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Effects of Different Levels of Protein, Energy and their Interaction on Growth Factors of Indian white Shrimp (*Fenneropenaeus indicus*) of Different Sizes

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Abstract: Three experiments of 28 days duration were conducted in three size groups (<1g, 1-5g and 5-10g) of Indian white shrimp, *Fenneropenaeus indicus*, to evaluate the appropriate protein: energy (GE) ratios in their diets. A common ingredient mixture consisting of fish meal, shrimp meal, ground nut oil cake, cholesterol, lecithin, vitamins and minerals blended with chicken egg albumin, tapioca flour and cellulose (filler) to formulate nine feeds with protein levels of 35%, 40% and 45% and GE levels of 380, 420 and 460 kcal/l00g at each level of protein (i.e:35:380, 35:420, 35:460; 40:380, 40:420, 40:460 and 45:380, 45:420 and 45:460). Nutritional responses in terms of weight gain (g/individual), RGR, FCR, FCE, PER and PPV indicated that the protein: GE combination of 45:460 to be best in samples of ≤lg size. Since weight increases were progressive, without indicating an optimum in this group of animals, protein levels beyond 45% needs to be explored. In the size group of l-5g the protein: GE combination of 40:420 was found to be appropriate, indicating a decline in the requirement of protein and energy as growth progresses. In the size group of 5-l0g, a further decline in the protein: GE combination was observed with the protein: GE combination of 35:380 performing the best among the levels tested. In this size group a protein level lower than 35% has to be investigated. The findings reported are discussed in the light of relevant reports.

Keywords : *Fenneropenaeus indicus*, Protein: Energy ration, Growth performances, Nutritional response

Introduction

Fenneropenaeus indicus, the Indian white shrimp is a penaeid shrimp, on which relatively less research has been carried out compared to *Penaeus monodon* and *Penaeus japonicus* in terms of nutrition. Macronutrient requirements are fairly well understood (Colvin, 1976; Gopal & Raj, 1990; Ali, 1990, 1994 and 1996, Akiyama *et al*., 1992, Chandge & Raj, 1997a, 1997b and Shiau, 1998). However, protein: energy ratios in the diets designed for this shrimp appears to be an area where very few reports are available. Abedian (2002) observed that at 35% proteins and 350 kcal /100gl table appropriate in *Penaeus indicus* weighing less than one gram. In *Penaeus monodon* Hajra *et. al*. (1988) reported an energy level of 412.6 kcal/100g at 46% protein in shrimps weighing<lg in near fresh water condition (5 ppt). Shiau & Chou (1991) had observed that at 36% protein, 330 kcal/100g GE to be appropriate and at 40% protein, 320 kcal/100 g GE to be appropriate in *Penaeus monodon* weighing 0.82±0.10g at salinity of 32-34ppt. Chuntapa *et al.* (1999) reported a lipid: carbohydrate ratio of 7:32 for *Penaeus monodon* using isonitrogenous (35%) and isocaloric diets (330 kcal/l00g). With this L:C ratio, 33-44% protein level and 263–331 kcal/l00g GE was reported to be appropriate in shrimps of <lg size. Inability of shrimps to utilize lipids beyond 12% (Chandge & Raj, 1997b) makes in imperative to examine comprehensively this shrimp's capability to utilize carbohydrates sparing protein for growth. The present investigation is an attempt to determine the appropriate protein: energy levels in feeds for three size groups of shrimps i.e., less than 1g between l and 5g and between 5 and 10g.

Materials and Methods

Three rearing experiments of 28 days duration were conducted in the wet laboratory of Central Marine Fisheries Research Institute, Cochin, India. For each experiment the Indian white shrimp, *Fenneropenaeus indicus*, was collected from hatcheries (post larvae of<1g) and from farms rearing hatchery brood stocks (shrimps between 1g and 5g is size) in and around Kochi and brought to the laboratory and acclimated to the lab conditions for 48hrs without feeding prior to the beginning of each experiment.

Diet design

Nine compounded diets were formulated for three levels of protein, i.e., 35%, 40% and 45%. For each protein level three GE levels chosen were 380 kcal/100g, 420 kcal/100g and 460 kcal/100g. The natural and purified feed ingredients used for the diet design which their proximate chemical compositions are shown in Table 1. All diets were formulated by using Lindo-Programm (1994).

Table 1: Proximate chemical composition of the natural and purified ingredients used for feed

Ingredients	CP	CF	EE	NFE	Ash
Shrimp meal (*Acetes spp.*)	37.98	II. 00	2.83	20.31	27.87
Fish meal {*Stolephorus spp.*)	61.75	---	5.39	16.83	15.55
Deoiled groundnut oil cake (DGNOC)	49.07	3.57	6.70	33.25	7.41
Clam meal	52.60	----	10.63	28.46	7.64
Tapioca flour	1.72	0.49	1.42	95.09	1.28
Albumin, chicken egg	94.00	------	------	1.50	4.50
Cellulose	0.65	92.56	0.28	0.00	0.31

CP= Crude protein (NX 625), CF=Crude fiber, EE=Ether extract, NFE=Nitrogen free extractives.

All the common natural ingredients were procured from the local market in one batch, dried, ground, sieved and stored in airtight containers. Chicken egg albumin was imported from M/s Sigma Aldrich, USA. Cellulose was procured from M/s Loba chemicals, Mumbai. Chicken egg albumin had the maximum protein content (95%) amongst all the ingredients used. This ingredient was used to adjust the protein levels in the experimental diets in conjunction with tapioca flour. Tapioca flour had a soluble carbohydrate (NFE) content of 95.09%, which was also the sole natural binder used. Clam meal, oven dried and pulverized powder of the black clam (*Villorita cyprinodis*) and fish meal were the other animal protein sources used and deoiled groundnut oil cake (DGNOC) with a protein content of 49.07% was the vegetable protein source used.

At first, a dry common ingredient mixture, (Basaldiet) was made, as shown in Table 2. This common ingredient mixture was fortified with vitamins and minerals.

Table 2: Ingredient composition of the common ingredient mixture (Basaldiet) and its proximate chemical composition

Ingredients	**parts/I 00**
Shrimp meal	20
Fish meal	20
Clam meal	20
DGNOC	20
Cod liver oil	6
Corn oil	6
Vitamin Mixture[a]	3
Mineral mixture[b]	2
Cholesterol[c]	1
Lecithin[c]	2

Proximate chemical composition (% as fed)

CP	CF	EE	NFE	Ash	AIA[d]
48.00	2.10	14.50	16.80	16.00	2.60

a: Thiamin HCL 120 mg/kg dry diet; riboflavin 40 mg/kg dry diet; pyridoxine HCL 120 mg/kg dry diet; nicotinic acid 150 mg/kg dry diet; calcium pantothenate 100 mg/kg dry diet; Folic acid 5 mg/kg dry diet; biotin 1 mg/kg dry diet; cyanocaobalamin 0.02 mg/kg dry diet; inositol 4000 mg/kg dry diet; choline chloride 1200 mg/kg dry diet; sodium ascorbate 5000 mg/kg dry diet; retinol 5000 I.U.. vitamin D 1000 I.U., vitamin E 200 I.U. (Kanazawa 1984 and He et al.. 1992).

b: U.S.P. Salt mixture XTV from M/s Sisco Research Laboratories. Mumbai.

c: Effect of lecithin and lipid sources Piedad (1986).

d: (A.I.A)Acid Insoluble Ash (Impuritis of Feed)

For the final diet design the aforementioned Basal diet was blended with chicken egg albumin (Sigma-Aldrich, USA), tapioca flour and cellulose in requisite quantities as shown in Table 3.

Table 3: Ingredient composition of the experimental diets

	Diet Nos								
Ingredients	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
CIM	45	35	30	30	30	30	30	35	35
Albumin	15	18	20	28	27	25	32	30	35
Tapiocaflour	22	40	48	23	35	45	17	25	30
Cellulose	18	7	2	19	8	-	21	10	-
CP	37.00	34.20	36.2	42.50	39.80	41.20	43.60	45.20	44.10
EE	4.00	5.60	5.80	5.10	4.90	4.30	3.80	4.20	4.10
CF	5.50	2.90	1.50	6.20	3.70	1.00	6.40	3.90	1.20
NFE	36.40	44.20	51.00	27.30	38.80	48.00	28.00	34.00	45.00
Ash	1.00	2.70	1.00	3.40	2.40	0.70	4.30	2.80	1.00
GE(kcal/100g)	380	420	461	390	422.5	462.4	388	426	464
P/E ration (mg protein/kcal)	95.12	86.19	78.52	108.97	94.20	89.10	112.11	106.10	95.04

GE contents when recalculated with analyzed values, were 389, 420 and 461 kcal/100g for the first three diets. For the diets numbered IV, V and VI the values were 390, 422.5 and 462.4 kcal/100g and for the diets numbered v11,v111 and Ix the GE values were 388, 426 and 464 kcal/100 respectively.

All the ground materials, sieved, dry ingredients were mixed well manually, except the tapioca flour. Tapioca flour was gelatinized with distilled water carefully and mixed with the dry ingredients so as to obtain stiff dough. This dough was steamed for 10-15 minutes without pressure and extruded through a kitchen noodle maker with a 2 mm die to obtain thin strands. These strands were dried in a hot air oven at 60°C for 24 hours, crumbled and stored in airtight containers for further use this procedure was followed uniformly for all nine feeds made.

Experiment set-ups

The first experiment was conducted using late post-larvae of <1g in weight; collected from the MPEDA Hatchery at Vallarpadom, Kochl Seawater collected at 20m depth off Kochi and brought to the laboratory filtered and stored in fibreglass tanks. Based on reported from several workers (Abedian, 2002), salinity for all the experiments were maintained at 25ppt in which the experimental animals were also acclimatised. Each dietary treatment had three replications. Shrimps were carefully weighed in an electronic balance (top loading with digital display) with 0.01g accuracy and transferred into 27 plastic tubs of 401 capacity, each. Each tub contained 20 animals of <1g and continuous aeration was provided through air stones from an air compressor (Table 4).

Table 4: Average quality parameters in experimental tanks

	Salinity (ppt)	Dissolved oxygen (ppm)	Temperature °C	pH	NH3 (ppm)
Experiments 1	25±2	4.2±1.00	28±2.00	8.6±0.5	0.025±0.004
Experiments 2	25±2	4.3±1.00	27.5±2.00	8.6±0.5	0.002±0.004
Experiments 3	25±3	4.4±1.00	26.6±3.00	8.8±0.5	0.003±0.005

Feed crushed and sieved through 212 μm, was provided at the rate of 20% of the body weight feed four timed daily (6a.m.; at 12.00p.m.; 16.00p.m. and 22.00p.m.). Exceed feed and faeces were removed next day morning, and one third of the water in each tub was changed daily.

The first experiment was terminated on day 28 and the final weights were recorded and the carcass was analyzed for crude protein. The next two experiments were also performed under similar conditions, except that, in the second experiment the animals used were between 1g and 5 g size and the stocking density was reduced to 15 animals per tub. In the third experiment animals in the size range between 5 and 10 g were stocked at density of 10 animals per tub. For experiment one, the food was granule type, while the other two experiments, were pelleted food.

Response parameters

1. Initial weights and final weights to the nearest 0.01 g were recorded

2. Absolute growth or weight gain was calculated using the formula Wt-Wi where, Wt = weight at time t and Wi = initial weight (Hopkins, 1992).
3. Percentage of Relative growth rate (RGR) was calculated using the formula, Wi-Wi /Wi x 100 where Wt= weight at time t and Wi = initial weight.
4. Food conversion ratio (FCR) = Dry feed consumed (g)*100 / wet weight gain (g)
5. Food conversion efficiency (FCE) = Wet weight gain / Dry food consumed (g); (Baker, 1986)
6. Protein efficiency ratio (PER) = Weight gain (g) / Protein consumed (g)
7. Productive protein value (PPV) = B - Bo /I where, B = final body W, Bo = Initial body W and I = W intake.
8. survival rate= final no / initial no x*100 .

Feed analysis

Feed samples were analyzed according to A.O.A.C. (1990) and GE was calculated according to ADCP (1983).

Statistical analysis

To test the effects of the factors such as the level of protein, level of GE and their two ways interaction, a 3 x 3 factorial experiment was carried out. Mean comparisons were also carried out in cases where the effects were found to be significant, following the method of least significant difference (Snedecor & Cochran, 1967).

Results

The percentage ingredient composition of the Basal' diet formulated is shown in Table 2. On as fed basis it contained 48% protein, 14.5% crude fat, 16.6% NFE, 2.1% CF and 16% ash. This Basal diet was made nutritionally complete with the addition of 2% of a vitamin mixture and 3% mineral mixture,1% Cholesterol and 2% lecithin. The nine experimental feeds were formulated with protein levels of

35%, 40% and 45% and three energy levels of 380 kcal/lg, 420 kcal/l00g and 460 kcal/l00g had crude protein values ranging from 30.08 to 45.50%. The gross energy content in these feeds when calculated according to ADCP (1983) varied from 377 kcal/l00g to 420 kcal/l00g.

Nutritional responses

In animal of <lg initial weights

The weight gain (g per individual), RGR, FCR, FCE, PER and PPV are shown in Table 5. In this experiment, with nine combinations of protein and energy in experimental feeds, two way interaction between the protein and energy levels differences were not significant statistically in terms of weight gain, RGR, FCR and PER. However, differences of PPV was statically significant at two way interaction ($P<0.05$). Protein levels significantly affected weight gain, RGR, FCR, PER and PPV ($P<0.01$). Energy levels solely did not have a significant effect, progressive increase in all the nutritional responses tested was observed without statistically significant ($p>0.01$).

When the means of weight gain, RGR, FCR, FCE, PER and PPV were compared in tables 6 and 7, only varying levels of protein illustrated significant differences ($P<0.05$). Energy levels significantly ($P<0.05$) affected the means of FCE at the energy levels of 420 kcal/100g and 460 kcal/100g, respectively. Moreover, means of PPV were significantly affected ($P<0.05$) at all the three levels of energy i.e., 380 kcal/100g, 420 kcal/100g and 460 kca/100g.

Table 5: Average values of weight gain, RGR, FCR, FCE, PER and PPV in shrimps (<lg) fed three levels of protein and three levels of gross energy.

Dietary protein%	Dietary energy	Weight gain (g/individual)	RGR	FCR	FCE	PER	PPV
35	380	0.0480	427.87	2.40	41.53	1.22	0.35
	420	0.0527	452.47	2.30	43.37	1.09	0.58
	460	0.0570	469.30	2.25	43.47	0.97	0.78
40	380	0.0480	429.07	2.41	41.62	1.18	0.51
	420	0.0530	454.50	2.29	43.58	1.07	0.61
	460	0.0573	473.77	2.22	44.87	0.97	0.92
45	380	0.0503	430.32	2.40	41.90	1.19	0.57
	420	0.0583	457.07	2.28	43.79	1.10	0.67
	460	0.0580	485.13	2.22	44.99	0.97	0.94

Table 6: Mean comparisons of shrimps (<1g) fed three levels of protein with reference to weight gain, RGR, FCR, FCE, PER and PPV (All values are based on nine observations)

Protein levels	35%	40%	45%
Weight gain(g)	0.049^{a}	0.0053^{b}	0.057^{c}
RGR	429.00^{a}	455.00^{b}	476.00^{c}
FCR	2.40^{a}	2.29^{b}	2.23^{c}
FCE	41.68^{a}	43.58^{b}	44.44^{c}
PER	1.29^{a}	1.09^{b}	0.97^{c}
PPV	0.48^{a}	0.62^{b}	0.88^{c}

Figures with same superscript do not differ significantly ($p > 0.01$)

Table 7: Mean comparisons of shrimps (< 1g) fed three levels of energy with reference to weight gain, RGR, FCR, FCE, PER and PPV. (All values are based on nine observations)

Energy levels(kcal/100g)	380	420	460
Weight gain(g)	0.053[a]	0.053[a]	0.0548[a]
RGR	450.00[a]	452.00[a]	458.00[a]
FCR	2.32[a]	2.31[a]	2.30[a]
FCE	42.79[a]	43.36[a]	43.56[ab]
PER	1.10[a]	1.07[a]	1.09[a]
PPV	0.57[a]	0.68[b]	0.73[c]

Figures with the same superscript do not differ significantly ($p > 0.01$)

In animals between 1g and 5g initial weight

In this experiment the weight gain (g/individual), RGR, FCR, FCE, PER and PPV were calculated in (Table 8). Two-way interaction between protein and energy levels were significantly different ($p<0.01$) in terms of weight gain, FCR, FCE and PER ($p<0.01$). When the trends were perused in terms of weight gain, the best protein: energy combination was 40% protein with 420 kcal/100g GE. Same trend was observed in terms of FCR and FCE with 40% protein and 420 kcal/100g GE resulting in the least (best) FCR and highest conversion efficiency ($p< 0.01$).

Table 8: Average values of weight gain, RGR, FCR, FCE, PER and PPV in shrimps (≥1g but <5g) fed three levels of protein and three levels of gross energy.

Dietary protein%	Dietary energy	Weight gain** (g/individual)	RGR**	FCR**	FCE**	PER**	PPV**
35	380	2.617	254.17	1.46	68.97	1.97	0.20
	420	2.732	252.67	1.33	74.83	1.86	0.20
	460	2.935	269.33	1.29	77.27	1.71	0.10
40	380	3.407	304.00	1.13	88.00	2.50	0.20
**	420	3.861	343.00	1.01	98.00	2.45	0.20
	460	3.517	314.00	1.09	91.73	2.03	0.10
45	380	2.906	266.77	1.29	77.87	2.26	0.20
	420	2.934	269.00	1.28	77.87	1.95	0.20
	460	2.689	251.00	1.39	71.67	1.60	0.10

****significant**

When the means were compared separately for protein and energy levels (Tables 9 and 10), diet with 40% protein produced significantly higher weight gain. RGR and FCR indicated no statistically difference results, whereas FCE was significantly higher at 40% protein level (P<0.01). In terms of GE levels, 420 kcal/l00g GE produced significantly higher weight gain (P<0.01). Even though, RGR and FCR were high and least respectively at the same energy level they were not significant different. FCE and PPV were significantly higher at 420 kcal/l00g GE (P<0.01), whereas PER, even though was high at the aforementioned energy level, was not significantly different.

Table 9: Mean comparisons of shrimps (1-5g) fed three levels of protein with reference to weight gain, RGR, FCR, FCE, PER and PPV (All values are based on nine observations)

Protein levels	35%	40%	45%
Weight gain(g)	2.977[a].	3.175[bc]	3.045[a]
RGR	271.98[a]	254.89[a]	278.11[a]
FCR	1.29[a]	1.21[a]	1.26[a]
FCE	78.28[a]	83.57[b]	80.22[c]
PER	2.24[a]	2.09[b]	1.78[c]
PPV	0.23[a]	0.22[ab]	0.18[c]

Figures with same superscript do not differ significantly($p>0.01$)

Table 10: Mean comparisons of shrimps (1-5g) fed three levels of energy with reference to weight gain, RGR, FCR, FCE, PER and PPV (All values are based on nine observations)

Energy levels(kcal/100g)	380	420	460
Weight gain(g)	2.761[a]	3.595[b]	2.841[a]
RGR	255.72[a]	287.00[a]	262.26[a]
FCR	1.36[a]	1.08[a]	1.32[a]
FCE	73.69[a]	92.58[b]	75.80[ab]
PER	1.85[a]	2.23[a]	1.94[a]
PPV	0.21[a]	0.22[ab]	0.20[c]

Figures with the same superscript do not differ significantly ($p>0.01$)

In animals of 5-10g initial weight

In this group of experimental animals, the best weight gain was noticed in animals fed with diet of 35% protein and 380 kcal/100g GE. Weight gain declined with increasing levels of protein and energy. Similarly, RGR, FCE and PER also decreased with increments in protein and energy levels (Table 11). These observations were not statistically significant, when the two-way interaction was tested. Significantly lower ($P<0.05$) FCR was obtained at 35% protein and 380 kcal/100gGE.

Table 11. Average values of weight gain, RGR, FCR, FCE, PER and PPV in shrimps (≥5-10g) fed three levels of protein and three levels of gross energy.

Dietary protein %	Dietary energy	Weight gain *(g/shrimp)	RGR*	FCR**	FCE*	PER*	PPV*
35	380	10.82 ^{a}	167.67 ^{ab}	1.56 ^{a}	64.27 ^{a}	1.83 ^{a}	0.09 ^{a}
	420	8.59 ^{ab}	144.00 ^{ab}	1.89 ^{b}	53.10 ^{ab}	1.32 ^{ab}	0.09 ^{ab}
	460	8.40 ^{ac}	144.67 ^{ac}	1.87 ^{c}	53.50 ^{ac}	1.19 ^{ac}	0.08 ^{ac}
40	380	9.60 ^{a}	152.67 ^{a}	1.71 ^{a}	58.17 ^{a}	1.65 ^{a}	0.09 ^{a}
	420	9.22 ^{ab}	133.33 ^{ab}	2.17 ^{b}	46.12 ^{ab}	1.15 ^{ab}	0.09 ^{ab}
	460	6.48 ^{ac}	118.00 ^{ac}	2.52 ^{c}	39.63 ^{ac}	0.88 ^{ac}	0.09 ^{ac}
45	380	8.01 ^{a}	140.00 ^{a}	1.97 ^{a}	50.93 ^{a}	1.45 ^{a}	0.10 ^{a}
	420	6.74 ^{ab}	121.67 ^{ab}	2.33 ^{b}	42.85 ^{ab}	1.06 ^{ab}	0.09 ^{ab}
	460	6.02 ^{ac}	112.00 ^{ac}	2.59 ^{c}	38.50 ^{ac}	0.85 ^{ac}	0.09 ^{ac}

***(It was not significant $p > 0.01$**

****It was significant $p < 0.01$**

Figures with the same superscrips do not differ significantly $P<0.01$).

When the means were compared for the effect of protein levels and energy levels in the feeds separately, the effect of protein' was significantly higher ($P < 0.01$) for weight gain at 35% protein level followed by 40% protein level (Table 12). Similarly, RGR and FCR were highest and lowest (indicating the best) respectively at 35% protein. FCE was significantly higher ($P<0.01$) at 35% protein, however, FCE's at 40 and 45% protein levels did not vary significantly. PER and PPV were also significantly higher at the lowest protein level of 35% followed by 40% and 45% levels of protein. When the energy levels were compared, weight gain, RGR, FCR, FCE and PER were significantly higher ($P<0.01$) at 380 kcal/loog (Table 13).

Table 12: Mean comparisons of shrimps (5-10g) fed three levels of protein with reference to weight gain, RGR, FCR, FCE, PER and PPV (All values are based on nine observations).

Protein levels	35%	40%	45%
Weight gain(g)	9.48^{a}	17.52^{b}	6.98^{bc}
RGR	153.44^{a}	133.00^{b}	124.89^{c}
FCR	1.75^{a}	2.13^{b}	2.33^{c}
FCE	57.79^{a}	47.36^{b}	43.88^{b}
PER	1.64^{a}	1.18^{b}	0.97^{c}
PPV	0.942^{a}	0.916^{b}	0.858^{c}

Figures with same superscript do not differ significantly ($p>0.01$)

Table 13: Mean comparisons of shrimps (5-l0g) fed three levels of energy with reference to weight gain, RGR, FCR, FCE, PER and PPV (All values are based on nine observations).

Energy levels(kcal/100g)	380	420	460
Weight gain(g)	9.28 [a]	7.76 [b]	6.92 [b]
RGR	152.11 [a]	134.67 [b]	124.56 [c]
FCR	1.77 [a]	2.13 [b]	2.30 [b]
FCE	56.96 [a]	49.97 [b]	44.10 [c]
PER	1.45 [a]	1.23 [b]	1.12 [b]
PPV	0.89 [a]	0.90 [a]	0.93 [a b]

Figures with the same superscript do not differ significantly P>0.01

Discussion

In terms of proximate composition, all the natural feed ingredients examined in present study had the proximate principles at a comparable level to those reported by Ali (1989). However; albumin used in this study was of better quality than that used by Ali (1989), which contained only 78.1% CP, 6.8% lipids, 0.73% carbohydrates and 7.56% ash. The difference could be due to the differences in the processing methods used by the manufacturer in the production of albumin.

Nutritional responses

In animals of< 1 g initial weight

The results in this experiment indicate that with progressive increase of dietary protein and energy all the nutritional responses worked out, i.e., weight gain, RGR, FCR, FCE, PER and PPV improved without statistical significance. In *Penaeus indicus*, in the literature there are no reports either to compare or contrast. However, Ali (1990) observed 414.75 kcal/100g dietary energy as the optimum with purified diets containing casein as the source of protein at 40%. Beyond this level, he reported a decline in terms of growth in length (%), growth in weight (%), FCR and survival % in *Penaeus indicus* having an average initial weight of 0.075g. In the light of the fact that adequate energy in the diet spares protein (Andrews *et al.*, 1972; Sick & Andrews, 1973; Ali, 1982) in shrimp feeds, there is a strong school of thought that high energy and low protein diets perform better nutritionally as well as economically. When the data from this experiment is perused it can be seen that a feed with 35% protein and 380 kcal/100 g energy, produced similar weight gain, RGR, FCR and FCE when compared with a feed containing 40% protein and 380 kcal/100 g dietary gross energy. This finding reiterates the fact that there is protein-sparing action at an appropriate energy level. In *Penaeus monodon*, Shiau & Chou (1991) reported that dietary protein requirements of tiger shrimp could be lowered from 40% to 36% when energy is supplied at a level of 330 kcal/100g. Whereas, at 40% level of protein 320 kcal/100g dietary energy would be sufficient at the extra cost of 4% protein. Such results were deduced using very small increments in energy at one or two levels of protein and analyzing the data by fitting a second order curve using a polynomial quadratic equation. Such an exercise was not possible in this investigation, because the data did not show an optimum; however, the trends indicate nutritional testing of a combination of the energy levels of 380, 420 and 460 kcal/100 g with protein levels higher than 45%. The aforementioned report of Shiau & Chou (1991) in *Penaeus monodon* is with semi-purified diets, as in the present study. However, Hajra *et al.*,(1988) reported in *Penaeus monodon* fed with natural diets of 46% protein and 412.5 kcal/100g dietary energy as the appropriate level when reared in near freshwater conditions i.e., 3.5-4.5ppt salinity. Thus, this experiment reveals that there exists a dietary protein level beyond 45% and more number of energy levels to be tested with smaller increments as reported by Shiau & Chou (1991) to ascertain the more productive use of energy at a lesser cost of protein in shrimp feeds.

Further, when the values of means were compared for the effect of protein levels alone, weight gain, RGR, FCR, FCE, PER and PPV were significantly different ($P<0.01$). This observation in the light of the preceding discussions emphasizes that in *Penaeus indicus* weighing less than 1g there is a protein requirement exceeding 45%. However, when the values of means of energy levels alone where compared, there were no statistically significant differences in terms of weight gain, RGR and FCR, but FCE, PER and PPV showed statistically significant variations at the energy levels of 420 and 460 kcal/100g.

Published reports on this subject were not available for comparison. Protein levels above 45% with same levels of energy at shorter increments of 10 kcal/100g or 20 kcal/100g, as reported by Shiau & Chou (1991) in *Penaeus monodon* provides some basis for future concrete experimental designs in *Penaeus indicus*.

In animals of 1-5g initial weight

In this experiment, significant ($P \leq 0.01$) protein and energy interaction was observed in terms of weight gain, FCR, FCE and PER 40% protein with 420 kcal/ 100g GE was observed to be the best protein: energy combination. This decline in the protein requirements when compared with the previous experiment in *Penaeus indicus* of lesser weight has not been reported, so far. However, Lee (1971) reported that the requirement of protein varies from 45 to 50% in *Penaeus monodon* in the size range of 0.5 to 1.8g. Later, Alava & Lim (1983) reported a protein requirement of 40% in *Penaeus monodon* of 1.3g. In *Penaeus ciztecus*, Venkatramiah *et al.* (1975) reported a requirement of 40% protein in the shrimps weighing 0.02 to 0.14g. Further, Smith *et al.* (1985) reported that the appropriate protein requirement of *Penaeus ocztecus* to be 30-36% for the size of 4g, 10g and 15g. Such reports of progressive education in protein requirements, ignoring the energy content of feeds, are many like Colvin & Brand (1977) in *Penaeus californiensis*, Deshimaru & Shigheno (1972) and Deshimaru & Yone (1978b) for *Penaeus japonicus*, Andrews *et al.* (1972) and Lee & Lawrence, (1985) for *Penaeus setiferus*. Based on these reports Akiyama et al. (1992) recommended 45% protein for shrimps weighing 1 g, 40% protein for 0.5 -3g, 38% for 3.0-15g, and 36% for 15-40g in commercial shrimp feeds. Yazdani (1995) reports diet with 40% portion performed best for white Indian shrimp.

The present results, very clearly, reaffirm that as shrimp growth progresses, there is a reduction in the requirement of protein in the diet. In case of *Penaeus indicus*, even though a protein requirement beyond 45% is indicated in shrimps weighing less than 1g, 40% protein with 420 kcal/100g gross energy appears to be the optimum requirement for shrimps weighing more than 1g. This finding agrees with the dietary levels recommended for omnivorous shrimp species under intensive culture by Tacon (1991). Hajra *et al*. (1988) however, reported that 46% protein with 412.6 kca/l00g energy as the appropriate protein: energy combination for *Penaeus monodon*. These results reveal the complexity of protein sparing and assert the imperativeness of studying calorie protein ratios, species-wise and size-wise with well defined experimental designs in order to compare the results and arrive at definite conclusions.

In the present experiments, comparison of means for the independent effects of protein and energy also show significantly higher weight gain, FCR, FCE and PER at 40% level of protein compared with that of 35% and 45% protein levels. However, with varying levels of gross energy, 420kcal/ 100g produced significantly higher ($P<0.01$) growth rate, FCE and PPV. Reports to compare such findings were not available in terms of energy levels in peaneid shrimps in general and *Penaeus indicus* in particular, especially in the size range of 1 to 5g.

In animals of between 5-10g initial weight

The combined effects of the three protein levels, i.e., 35, 40 and 45% and three energy levels 380, 420 and 460 kcal/100g at each protein level on weight gain, RGR, FCR, FCE, PER and PPV were calculated. Significantly high body weight gain ($P<0.01$) was recorded with 35% protein and 380 kca/l00g GE. There is a further lowering of the requirement of protein as well as energy when compared with the previous experiments. Reports of similar experiments, i.e., impact of protein and energy on growth of shrimps more than 5g to compare with those in *Penaeus indicus* are not available. In *Penaeus vannamei*, Smith *et al*., (1985) evaluated protein levels of 22%, 24%, 30%, 32%, 37% and 41% in shrimps being 4g, 9.8g and 20.8g. They reported that shrimps fed diet containing 37%

protein and 41% protein levels, gained almost same weights daily, i.e., 0.20g and 0.21g, respectively. Similarly, Andrews *et al.* (1972), evaluating a protein range of 14-15% in *Penaeus setiferus* (4g size), recommended a protein level of 28-32% in the diet. The present evaluation of protein and energy required in feeds of *Penaeus indicus* of sizes more than 5g appears to be the first attempt. Protein requirements reported by Smith *et al.*(1985) for *Penaeus vannamei* and Andrews *et al.*, (1972) for *Penaeus setiferus* in shrimps weighing more than 5g is comparable. The results clearly agree with the recommended protein levels by Tacon (1991). However, energy levels in combination with protein in the aforementioned 5g size shrimps are still an area that remains to be studied. The present evaluation suggests a lower energy requirement of the lowest level of GE tested, i.e., 380 kcal/l00g as the best with 35% protein. However, gross energy indicates only the energy available to the animal. In such a scheme of research, the present results are a beginning in terms of the knowledge of a range of protein and energy to be contained in shrimp feeds meant for testing in sizes more than 5g.

Further, when means were compared for the independent effects of protein and energy, level of 35% protein produced the highest weight gain and RGR, best FCR and highest FCE, PER and PPV ($P<0.01$). These findings are comparable with those in reports of Andrews *et al.* (1972) and Smith *et al.* (1985). However, a protein level below the 35% protein tested in this experiment, needs further probing. Energy at 380 kcal/100g is also the lowest level tested, which was significantly the best ($P<0.01$) in this experiment.

Macronutrient interactions in shrimp is an area in shrimp nutrition where reports are scant. The present investigation in *Fenneropenaeus indicus* indicates that a protein level of 35% and 40% GE level of 420 kcal/l00g to be optimum in the size range of 1-5g . However, protein: GE combinations of 45:460 for the size range of <1g has to be probed further for an optimum level. Similarly, the level of 35:380 observed to be the best among the levels tested in the size group of 5-10g, but this also needs to be examined elaborately for an optimum combination. Extension of such studies on farm could facilitate reduction in cost of nutritional inputs.

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