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Nutritional evaluation of varying protein: energy ratios in feeds for Indian white shrimp *Penaeus (Fenneropenaeus) indicus*

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Abstract

Six experiments of six weeks duration were conducted in controlled conditions in the Indian white shrimp *Fenneropenaeus indicus* early juveniles (~0.040 mg initial weight). A common ingredient mixture (CIM) consisting of albumin (chicken egg), fish meal, shrimp meal, clam meal and deoiled ground nut oil cake was formulated and incorporated in all feeds at varying levels to obtain the requisite protein: energy combinations. Eight feeds tested in each experiment were formulated by incorporating CIM, cellulose (filler), tapioca flour (starch, binder), oil, mineral mixture, vitamin mixture and other additives viz., cholesterol and lecithin. The six protein levels fixed were 250 g kg^{-1} to 500 g kg^{-1} with 50 g kg^{-1} increments for each experiment. The gross energy (GE) levels in the eight dietary treatments for each level of protein varied from 290 kcal $100g^{-1}$ to 430 kcal $100g^{-1}$ and the digestible energy levels varied from198 – 300 kcal $100g^{-1}$. Growth, relative growth rate (RGR), absolute growth rate (AGR), food conversion efficiency (FCE), food conversion ratio (FCR), survival and initial and final body composition were monitored and analysed. One-way analysis of variance (ANOVA) followed by comparison of means was done to examine statistically significant differences between treatments and second degree polynomial regression of the from $y = a + bx + cx^2$ was fitted with the data for RGR on P/E ratio (mg protein $kcal^{-1}$), RGR on GE and RGR on DE to derive the optimum RGR, GE, DE and P/E. The results showed that growth of shrimp was highest with 450 g kg^{-1} protein and 363 kcal $100g^{-1}$ GE, 276 kcal $100g^{-1}$ DE with a P/E of 124. Theoretical optima derived confirmed this observation with optimal values of 360 kcal $100g^{-1}$ GE, 275 kcal $100g^{-1}$ DE and a P/E ratio of 125. However, similar response in growth with feeds containing 350 - 450 g kg^{-1} protein indicating GE requirement of 362 – 371 kcal $100g^{-1}$ and DE requirement of 262 – 276 kcal$100g^{-1}$ suggested a protein sufficiency of 350 g kg^{-1} with a P/E ratio of 98- 103 without major variations in the whole body composition.

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Introduction

Energy requirement in shrimp cannot be examined in isolation. Energy requirement in the Indian white shrimp is the problem addressed here in the backdrop of the following reports available. Energy requirements have been reported for a number of shrimp and the ranges are, 350 – 450 kcal 100g^{-1} GE and 250 – 350 kcal 100g^{-1} DE (Colvin 1976, in *F. indicus*; Sedgwick, 1979, in *Penaeus merguiensis*; Aquacop, 1977, Bautista, 1986, Shiau and Peng, 1992, Hajra, et al., 1988, Shiau and Chou, 1991 and Chuntapa, et al., 1999, in *Penaeus monodon*). Commercially produced feeds contain the levels of total energy more than the aforementioned reports i.e., 366 – 414 kcal 100g^{-1} (Devreese, 1995) and 382 – 597 kcal 100g^{-1} (Epa et al., 2007). This is probably the result of the absence of information regarding the energy available to the animals from natural food organisms under practical farming conditions (Tacon, 2002). Since the reported ranges are found to be wide, an attempt is made to discern the apparent total energy levels required in the feeds of early juveniles of the Indian white shrimp.

Materials and methods

Shrimp post larvae from a single brood were procured separately for each experiment from M/s SS Hatchery, Kodungallur, Cochin. The post larvae were reared in the wet laboratory to mean average weight 0.040 – 0.050g using a commercial post larval feed. The animals were hand sorted and weighed individually and stocked in the culture units (circular Perspex tanks of 50 cm diameter x 25 cm h; 45-liter water volume) at the rate of 15 animals. The calculated densities of shrimp in each of these experimental units equal 75 m^{-2}, in triplicate. Seawater diluted to 25‰ was used in all the experiments. Ninety percent of water exchange was done in all the experimental units daily and 100% water exchange and mild scrubbing of the tubs with minimum disturbance to the animals weekly. Sampling of seawater for analysis for pH, dissoloved oxygen (D.O.) and salinity was done fortnightly and temperature was recorded daily.

Diets and feeding protocol

Six experiments performed were by using a uniform diet design. For experiments 1-6 the protein content in the diets were 250, 300, 350, 400, 450 and 500 g kg^{-1} respectively. GE levels varied from 280 kcal 100g^{-1} to 450 kcal 100g^{-1}. All the feeds contained a common ingredient mixture (CIM). By varying mainly the content of CIM and starch (tapioca flour) content the variations protein and GE and thereby DE was brought about. Wherever, desirable variation in energy was not obtained lipid levels were adjusted to obtain them. In diets where tapioca flour was less than 100g kg^{-1} or avoided, carboxymethylcellulose (CMC) was used as a binder. Cellulose was used as the filler. CIM was blended separately. Tapioca flour and CMC were gelatinised in water and CIM and cellulose were mixed and blended to form the dough for hand pelleting using a kitchen noodle maker with a 2 mm die. The pellets were air dried first and oven-dried at 55^{o}C, crumbled, crushed using a food mixer and sieved through 0.5 mm and stored in airtight containers in a refrigerator and used. Experiment-wise, the composition feed ingredients used; CIM, and the ingredient composition of the experimental diets are shown in Tables 1, 2, 3, 6, 9, 12, 15 and 18 respectively.

Table 1. Proximate composition of the natural and purified feed ingredients used for experimental diet compounding (Exp.1) % on DM basis

	DM	CP	EE	CF	NFE	Ash	AIA
Fish meal	98.28	70.58	3.09	0.93	0.36	23.32	11.52
Shrimp meal	92.51	67.45	3.29	0.00	5.27	16.50	4.39
Fish meal	98.28	70.58	3.09	0.93	0.36	23.32	11.52
Shrimp meal	92.51	67.45	3.29	0.00	5.27	16.50	4.39
Clam meal	94.37	59.79	13.01	0.00	15.10	6.47	1.94
GNOC	94.55	43.75	8.13	5.49	30.10	7.08	2.36
Tapioca flour	87.18	2.82	0.29	1.79	80.26	2.02	0.10
Cellulose	93.80	0.65	0.28	92.56	0.00	0.31	0.00
Albumin	92.91	80.50	0.00	0.00	5.97	6.44	0.00

DM – Dry matter, CP – Crude protein, EE -– Ether extract, CF – Crude fibre, NFE – Nitrogen free extract, AIA – Acid insoluble ash

Feeding was carried out at the rate of 15% of the body weight in two doses. Pre-weighed Petri dishes containing 40% of the feed ration was provided at 10:00 h and 60% was provided at 16:00 h. Feed residue and faecal matter was removed daily prior to water-exchange. Feeding rates were adjusted based on daily observations to compensate mortality if any, and reduce feed residues to minimum. Daily record of mortality was also maintained. On termination of the experiment shrimps were weighed and dried and pooled treatment wise for chemical analyses.

Growth was measured as biomass gain shrimp^{-1} (g), absolute growth rate (AGR), relative growth rate (RGR) and specific growth rate (SGR). Protein efficiency ratio (PER), food conversion ratio (FCR), food conversion efficiency (FCE) and survival % were also calculated.

Chemical analyses of diets, water and shrimp

Feed ingredients, CIM and all experimental feeds were analysed for their proximate chemical compositions according to A.O.A.C. (1990). GE and DE were calculated using the conversion factor according to ADCP (1983). Seawater was analysed according to the standard methods of Strickland and Parsons (1972). Shrimp dried and pooled treatment wise were analysed for moisture, CP and EE and ash.

Experiment 1: Protein levels in the diet were 250 g kg^{-1} and GE levels varied from 290.06 kcal $100g^{-1}$ to 426.16 kcal $100g^{-1}$. DE levels ranged from 197.84 to 286.30 kcal $100g^{-1}$ and P/E ratios ranged from 59.63 to 85.94 (Table 4). The eight feeds in this experiment contained 350g kg^{-1} CIM, 0 – 300g kg^{-1} cellulose (filler) and 300–540g kg^{-1} of tapioca flour (Table 3).

Table 3. Ingredient composition of the experimental feeds (g kg^{-1}) (Exp. 1)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed5	Feed 6	Feed 7	Feed 8
CIM	350	350	350	350	350	350	350	350
Cellulose	300	250	190	130	70	10	0	0
Tapioca flour	300	350	410	470	530	590	570	540
Oil[1]	0	0	0	0	0	0	30	60
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture[2]	20	20	20	20	20	20	20	20
Vitamin mixture[3]	20	20	20	20	20	20	20	20

[1]Codliver oil and groundnut oil mixed in the ratio 1:1

[2] U.S.P. XIV (1950) Salt mixture M/s Sisco Research Laboratories**,** Mumbai. As required in the various biological test diets listed U.S.P. XIV p.789. % Composition: Calcium carbonate 6.86000, Calcium citrate 30.83000, Calcium phosphate monobasic 11.28000, Magnesium sulphate.$7H_2O$ 3.83000, Manganese carbonate 3.52000, Potassium chloride 12.47000, Dipotassium phosphate 21.88000, Sodium chloride 7.71000, Copper sulphate.$5H_2O$ 0.00777, Ferric citrate (16-17% Fe) 1.52815, Manganese sulphate.H_2O 0.02008,Potassium aluminium sulphate 0.00923, Potassium Iodide 0.00405, Sodium flouride 0.05070.

[3]According to recommended levels of vitamins for shrimp by Conklin (1997)

Vitamin premix to supply mg or IU kg^{-1} diet. Thiamin 60 mg, Riboflavin 25 mg, Niacin 40 mg, Pyridoxine 50 mg, Pantothenic acid 75 mg, Biotin 1 mg, Folic acid 10 mg, Cyanocobalamin 0.2 mg, choline 600 mg, Myo-inositol 400 mg, Ascorbic acid polyphosphate 200 mg, Retinol 5000 IU, Vitamin E 100 mg, Vitamin D_3 0.1 mg and Vitamin K 5 mg.

Table 4. Nutrient composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. 1)

Nutrientsand energy	Diet Nos.							
	1	2	3	4	5	6	7	8
DM	90.06	89.73	89.34	88.94	88.54	88.14	88.46	88.85
CP	24.93	25.04	25.17	25.30	25.43	25.56	25.49	25.41
EE	4.85	4.85	4.85	4.85	4.86	4.86	7.85	10.84
NFE	26.54	30.55	35.36	40.18	44.99	49.81	48.21	45.80
Ash	5.33	5.42	5.52	5.62	5.72	5.83	5.78	5.72
AIA	0.42	0.42	0.43	0.43	0.44	0.44	0.44	0.44
GE kcal $100g^{-1}$	290.06	307.12	327.58	348.05	368.51	388.98	409.27	426.16
DE kcal $100g^{-1}$	197.84	206.33	216.52	226.71	236.90	247.09	267.54	286.30
P/E ratio (mg protein $kcal^{-1}$)	85.94	81.52	76.83	72.68	69.00	65.70	62.29	59.63
L: C (% weight)	5:27	5:31	5:35	5:40	5:45	5:50	8:48	11:46
EE+NFE	31.39	35.40	40.22	45.03	49.85	54.67	56.05	56.64

GE and DE calculated according to ADCP (1983) as shown below Table 2.

Table 2. Ingredient composition, proximate analysis (% on DM basis) and calculated values of gross energy (GE) and digestible energy (DE) in common ingredient mixture (CIM) (Exp. 1)

Ingredients	g kg^{-1}	CP	EE	CF	NFE	Ash	AIA
Fish meal	50	3.53	0.15	0.00	0.02	1.17	0.58
Shrimp meal	50	3.37	0.16	0.00	0.26	0.83	0.22
Clam meal	50	2.99	0.65	0.00	0.76	0.32	0.10
GNOC	50	2.19	0.41	0.27	1.51	0.35	0.12
Oil[1]	90		9.00				
Albumin	710	57.16			4.24	4.57	
Calculated	1000	69.23	10.38	0.27	6.78	7.24	1.01
Analysed		68.25	10.52	0.32	7.02	7.52	1.10
GE kcal 100 g^{-1*}		380.78	94.42		28.78		503.99
DE kcal 100 g^{-1**}		290.06	84.16		14.04		388.26

[1]Codliver oil and groundnut oil mixed in the ratio 1:1

*Analysed values for protein, EE and NFE multiplied by 5.5, 9.1 and 4.1kcal g^{-1} respectively (ADCP1983)

**Analysed values for animal protein x 4.25, vegetable protein x 3.8, EE x 8, animal NFE x 3 and vegetable NFE x 2 kcal g^{-1} respectively (ADCP 1983)

Lipid :carbohydrate (L:C)

Lipid content in diets 7 and 8 were 7.85 and 10.84% respectively due to incorporation of additional oil at the level of 3 and 6 % to obtain higher levels of energy.

Experiment 2: In this experiment protein level of 300 g kg^{-1} was obtained by incorporating 400 g kg^{-1} of CIM. Filler levels varied from 0 to 320 g kg^{-1}. Oil at the levels of 10 g kg^{-1} and 20 g kg^{-1} was added to obtain higher energy levels in diets 7 and 8 respectively (Table 6).

Table 6. Ingredient composition of the experimental feeds (g kg^{-1}) (Exp. 2)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	400	400	400	400	400	400	390	390
Tapioca flour	210	270	330	390	450	500	550	530
Cellulose	340	280	220	160	100	50	0	10
Oil[1]	0	0	0	0	0	0	10	20
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture[2]	20	20	20	20	20	20	20	20
Vitamin mixture[3]	20	20	20	20	20	20	20	20

1, 2 and 3 are as shown below Table 3

GE levels in this experiment varied between 289.67 kcal $100g^{-1}$ and 430.14 kcal $100g^{-1}$. DE levels were between 208.39 kcal $100g^{-1}$ and 290.73 kcal $100g^{-1}$ (Table 7).

Table 7. Proximate chemical composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. - 2)

Nutrients and energy	Diet Nos.							
	1	2	3	4	5	6	7	8
DM	89.12	90.61	90.22	89.83	89.44	89.11	88.84	91.03
CP	30.01	30.15	30.28	30.41	30.54	30.65	30.03	29.98
EE	5.63	5.64	5.64	5.64	5.64	5.64	6.53	9.53
NFE	17.89	22.71	27.52	32.34	37.15	41.17	45.15	43.55
Ash	5.18	5.29	5.39	5.50	5.60	5.69	5.70	5.67
AIA	0.24	0.24	0.25	0.26	0.26	0.27	0.27	0.26
GE kcal $100g^{-1}$	289.67	310.26	330.72	351.19	371.66	388.71	409.72	430.14
DE kcal $100g^{-1}$	208.39	218.68	228.87	239.06	249.25	257.74	270.18	290.73
P/E ratio (mg protein $kcal^{-1}$)	103.59	97.18	91.56	86.60	82.18	78.85	73.29	69.70
L:C (% weight)	6:18	6:23	6:28	6:32	6:37	6:41	7:45	10:44
EE+NFE	23.53	28.35	33.16	37.98	42.80	46.81	51.68	53.08

Experiment 3: Protein levels of 350 g kg^{-1} were obtained in the experimental diets in this experiment by incorporating 470 - 480 g kg^{-1} CIM. Filler levels varied between 20 – 360 g kg^{-1} and tapioca flour levels were varied between 90 g kg^{-1} and 440 g kg^{-1} to obtain the desired energy levels (Table 9).

Table 9. Ingredient composition of the experimental feeds (g $kg^{-}1$) (Exp. 3)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	480	480	480	480	480	470	470	470
Tapioca flour	90	150	210	270	330	400	450	440
Cellulose	360	320	260	200	140	80	30	20
CMC	20	0	0	0	0	0	0	0
Oil	0	0	0	0	0	0	0	20
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture	20	20	20	20	20	20	20	20
Vitamin mixture	20	20	20	20	20	20	20	20

GE levels varied between 289.30 kcal $100g^{-1}$ and 421.77 kcal $100g^{-1}$ and DE levels varied between 220.03 kcal $100g^{-1}$ and 291.36 kcal $100g^{-1}$ (Table 10).

Table 10. Proximate chemical composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. 3)

Nutrients and energy	Diet Nos.							
	1	2	3	4	5	6	7	8
DM	89.96	91.44	91.05	90.66	90.27	89.81	89.48	87.79
CP	35.54	35.68	35.81	35.94	36.07	35.50	35.61	35.56
EE	6.51	6.51	6.51	6.52	6.52	6.41	6.41	8.40
NFE	8.47	13.28	18.10	22.91	27.73	33.32	37.33	36.53
Ash	5.49	5.59	5.70	5.80	5.90	5.96	6.04	6.01
AIA	0.27	0.27	0.28	0.29	0.29	0.29	0.30	0.30
GE kcal $100g^{-1}$	289.39	309.98	330.44	350.91	371.37	390.17	407.22	421.77
DE kcal $100g^{-1}$	220.03	230.32	240.51	250.70	260.89	268.77	277.26	291.36
P/E ratio (mg protein $kcal^{-1}$)	122.80	115.11	108.37	102.42	97.13	90.98	87.44	84.31
L:C (% weight)	7:8	7:13	7:18	7:23	7:27	6:33	6:37	8:37
EE+NFE	14.97	19.80	24.61	29.43	34.24	39.73	43.74	44.93

Experiment 4: Protein levels of 400 g kg^{-1} were obtained in the experimental diets in this experiment by incorporating 540 g kg^{-1} CIM. Filler levels varied between 30 – 370 g kg^{-1} and tapioca flour levels were varied between 20 g kg^{-1} and 380 g kg^{-1} to obtain the desired energy levels (Table 12).

Table 12. Ingredient composition of the experimental feeds (g kg^{-1}) (Exp. 4)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	540	540	540	540	540	540	540	540
Tapioca flour	20	60	120	180	240	300	360	380
Cellulose	370	330	290	230	170	110	50	30
Oil	0	0	0	0	0	0	0	0
CMC	20	20	0	0	0	0	0	0
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5

Mineral mixture	20	20	20	20	20	20	20	20
Vitamin mixture	20	20	20	20	20	20	20	20

GE levels varied between 296.12 kcal 100g^{-1} and 418.91 kcal 100g^{-1} and DE levels varied between 232.25 kcal 100g^{-1} and 293.39 kcal 100g^{-1} (Table 13).

Table 13. Proximate chemical composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. 4)

Nutrients and energy	Diet Nos							
	1	2	3	4	5	6	7	8
DM	92.33	92.07	91.68	91.29	90.90	90.51	90.11	89.98
CP	39.74	39.83	39.96	40.09	40.22	40.35	40.48	40.52
EE	7.17	7.17	7.17	7.17	7.17	7.17	7.17	7.17
NFE	3.00	6.21	11.03	15.85	20.66	25.48	30.29	31.90
Ash	5.75	5.82	5.92	6.03	6.13	6.23	6.33	6.37
AIA	0.29	0.30	0.30	0.31	0.32	0.32	0.33	0.33
GE kcal 100g^{-1}	296.12	309.77	330.23	350.70	371.16	391.63	412.09	418.91
DE kcal 100g^{-1}	232.25	239.05	249.24	259.42	269.61	279.80	289.99	293.39
P/E ratio (mg protein kcal^{-1})	134.20	128.57	121.00	114.31	108.36	103.03	98.23	96.73
L:C (% weight)	7:3	7:6	7:11	7:16	7:21	7:25	7:30	7:32
EE+NFE	10.17	13.38	18.20	23.02	27.83	32.65	37.46	39.07

Experiment 5: Protein levels of 450 g kg^{-1} were obtained in the experimental diets in this experiment by incorporating 610 g kg^{-1} CIM. Filler levels varied between 60–340 g kg^{-1} and tapioca flour levels were varied between 0 g kg^{-1} and 280 g kg^{-1} to obtain the desired energy levels (Table 15).

Table 15. Ingredient composition of the experimental feeds (g kg^{-1}) (Exp. 5)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	610	610	610	610	610	610	610	610
Tapioca flour	0	50	80	110	140	170	200	280
Cellulose	320	270	240	230	200	170	140	60
Oil	0	0	0	0	0	0	0	0
CMC	20	20	20	0	0	0	0	0

Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture	20	20	20	20	20	20	20	20
Vitamin mixture	20	20	20	20	20	20	20	20

GE levels varied between 324.87 kcal $100g^{-1}$ and 420.37 kcal $100g^{-1}$ and DE levels varied between 256.87 kcal $100g^{-1}$ and 304.42 kcal $100g^{-1}$ (Table 16).

Table 16. Proximate chemical composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. 5)

Nutrients and energy	Diet Nos							
	1	2	3	4	5	6	7	8
DM	92.51	92.18	91.98	91.79	91.59	91.40	91.20	90.68
CP	44.76	44.87	44.94	45.00	45.07	45.13	45.20	45.37
EE	7.93	7.93	7.93	7.93	7.93	7.94	7.94	7.94
NFE	1.58	5.59	8.00	10.41	12.82	15.22	17.63	24.05
Ash	6.16	6.25	6.30	6.35	6.40	6.45	6.50	6.64
AIA	0.33	0.33	0.34	0.34	0.34	0.35	0.35	0.36
GE kcal $100g^{-1}$	324.87	341.92	352.16	362.39	372.62	382.85	393.09	420.37
DE kcal $100g^{-1}$	256.87	265.36	270.46	275.55	280.64	285.74	290.83	304.42
P/E ratio (mg protein $kcal^{-1}$)	137.79	131.23	127.60	124.18	120.95	117.88	114.98	107.93
L:C (% weight)	8:2	8:6	8:8	8:10	8:13	8:15	8:18	8:24
EE+NFE	9.51	13.53	15.93	18.34	20.75	23.16	25.57	31.99

Experiment 6: Protein levels of 500 g kg^{-1} were obtained in the experimental diets in this experiment by incorporating 680 g kg^{-1} CIM. Filler levels varied between 0 – 250 g kg^{-1} and tapioca flour levels were varied between 0 g kg^{-1} and 270 g kg^{-1} to obtain the desired energy levels (Table 18).

Table 18. Ingredient composition and proximate composition of the experimental feeds (g kg-1) (Exp. 6)

Ingredients	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	Feed 7	Feed 8
CIM	680	680	680	680	680	680	680	680
Tapioca flour	0	50	80	110	140	170	200	270
Cellulose	250	200	170	160	130	100	70	0
Oil	0	0	0	0	0	0	0	0
CMC	20	20	20	0	0	0	0	0
Lecithin	5	5	5	5	5	5	5	5
Cholesterol	5	5	5	5	5	5	5	5
Mineral mixture	20	20	20	20	20	20	20	20
Vitamin mixture	20	20	20	20	20	20	20	20

GE levels varied between 360.31 kcal $100g^{-1}$ and 452.53 kcal $100g^{-1}$ and DE levels varied between 284.78 kcal $100g^{-1}$ and 330.74 kcal $100g^{-1}$ (Table 19).

Table 19. Proximate chemical composition of the experimental diets (% on dry matter basis) and their energy contents and ratios of non-protein energy yielding nutrients (Exp. 6)

Nutrients and energy	Diet Nos.							
	1	2	3	4	5	6	7	8
DM	90.68	92.23	92.03	91.83	91.64	91.44	91.25	90.79
CP	49.82	49.94	50.00	50.07	50.13	50.20	50.26	50.42
EE	8.69	8.70	8.70	8.70	8.70	8.70	8.70	8.70
NFE	1.76	5.77	8.18	10.59	13.00	15.41	17.81	23.43
Ash	6.60	6.69	6.74	6.79	6.85	6.90	6.95	7.07
AIA	0.37	0.37	0.38	0.38	0.38	0.38	0.39	0.39
GE kcal $100g^{-1}$	360.31	377.49	387.72	397.96	408.19	418.42	428.65	452.53
DE kcal $100g^{-1}$	284.78	293.37	298.47	303.56	308.66	313.75	318.85	330.74
P/E ratio (mg protein $kcal^{-1}$)	138.26	132.29	128.96	125.81	122.82	119.97	117.26	111.41
L:C (% weight)	9:2	9:6	9:8	9:11	9:13	9:15	9:18	9:23
EE+NFE	10.45	14.47	16.88	19.29	21.70	24.11	26.51	32.13

Statistics

Comparison of means and analysis of variance (ANOVA) of the data were done according to Snedecor and Cochran (1973) using SPSS software. Using critical difference values, 'Student's t-test' for equality of means was used to compare the differences between means (P<0.05). To estimate the optimum levels of protein and GE second-degree polynomials of the form $y = a + bx + cx^2$ were fitted. The significance of the second order regression was also tested here using the 't-test'.

Results

Experiment -1

Growth of shrimp was significantly high (P <0.05) with feeds 6 and 7 (297.62 and 306.98 respectively in terms of RGR) containing 388.98 and 409.27 kcal $100g^{-1}$ GE, 247.09 and 267.54 kcal $100g^{-1}$ DE. P/E ratios of these feeds were 65.70 and 62.29. RGR, SGR, PER, FCR, FCE and survival were significantly higher (P <0.05) with diet 7 (Table 5).

Table 5. Average values of initial and final biomass, biomass gain, AGR, RGR, SGR, PER, FCR and FCE when fed test diets (Exp. 1). Means with the same superscript in columns do not differ significantly (P <0.05)

Diet Nos.	Initial g biomass $shrimp^{-1}$	Final biomass $shrimp^{-1}g$	Biomass gain $shrimp^{-1}$ g	AGR	RGR	SGR	PER	FCR	FCE	Survival %
1	0.041	0.125^{a}	0.084^{a}	0.0020^{a}	204.88^{a}	2.66^{a}	1.22^{a}	3.75^{a}	26.72^{a}	72^{a}
2	0.042	0.134^{b}	0.092^{b}	0.0022^{b}	219.05^{ab}	2.74^{ab}	1.33^{b}	3.07^{b}	32.65^{b}	77^{ab}
3	0.042	0.142^{c}	0.100^{c}	0.0024^{c}	238.10^{bc}	2.90^{bc}	1.43^{c}	2.43^{c}	41.22^{c}	83^{bc}
4	0.043	0.145^{c}	0.102^{c}	0.0024^{c}	237.21^{bcd}	2.90^{bcd}	1.55^{d}	2.24^{c}	44.73^{d}	90^{d}
5	0.042	0.157^{d}	0.114^{d}	0.0027^{d}	271.43^{e}	3.12^{e}	1.62^{e}	2.24^{c}	44.60^{de}	92^{de}
6	0.042	0.167^{e}	0.125^{e}	0.0030^{e}	297.62^{f}	3.27^{f}	1.75^{f}	1.86^{d}	53.92^{f}	95^{e}
7	0.043	0.175^{f}	0.132^{f}	0.0031^{f}	306.98^{f}	3.36^{f}	1.82^{g}	1.75^{e}	57.23^{g}	99^{f}
8	0.043	0.154^{d}	0.111^{d}	0.0027^{d}	258.14^{cde}	3.04^{de}	1.71^{h}	2.37^{c}	42.17^{ce}	89^{cde}

AGR = Wt. gain day^{-1}, RGR = Final wt. – Initial wt./ Initial wt. X 100, SGR = Ln. final wt. – Ln. initial wt./ No. of days x 100, PER = Wet wt. Gain/ Dry wt. of protein consumed

FCR = Dry wt. of feed consumed/ Wet wt. Gain, FCE = Wet wt. gain/ Dry wt. of feed consumed.

Regressions of RGR on P/E, GE and DE indicated the RGR optimum between 288.36 – 292.68, GE 417.89 kcal $100g^{-1}$and DE 261.21 kcal $100g^{-1}$. The optimum P/E derived was 51.54.

Experiment - 2

Growth of shrimps was significantly higher with diet 5 (395.45 % in terms of RGR) with a GE of 371.66 kcal 100g-1 and DE of 249.55 kcal 100g-1. P/E ratio of this feed was 82.18. RGR, SGR, PER FCR, FCE and survival were also significantly higher (P <0.05) with this diet (Table 8)

Table 8. Average values of initial and final biomass, biomass gain, AGR, RGR, SGR, PER, FCR and FCE fed test diets (Exp. 2). Means with the same superscript in columns do not differ significantly (P <0.05)

Diet Nos.	Initial g biomass shrimp^{-1}	Final biomass shrimp^{-1}g	Biomass gain shrimp^{-1} g	AGR	RGR	SGR	PER	FCR	FCE	Survival %
1	0.045	0.181^{a}	0.136^{a}	0.003^{a}	302.22^{a}	3.33^{a}	1.11^{a}	3.18^{a}	31.39^{a}	73^{a}
2	0.047	0.197^{b}	0.150^{ab}	0.004^{ab}	319.15^{ab}	3.41^{ab}	1.22^{b}	2.77^{b}	36.06^{b}	78^{ab}
3	0.045	0.198^{bc}	0.153^{bc}	0.004^{c}	340.00^{ab}	3.53^{abc}	1.32^{c}	2.39^{c}	41.79^{c}	83^{bc}
4	0.044	0.203^{bcd}	0.159^{d}	0.004^{bc}	361.36^{bc}	3.65^{bc}	1.45^{d}	1.98^{d}	50.41^{d}	87^{c}
5	0.044	0.218^{d}	0.174^{e}	0.004^{d}	395.45^{c}	3.79^{c}	1.54^{e}	1.79^{e}	55.86^{e}	95^{d}
6	0.045	0.175^{e}	0.130^{af}	0.003^{e}	288.89^{ab}	3.23^{ab}	1.45^{d}	2.23^{c}	44.67^{f}	93^{d}
7	0.044	0.163^{ef}	0.118^{cf}	0.003^{e}	268.18^{a}	3.10^{d}	1.34^{c}	2.43^{c}	41.10^{c}	87^{c}
8	0.046	0.156^{f}	0.110^{b}	0.003^{f}	239.13^{d}	2.90^{d}	1.25^{b}	2.75^{b}	36.35^{b}	82^{bc}

Regressions of RGR on P/E, GE and DE indicated the RGR optimum between 346.58 – 357.98, GE 346.49 kcal 100g^{-1}and DE 237.84 kcal 100g^{-1}. The optimum P/E derived was 89.35.

Experiment - 3

Growth of shrimp was significantly higher with diet 5 (676.74 % in terms of RGR) with a GE of 371.37 kcal 100g^{-1} and DE of 260.89 kcal 100g^{-1}. P/E ratio of this feed was 97.13. RGR, SGR, PER FCR, FCE and survival were also significantly higher (P <0.05) with this diet (Table 11).

Table 11. Average values of initial and final biomass, biomass gain, AGR, RGR, SGR, PER, FCR and FCE fed test diets (Exp. 3). Means with the same superscript in columns do not differ significantly (*P* <0.05)

Diet Nos.	Initial g biomass shrimp^{-1}	Final biomass shrimp^{-1}g	Biomass gain shrimp^{-1} g	AGR	RGR	SGR	PER	FCR	FCE	Survival %
1	0.046	0.640^{a}	0.501^{a}	0.0040^{a}	363.04^{a}	3.63^{a}	0.79^{a}	2.77^{a}	36.05^{a}	84^{a}
2	0.049	0.743^{b}	0.597^{b}	0.0047^{b}	406.12^{ab}	3.87^{ab}	0.83^{a}	2.77^{a}	36.06^{a}	84^{a}
3	0.043	0.710^{b}	0.580^{c}	0.0046^{b}	448.83^{b}	4.04^{bc}	0.90^{a}	2.12^{b}	47.12^{b}	83^{a}
4	0.051	0.930^{c}	0.778^{d}	0.0062^{c}	507.84^{c}	4.31^{c}	0.96^{a}	1.82^{c}	55.07^{c}	87^{ab}
5	0.043	1.003^{d}	0.874^{e}	0.0069^{d}	676.74^{d}	4.89^{d}	1.35^{b}	1.79^{c}	55.86^{cd}	95^{c}
6	0.045	0.694^{b}	0.558^{bc}	0.0044^{b}	413.33^{ab}	3.88^{abc}	0.94^{a}	2.24^{d}	44.67^{e}	93^{bc}
7	0.046	0.616^{a}	0.479^{a}	0.0038^{a}	347.83^{a}	3.60^{ab}	0.86^{a}	2.43^{d}	41.10^{f}	87^{ab}
8	0.045	0.469^{e}	0.335^{f}	0.0027^{e}	248.88^{e}	2.99^{e}	0.85^{a}	2.75^{a}	36.35^{a}	82^{ab}

Regressions of RGR on P/E, GE and DE indicated the RGR optimum of 527.85 – 543.92, GE 352.03 kcal 100g^{-1} and DE 252.20 kcal 100g^{-1}. The optimum P/E derived was 103.98.

Experiment - 4

Growth of shrimp was significantly higher with diet 5 (676.14 % in terms of RGR) with a GE of 371.16 kcal 100g^{-1} and DE of 269.61 kcal 100g^{-1}. P/E ratio of this feed was 108.36. RGR, SGR, PER FCR, FCE and survival were also significantly higher (*P* <0.05) with this diet (Table 14).

Table 14. Average values of initial and final biomass, biomass gain, AGR, RGR, SGR, PER, FCR and FCE fed test diets (Exp. 4). Means with the same superscript in columns do not differ significantly (*P* <0.05)

Diet Nos.	Initial g biomass shrimp^{-1}	Final biomass shrimp^{-1}g	Biomass gain shrimp^{-1} g	AGR	RGR	SGR	PER	FCR	FCE	Survival %
1	0.046	0.251^{a}	0.204^{a}	0.0049^{a}	443.48^{a}	4.02^{a}	0.56^{a}	2.58^{a}	38.69^{a}	84^{a}
2	0.049	0.276^{ab}	0.227^{b}	0.0054^{b}	463.27^{a}	4.13^{a}	0.65^{b}	2.37^{a}	42.26^{b}	83^{a}
3	0.043	0.273^{ab}	0.230^{b}	0.0055^{b}	534.88^{b}	4.38^{b}	0.73^{c}	2.12^{b}	47.12^{c}	87^{ab}
4	0.051	0.353^{a}	0.302^{c}	0.0072^{c}	592.16^{c}	4.62^{c}	0.82^{d}	1.82^{c}	55.07^{d}	93^{bc}
5	0.043	0.334^{a}	0.291^{c}	0.0069^{c}	676.14^{d}	4.89^{d}	0.97^{e}	1.79^{c}	55.86^{d}	95^{c}

6	0.045	0.278^{b}	0.232^{b}	0.0055^{b}	575.55^{b}	4.31^{b}	0.87^{f}	2.24^{a}	44.67^{e}	93^{bc}
7	0.046	0.240^{a}	0.194^{a}	0.0046^{a}	421.74^{a}	3.96^{a}	0.79^{d}	2.43^{a}	41.10^{b}	87^{abc}
8	0.045	0.230^{a}	0.186^{a}	0.0044^{a}	413.33^{a}	3.91^{a}	0.65^{b}	2.75^{c}	36.35^{f}	77^{a}

Regressions of RGR on P/E, GE, DE indicated the RGR optimum of 603.61 – 608.50, GE 357.12 kcal 100g^{-1}and DE 262.57 kcal 100g^{-1}. The optimum P/E derived was 114.95.

Experiment - 5

Growth of shrimp was significantly higher with diet 4 (673.46 % in terms of RGR) with a GE of 362.39 kcal 100g^{-1} and DE of 275.55 kcal 100g^{-1}. P/E ratio of this feed was 124.18. RGR, SGR, PER FCR, FCE and survival were also significantly higher (P <0.05) with this diet (Table 17).

Table 17. Average values of initial and final biomass, biomass gain, AGR, RGR, SGR, PER, FCR and FCE fed test diets (Exp. B-5). Means with the same superscript in columns do not differ significantly (P <0.05)

Diet Nos.	Initial g biomass shrimp^{-1}	Final biomass shrimp^{-1}g	Biomass gain shrimp^{-1} g	AGR	RGR	SGR	PER	FCR	FCE	Survival %
1	0.043	0.278^{a}	0.235^{a}	0.0056^{a}	546.51^{a}	4.45^{a}	0.43^{a}	2.30^{a}	43.41^{a}	57^{a}
2	0.047	0.306^{b}	0.259^{b}	0.0062^{b}	551.06^{a}	4.45^{a}	0.55^{b}	2.07^{b}	48.35^{b}	60^{ab}
3	0.043	0.313^{b}	0.269^{b}	0.0064^{b}	625.58^{b}	4.71^{b}	0.64^{c}	1.81^{c}	55.18^{c}	64^{ab}
4	0.049	0.379^{c}	0.330^{c}	0.0079^{c}	673.46^{c}	4.89^{c}	0.76^{d}	1.73^{c}	57.86^{d}	84^{c}
5	0.043	0.318^{b}	0.275^{b}	0.0065^{b}	639.53^{b}	4.75^{b}	0.64^{c}	1.79^{c}	55.86^{cd}	78^{c}
6	0.045	0.287^{ab}	0.241^{ab}	0.0057^{ab}	535.56^{ad}	4.39^{ad}	0.55^{b}	2.19^{b}	45.63^{a}	68^{b}
7	0.042	0.254^{d}	0.213^{d}	0.0051^{d}	507.14^{de}	4.31^{de}	0.43^{a}	2.27^{a}	44.11^{a}	61^{ab}
8	0.044	0.253^{d}	0.209^{d}	0.0050^{d}	475.00^{e}	4.15^{e}	0.35^{e}	2.58^{d}	38.58^{e}	50^{ab}

Regressions of RGR on P/E, GE and DE indicated the RGR optimum between 607.60 –613.52, GE 360.11 kcal 100g^{-1} and DE 274.67 kcal 100g^{-1}. The optimum P/E ranged derived was 125.83.

Experiment - 6

Growth of shrimp was significantly higher with diet 4 (530.61 % in terms of RGR) with a GE of 397.96 kcal 100g^{-1} and DE of 303.56 kcal 100g^{-1}. P/E ratio of this feed was 125.81. RGR, SGR, PER FCR, FCE and survival were also significantly higher (P <0.05) with this diet (Table 20).

Table 20. Average values of initial and final biomass, biomass gain, AGR, RGR, SGR, PER, FCR and FCE fed test diets (Exp. B-6). Means with the same superscript in columns do not differ significantly (P <0.05)

Diet Nos.	Initial g biomass shrimp^{-1}	Final biomass shrimp^{-1}g	Biomass gain shrimp^{-1} g	AGR	RGR	SGR	PER	FCR	FCE	Survival %
1	0.043	0.237^{a}	0.194^{a}	0.0046^{a}	451.16^{a}	4.07^{a}	0.35^{a}	2.30^{a}	43.41^{a}	84^{a}
2	0.047	0.266^{b}	0.219^{b}	0.0052^{b}	465.95^{a}	4.11^{ab}	0.43^{b}	2.07^{a}	48.35^{b}	87^{ab}
3	0.043	0.255^{bc}	0.212^{bc}	0.0050^{bc}	493.02^{b}	4.22^{abc}	0.53^{c}	1.81^{b}	55.18^{c}	90^{abc}
4	0.049	0.309^{d}	0.260^{d}	0.0062^{d}	530.61^{c}	4.40^{cd}	0.62^{d}	1.73^{b}	57.86^{d}	98^{d}
5	0.043	0.247^{bc}	0.204^{ac}	0.0048^{ac}	474.42^{b}	4.15^{abc}	0.50^{c}	1.79^{b}	55.86^{cd}	97^{d}
6	0.045	0.253^{bc}	0.208^{bc}	0.0050^{bc}	462.22^{a}	4.10^{abc}	0.39^{b}	2.19^{a}	45.63^{a}	93^{bc}
7	0.042	0.232^{a}	0.190^{a}	0.0045^{a}	452.38^{d}	4.08^{abc}	0.33^{a}	2.27^{a}	44.11^{a}	87^{abc}
8	0.044	0.243^{ac}	0.199^{ac}	0.0047^{ac}	452.27^{e}	4.05^{abc}	0.29^{e}	2.59^{c}	38.58^{e}	77^{ab}

Regressions of RGR on P/E, GE and DE indicated optimum of 486.19 – 487.92, GE e 400.54 kcal 100g^{-1} and DE 304.83 kcal 100g^{-1}. The optimum P/E derived was 126.07.

The observed maxima and derived optima are presented in Table 21.

Table 21. Observed maximum and derived optimum growth and energy requirement in *F. indicus* (Experiments 1-6)

Protein g kg^{-1}	250	300	350	400	450	500
ObservedGE kcal 100g^{-1}	389-409	351-372	371	371	362	398
Derived GE kcal 100g^{-1}	418	346	352	357	360	396
ObservedDE kcal 100g^{-1}	247-268	239-249	261	270	276	304
DerivedDE kcal 100g^{-1}	261	238	252	263	275	302
Observed RGR %	298-307	361-395	678	677	673	531
Derived RGR	284-293	347-358	527-543	604-609	607-613	486-488
Observed P/E (mg	62-66	82-87	97	108	124	126

protein kcal^{-1})						
Derived P/E	51-65	82-89	98-103	112-114	120-125	120-126

Whole body composition and water quality

Whole body composition of the experimental animals before and after the experiments in terms of moisture, CP, EE and ash is depicted in Tables 22. Water quality in all the succeeding six experiments was within the acceptable range for aquatic life (Table 23).

Table 23. Means of temperature, pH and salinity in the culture containers

Experiment No.	1	2	3	4	5	6
Temperature °C	28.52±0.17	28.67±0.17	29.47±0.17	28.77±0.14	28.87±0.06	29.00±0.04
D.O. (mg L^{-1})	4.52±0.17	4.77±0.31	5.65±0.22	5.58±0.27	5.63±0.15	5.25±0.18
pH	8.17±0.09	8.08±0.20	8.17±0.13	8.05±0.06	7.95±0.06	8.05±0.06
Salinity (g L^{-1})	25.25±0.06	25.55±0.18	25.37±0.10	25.35±0.13	25.30±0.04	25.13±0.05

Discussion

These six experiments were conducted with diet designs modified after Shiau and Chou (1991). The CIM provided the complement of natural feed ingredients such as fish meal, shrimp meal, clam meal and deoiled groundnut oil cake and oil. Chicken egg albumin rated to be the best purified animal protein source by Ali (1994) for *F. indicus* was the other major source of protein incorporated in the CIM. The results of these six experiments demonstrated that shrimp fed diets with 250 g kg^{-1} to 300 g kg^{-1} at all energy levels showed a lower growth rate compared with shrimp fed higher protein levels; protein levels below 300 g kg^{-1} appear to be insufficient for optimal growth.

Colvin (1976) while estimating protein requirement of *F. indicus* tested protein (g kg^{-1}): GE (kcal 100g^{-1}) combinations of 213:450, 334:460, 428:470 and 530: 480 respectively found 428: 470 to be the most appropriate combination. Ali (1990) was the next to report that in *F. indicus* with a diet containing 400g kg^{-1} protein, 50 g kg^{-1} lipid and 350 g kg^{-1} carbohydrate 414 kcal 100g^{-1} GE as the optimum.

Further, Ali (1996) reported that with 348 g kg^{-1} protein and 70g kg^{-1} lipid; maximum growth was at 348 kcal 100g^{-1} DE (whether estimated or calculated was not mentioned and from the values reported DE appears to be GE) in *F. indicus*. With the same lipid level (70g kg^{-1}), and protein levels ranging from 220 g kg^{-1} to 510 g kg^{-1} maximum growth was registered at 400 kcal 100g^{-1}. Again, with 348 g kg^{-1} protein, lipid level ranging from 15 g kg^{-1} to 178 g kg^{-1}, maximum growth was at 392 kcal 100g^{-1}. This observation of Ali

(1996), ascribing the preferential utilisation of carbohydrate as high as 530g kg^{-1} in a protein deficient (220 g kg^{-1}) situation was also reported to cause poor survival. In this study, it is observed that in Exp. 1 with 250g kg^{-1} protein the GE of 389-409 kcal $100g^{-1}$ recorded maximum growth and survival. The effect was manifested as poorest growth recorded among the six experiments. Protein sufficiency in formulated feeds in this research is found ensured only in Experiments 3-6. Similar and superior growth resulted (673-678 % RGR), with protein levels of 350, 400 and 450 g kg^{-1}. The potential of manipulating energy levels by altering the inclusion levels of non-protein dietary constituents to reduce protein level to the extent of not having an impacting growth is thus imminent. In *P. monodon* AQUACOP (1977) estimated that a total dietary energy content of 330 kcal $100g^{-1}$ was required for optimal growth at 400 g kg^{-1} protein. Hajra et al. (1988) reported that a GE level of 413 kcal $100g^{-1}$ to be the optimum at 460g kg^{-1} protein with feeds compounded using natural ingredients and shrimp reared in near freshwater conditions. In their review Cuzon and Guillaume (1997) found that the GE levels in crustacean diets generally ranged from 310 to 410 kcal $100g^{-1}$. While attempting to discern the most appropriate range in this work, it is clear that there is a threshold level for protein (350g kg^{-1} here), which is responsible for optimum growth. GE level of 371 kcal $100g^{-1}$ required to sustain this is derived from a Lipid: Carbohydrate (L: C) % weight ratio of 7:27. Bautista (1986) reported that the *P. monodon* (0.60-0.80 g) fed with 300g kg^{-1} protein and GE ranging from 205-335 kcal $100g^{-1}$ had lower growth rates compared with shrimp fed on diets containing 350-450 g kg^{-1} protein at all energy levels. Shiau and Chou (1991) in their work on *P. monodon* reported that at 400 g kg^{-1} protein the optimum GE level was 320 kcal $100g^{-1}$ and at 360 g kg^{-1} protein the GE level was 330 kcal $100g^{-1}$. In *P .monodon,* Chuntapa et al. (1999) documented observations similar to the present study. Low growth at energy levels ranging from 203-339 kcal $100g^{-1}$ with protein levels below 330g kg^{-1} In shrimp fed on diets containing 330 – 440 g kg^{-1} protein and GE levels ranging from 223 – 459 kcal $100g^{-1}$ had greater growth. Further, growth was reported to be similar with 340 g kg^{-1} protein and GE levels of 223 and 331 kcal $100g^{-1}$. At 330 g kg^{-1} protein with GE of 439 kcal $100g^{-1}$-growth rates tended to decrease. However, at 360 g kg^{-1} protein and 459 kcal $100g^{-1}$ GE, growth rate was similar in diets containing 330-440 g kg^{-1} protein at all GE levels. At 440 g kg^{-1} protein and GE levels of 263 – 371 kcal $100g^{-1}$ growth is again reported to match the levels of growth observed at 330 - 440 g kg^{-1} protein. Using regression analysis with this data they (Chuntappa et al., 1999) derived the optimum P/E ratio as 146-150 mg protein $kcal^{-1}$. This trend is observed in the present work also, however, the GE values corresponding to 350, 400 and 450 g kg^{-1} protein in the diets where maximum and similar growth was observed were 362 – 371 kcal $100g^{-1}$ and P/E ranged from 97-124 mg protein $kcal^{-1}$. With regression analysis these GE values ranged between 353 – 360 kcal $100g^{-1}$ and P/E ranged from 103-125 mg protein $kcal^{-1}$.

Thus, the optimum protein requirement in *F. indicus* in this study does conform to the earlier reports on this species by Colvin (1976) and Gopal and Raj (1990). The energy requirement even though decreases with an increase in the protein content in the diets the protein sparing capability in this species appears to be lower when compared with the report on *P. monodon* (Shiau and Chou 1991). P/E ratio (103-125 mg protein $kcal^{-1}$) is also lower implying cheaper and more cost effective feeds can be formulated for this species.

L: C as a ratio in feed by weight is another important parameter which was 7:27, 7:21 and 8:13 by weight for the diets containing 350, 400 and 450 g kg^{-1} protein respectively. This ratio of non-protein energy constituents indicates the gross tolerance level of this organism towards unnatural levels of fat and carbohydrates without ignoring the fact that the natural disposition of shrimp in general is towards a protein rich food. The ratio reported for P. *monodon* is 7:32 by weight by Chuntapa et al. (1999). Ali (1990) in *F. indicus* reports this ratio to be 5:35 for the diet, which resulted in the optimum growth. The current research shows that 7:27, 7:21 and 8:13 to be the appropriate ratios for optimum growth for diets containing 350, 400 and 450 g kg^{-1} protein respectively. Moreover, these ratios recorded higher growth compared with the work of Ali (1990) who had not tested lipid level beyond 6.25% because his own finding that 6% gross lipid level was optimal. Chandge and Raj (1997) reported a range of 8-12% for the same species. L: C ratios of 8:48, 6:37 and 9:11 at protein levels of 250, 300 and 500 g kg^{-1} respectively produced sub-optimal growth (Tables 5, 8 and 20). This indicated threshold levels of fat and carbohydrate beyond which abnormally high levels of these nutrients indirectly affecting protein deposition (growth). SGR, PER, FCR FCE and survival are the other nutritional indices which conformed to the optimal values of growth in all the six experiments 1-6 conducted. Significantly higher values (P <0.05) values for SGR, PER, FCE and significantly least values for FCR support the findings discussed. Varying levels of protein and energy in feed did not impact the body composition of the animals (Table 22).

Table 22. Mean whole body proximate compositions of the experimental shrimp initially and finally (% on dry matter basis)

	Initial					
Experiment Nos.	1	2	3	4	5	6
Moisture	77.2	77.22	76.9	72.75	73.24	72.14
Crude protein	61.93	66.43	66.96	68.94	69.68	69.85
Ether extract	11.01	6.83	6.14	4.85	4.85	4.86
Ash	16.02	16.44	16.83	19.37	19.38	19.84
	Final					
Moisture	73.40	73.96	72.66	73.37	73.17	73.32
Crude protein	65.89	69.57	69.05	69.12	69.64	69.93

Ether extract	8.46	4.89	4.85	4.55	5.02	5.03
Ash	18.78	19.56	19.55	20.02	20.11	20.11

Conclusion

Ratio of protein, carbohydrate and lipid in the feeds of shrimp play an important role in formulation of cost effective feeds. Absolute requirements become dynamic with the alterations in their ratio and knowledge of their interactions can be applied in reducing the cost of shrimp production.

Experiments 1-6 with feeds compounded with purified ingredients mainly (semi-purified diet), showed that the optimum range of protein is required in the feed to realise maximum growth at 350 to 450g kg^{-1}. The energy levels, which sustained this growth, were 362 – 371 kcal $100g^{-1}$ GE and 262 – 276 kcal $100g^{-1}$ DE. The optima derived through regression analysis were 353 – 360 kcal $100g^{-1}$ GE and 252 – 274 kcal $100g^{-1}$ DE. Within this range energy can be manipulated to lower the protein inclusion in the feed. However, further precision in energy requirement data can only be achieved if the DE and ME values are available for shrimp. The future course of work should be on those lines examined in along with environmental interactions.

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