [ab]
P4	40A – 60V	9	59.92 ± 2.57 [a]	3.77 ± 1.17 [bc]	4.82 ± 0.54 [ab]	9.70 ± 2.06 [a]	10.10 ± 1.19 [a]	11.63 ± 2.04 [a]	47.98 ± 9.66 [ab]
P5	20A – 80V	9	58.76 ± 2.71 [a]	4.33 ± 1.77 [abc]	4.61 ± 0.63 [b]	9.52 ± 3.85 [a]	10.66 ± 1.80 [a]	12.35 ± 2.50 [a]	55.58 ± 9.32 [a]
P6	0A – 100V	9	59.65 ± 3.55 [a]	3.12 ± 0.87 [c]	5.44 ± 0.79 [a]	9.25 ± 2.28 [a]	10.17 ± 0.87 [a]	12.12 ± 2.64 [a]	54.59 ± 10.92 [a]

Table 10: Effect of different energy levels on chemical body composition of pacific white shrimp (*Litopenaeus vannamei*)

Treatment	Treatment name (Kcal/100gr food)	Number of samples	Protein (%)	Lipid (%)	Fiber (%)	NFE	Ash (%)	Moisture (%)	ANPU
E1	262	18	59.96 ± 1.70 [a]	4.11 ± 1.30 [a]	4.97 ± 0.46 [a]	8.46 ± 1.79 [a]	10.65 ± 1.16 [a]	12.23 ± 1.67 [a]	52.91 ± 10.02 [a]
E2	312	18	59.57 ± 3.30 [a]	4.31 ± 1.30 [a]	5.10 ± 0.72 [a]	8.60 ± 3.14 [a]	10.16 ± 1.26 [a]	11.80 ± 2.52 [a]	52.15 ± 5.75 [a]
E3	362	18	58.87 ± 2.82 [a]	4.77 ± 1.84 [a]	4.79 ± 0.81 [a]	9.24 ± 2.91 [a]	10.64 ± 1.29 [a]	12.01 ± 2.26 [a]	47.97 ± 9.54 [a]

ANPU= apparent net protein utilization

NFE= nitrogen free extract

But most of this parameters such as crude protein, ash content, moisture content and NFE were not significantly affected ($P \geq 0.05$). Maximum crude lipid (5.30±1.61) and crude fibre (5.44±0.79) were observed in shrimp fed P1 and P6 treatments respectively that had significant differences with (P4 and P6) and (P1and P5) treatments respectively ($P< 0.05$).

Maximum crude protein (60.66±1.76), ash content (10.66±0.76), moisture content (12.47±1.82) and NFE (9.70±2.06) were observed in shrimp fed P3, (P3 and P4), P2 and P4 treatments respectively that had no significant difference with other treatments ($P\geq0.05$). Results presented in table 10 indicated that all chemical body composition parameters were not significantly affected by different energy levels ($P \geq 0.05$). During the experimental period, temperature and pH ranged from 24.2 to 30.1 and 7.42 – 8.85 respectively.

Discussion

The results obtained in this study indicated that the best growth performance and feeding parameters of juvenile pacific white shrimp occurred in P6E1 and P5E1 treatments and also in P6 and P5 protein ratios and mostly in E1 energy level. Maximum survival rate occurred in P1E1, P1E2, P3E3 and P5E3 treatments and also in P1 protein ratio and E3 energy level. The most important problems of application soybean products in aquaculture diets are deficiency or imbalance of essential amino acids, mainly methionine, lysine and threonine, lack of n-3 marine fatty acids EPA and DHA and presence of anti-nutritional factors such as protease inhibitors and glycosides. Additionally only 30-40% of the total phosphorus content is considered to be available for this shrimp (Hertrampf and Piedad-Pascual, 2000). If the replacement strategy considers shifts in essential nutrients, it also appears that fish meal can be removed from shrimp formulations if suitable alternative sources of protein and lipids are provided to meet the nutritional requirements of the animal (Amaya et al., 2007).

These results showed that the treatments having the best effect on growth performance and feeding parameters of pacific white shrimp have minimum fish meal and maximum soybean meal proportion and contain minimum energy level and these priorities had significant difference for most parameters. This confirmed favorability of feeds containing high plant protein for feeding pacific white shrimp and showing high ability of this shrimp in consumption of plant protein sources in diets. These important results suggested that with fish meal complete remove from diets of this shrimp or considering minimum proportion of this important and expensive ingredient in diets and obtaining maximum production, shrimp producers will obtain considerable profit.

The highest survival rate was observed in diets containing the highest fish meal levels but had no significant difference with diets containing maximum soybean meal levels. The high survival during the growth trial indicated the good health condition of the shrimp and confirmed the absence any nutrient deficiency. Results showed that most of growth parameters decreased with increasing digestible energy levels. This is because of high digestible energy in diets can lead to decreasing protein and feed intake in shrimps. On the other hand high lipid contents in diets containing highest energy levels diminish the pellet hardness and stability in water due to the reduction in the compression capacity of the press pellet machine resulting in decreasing daily feed intake and finally can explain decreasing growth performance in these treatments.

ANPU (apparent net protein utilization) usually increase with increasing animal protein content of diets but in this study maximum ANPU observed in diets containing highest plant protein value showing suitable protein sources and high quality of dietary plant protein in these diets. The favorable response of the shrimp to diets used in the present study is probably due to the high quality of the ingredients used in terms of nutrient profile and possibly digestibility as well as lack of apparent palatability problems. Some of the studies conducted on this species regarding replacement of fish meal with different plant and animal sources confirm results of this study. According to the findings of Akiyama (1988), soybean meal was favorable protein source in proportion to fish meal for *p.durarum* also this author stated that we can utilize soybean meal in culture of tiger shrimp (*penaeus monodon*) up to 35 % diet in density of 20 shrimp / m^2 and up to 45 % diet in density of 10 shrimp / m^2 and shrimp grows well, also according to these findings, complete replacement of fish meal with soybean meal in *palaemon serratus* caused reduction in growth. In another study, Swick et al. (1995) concluded that use of soybean meal in 20 to 40 % proportions in cultured shrimp lead to optimum results. Lim et al. (1997) evaluate nutritive values of low and high fibre canola meals for pacific white shrimp. It is concluded that commercial high-fibre canola meal can constitute 300 g kg^{-1} of the dietary protein of juvenile shrimp without compromising growth, feed intake and feed and protein utilization. Davis and Arnold (2000) reported that up to 80 % of the fish meal in diets for pacific white shrimp can be substituted by co-extruded soybean poultry by-product meal containing egg supplement or poultry by-product meal without any apparent effect on survival, growth and feed palatability. Mendoza et al. (2001) evaluate fish meal replacement with feather-enzymatic hydrolysates co-extruded with soybean meal in practical diets for the pacific white shrimp. Feather meal processed in two forms: one, with using of steam and the other, with using of enzymatical hydrolysis. These two products were blended with soybean meal in a 1:1 ratio. The shrimp fed on first co-extruded product gained less weight compared with control diet (diet including only fish meal) but weight gain in shrimp fed on second co-extruded product did not differ from that of shrimp fed on the fish meal control diet. Davis et al. (2002) concluded that pea meal had potential as an alternative feed ingredient in this shrimp feeds and there appear to be no adverse effects on shrimp growth, survival and FE values at the inclusion level tested. Forster et al. (2003) reported that meat and bone meal (MBM) depending on utilized source, can effectively replace fish meal in 25 to 75 % white leg shrimp diet. Samocha et al (2004) evaluate the use of a co-extruded soybean poultry by-product meal with egg supplement as a substitute for fish meal in a practical diet for pacific white shrimp. Inclusion levels varied from 0% (30 gr fish meal / 100 gr diet) to 100% (0 gr fish meal / 100 gr diet). At the conclusion of growth trial, survival, final weight, weight gain percent and feed efficiency were not significantly different among treatments. Based on the results, these authors concluded that co-extruded soybean poultry by-product meal with egg supplement appears suitable as a substitute for fish meal in this shrimp diets. Amaya et al. (2007) evaluate plant proteins as replacement ingredients to animal protein sources in the diets of juvenile pacific white shrimp in an outdoor tanks system. This study demonstrated that fish meal can be removed from commercially manufactured shrimp diets including 16% poultry by-product meal using vegetable protein sources with no adverse effect on the productive performance of this shrimp reared in green water environments. Suarez et al. (2009) concluded that pacific white shrimp can be fed plant meals (70% soybean – 30% canola) thereby reducing the quantity of fish meal from 30 to 6 g / 100 g dry weight (corresponding to a reduction of 80%). Nevertheless results obtained in some of the studies regarding feeding of these species partly differ from present study. Lim and Dominy (1990) evaluate soybean meal as a replacement for marine animal protein in diets for pacific white shrimp and concluded that shrimp fed on the three lowest dietary levels of soybean meal (0, 14 and 28 %) had similar weight gains, and weight gains declined significantly as the dietary soybean levels increased. There were significant differences ($P < 0.05$) among the survival rates; however these differences could not be attributed to the dietary levels of soybean meal. Feed conversion ratio, protein efficiency ratio and apparent protein utilization were similar for diets having 0 to 56 % soybean meal. The 70 % soybean meal diet was utilized very poorly by the shrimp. Ghorbani vagheie et al. (2007) assessed the influence of different dietary levels of plant protein (30, 50 and 70 %) on growth and feeding indices of pacific white shrimp (with initial average weight 10 gr) fed with an original 38 percent protein and compared with that of the commercial shrimp diet as control diet (include plant protein 20 %). FCR, PER, SGR and average daily weight gain indices were better in the control diet compared to the treatments but no significant difference was found among the treatments and between the treatments and the control diet for this indices ($P \geq 0.05$).

Results obtained in above studies showed that there is no doubt about effectiveness of replacement of a part of fish meal with soybean meal in diets of different shrimps (particularly this species) and the doubt is mainly about replacement proportion that some of researchers found this proportion in low extent and some of authors found this proportion in high extent that this difference may results from factors such as age and size of shrimp, the compositions of ingredients and experimental conditions. In consideration of results obtained in this study, juvenile pacific white shrimp prefers soybean meal to fish meal in its diet so that up to 80 % fish meal in this shrimp diet can replace the soybean meal while obtaining suitable growth and feeding indices.

Undoubtedly complete or main part replacement of fish meal with less expensive plant protein sources such as soybean meal can highly affect the improvement of profitability and development of aquaculture industry (particularly shrimp culture industry that is mainly based on fish meal protein source) and increases its efficiency.

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