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Evaluation of compounded feeds with varying protein: energy ratios for the Indian white shrimp *Fenneropenaeus indicus*

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Abstract

The present investigation is on the performance of six feeds compounded using fishmeal, shrimp meal, groundnut oil cake and clam meal to determine the appropriate protein: calorie ratios. Six protein (%): gross energy (kcal.100 g^{-1}) combinations (22.43: 413.69, 31.99: 451.63, 35.71: 456.02, 43.28: 407.68, 47.65:451.80 and 52.68:469.76) were evaluated in the early juveniles of the Indian white shrimp *Fenneropenaeus indicus* (average initial weight: 0.43 $\pm$ 0.03 g) . The P/E ratio *i.e.,* mg protein. $kcal^{-1}$ of these diets was 54.22, 70.83, 78.31, 106.16, 105.47 and 112.14. Weight gain, relative growth rate (RGR), specific growth rate (SGR), protein efficiency ratio (PER) and food conversion ratio (FCR), apparent dry matter digestibility (ADMD) and apparent protein digestibility (APD) were assessed. Second degree polynomials were fitted with the data set to derive optimum levels of protein and GE. The best performance was observed with the protein: GE combinations 2 and 5 respectively suggesting a protein sparing of 15% with an increase of 30% non-protein energy constituents in the diet. Optimum levels of protein and GE were 31.74% and 430.95 kcal 100g^{-1} with a P/E ratio of 86.18. Nursery feeds with the above derived protein: energy density can be formulated with locally available feed ingredients and applied in nursery rearing systems of the Indian white shrimp. *F. indicus* is found capable of utilizing high amount of carbohydrates compared to closely related penaeid shrimps.

Keywords: *Fenneropenaeus indicus*, calorie: protein ratios, gross energy, digestible energy

Introduction

Farm-made aquafeeds (New *et al.,* 1993) using locally available feed ingredients had moved along with the availability of manufactured shrimp feed because of their effectiveness in backyard type of hatchery and nursery systems in the southeast Asian region. Improvement in the knowledge of nutritional requirements has helped the organized feed manufacturers in bringing out better quality feeds in the market. Postlarval (PL) and juvenile shrimp feeds are required in less quantity compared to grow-out feeds. The prices of feeds for PL are high. Feed formulations using locally available ingredients, including and excluding fish and shrimp meal containing varying protein: energy ratios were tested in this investigation and the results are discussed.

Material and methods

Experimental diets: Six experimental diets were formulated using natural feed ingredients available locally around Cochin. The proximate composition of the feed ingredients was determined prior to the experiment (Table 1). The ingredient composition of the experimental diets is shown in Table 2. Ascending levels of protein were obtained (Table 3) in the experimental feeds formulated by varying the major protein sources, fish meal (dried,

Table 1. Proximate composition (%) of feed ingredients

Ingredients	DM	OM	CP	CF	EE	NFE	Ash	AIA
Fish meal	84.06	68.51	61.75	-	5.39	16.83	15.55	0.90
Shrimp meal	89.06	61.17	37.98	11.00	2.83	20.31	27.87	0.31
GNOC	92.78	85.37	49.07	3.57	6.70	33.25	7.41	0.48
Tapioca flour	89.95	88.68	1.72	1.42	0.49	95.09	1.28	0.16
Clam meal	94.33	86.69	52.60	-	10.63	28.46	7.64	2.57

DM = Dry matter, OM = Organic matter, CP = Crude protein, CF = Crude fiber, EE = Ether extract, NFE = Nitrogen free extractives, AIA = Acid insoluble ash.

Table 2. Composition (%) of experimental diets

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish meal	-	15	-	16	20	20
Shrimp meal	20	-	-	26	20	20
Groundnut oil cake	20	15	30	26	20	20
Tapioca flour	44	42	33	17	10	5
Clam meal	6	18	27	5	20	25
Oil*	6	4	2	2	4	6
Carboxymehtylcellulose	-	2	4	4	2	-
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mixture**	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mixture***	2.0	2.0	2.0	2.0	2.0	2.0
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5

* Codliver oil and groundnut oil mixed in the ratio 1:1

** Contains Vitamin B_1 - 10 mg; Vitamin B_2 - 10 mg; Vitamin B_6 - 3 mg; Nicotinamide - 110 mg; Calcium pantothenate - 50 mg; Folic acid - 1500 mcg; Vitamin B_{12} - 15 mcg ; Vitamin C - 150 mg ; Choline chloride - 1200mg and Innositol - 4000 mg

*** Salt mixture USP XIV from M/s Sisco Research Laboratories, Mumbai.

Table 3. Proximate composition of the experimental diets (% on dry matter basis) and their gross energy content

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Dry matter	97.47	97.69	98.03	98.01	97.67	94.05
Organic matter	86.31	88.89	96.91	80.87	82.86	79.79
Crude Protein	22.43	31.99	35.71	43.28	47.65	52.68
Ether Extract	6.20	7.74	7.34	4.26	9.31	10.69
Crude fiber	3.14	1.41	1.76	3.40	2.61	2.19
Nitrogen free extract	57.05	50.06	47.03	31.92	25.61	20.18
Ash	11.18	8.80	8.16	17.64	14.82	14.26
Acid insoluble ash	0.64	1.41	1.42	0.36	0.74	1.17
NFE + EE	63.25	57.80	54.33	36.18	28.22	30.87
GE kcal. 100 g^{-1}	413.69	451.63	456.02	407.68	451.80	469.76
P/E mg protein $kcal^{-1}$	54.22	70.83	78.31	106.16	105.47	112.14

unsalted anchovies), shrimp meal (dried *Parapenaeopsis stylifera*), deoiled groundnut oil cake and clam meal (*Villorita cyprinoides*).

The gross energy (GE) was calculated from the values reported by ADCP (1983) *i.e.,* 5.5 kcal g^{-1} for protein, 4.1 kcal g^{-1} for carbohydrate (excluding crude fiber) and 9.1 kcal g^{-1} for fat. Thus, six known protein: energy combinations formed the treatments that were tested in these experiments. The P/E *i.e.*, mg protein. $kcal^{-1}$ of the experimental diets was also calculated. Chromic oxide was incorporated at 0.5% level in all the feeds for estimating the apparent dry matter digestibility (ADMD) and apparent protein digestibility (APD).

All the ingredients were pulverized and sieved through 200 µ mesh to obtain uniform particle size. The dry ingredients except tapioca flour and carboxymethylcellulose (CMC) were weighed and mixed well and blended with oil manually. Tapioca flour and CMC were gelatinized in 200 ml water for 500 g dry mix and subsequently mixed with other ingredients into thick dough. The dough was pelletized using a hand pelletizer with a 2 mm diameter die and dried in a hot air oven at constant temperature (65± 2°C). The dry pellets were then crumbled and stored in airtight containers for subsequent chemical analyses and feeding.

***Feeding**:* A feeding trial was conducted for 28 days with early juveniles of the Indian white shrimp *F. indicus* in the wet laboratory of Central Marine Fisheries Research Institute, Cochin. Shrimps of average weight 0.43 ± 0.03 g (0.38 g - 0.48 g) were segregated into 18 groups of 10 animals each and stocked in non-toxic plastic tubs (40 l) in triplicates for each treatment. After acclimatization and conditioning of the experimental animals for three days, initial weights were recorded using an electronic balance. Shrimps were fed at the rate of 10% in two divided doses at 10 am (40%) and 5 pm (60%) every day. The tubs were cleaned daily. Fecal strands and leftover feed from each tub was siphoned out and collected daily with the help of a thin tube and bolting silk and rinsed with distilled water to remove traces of adhering salts. Feed residue and fecal output were quantified and dried in a hot air oven at 65 ± 2°C and pooled for analyses. The water quality parameters were monitored as salinity: 25‰, dissolved oxygen: 4 mg l^{-1}, pH: 8.4 ± 0.2 and temperature: 28.5 ± 0.5°C.

Growth was measured as biomass gain. $shrimp^{-1}$ (g), relative growth rate (RGR), specific growth rate (SGR), protein efficiency ratio (PER), food conversion ratio (FCR) using the following formulae:

RGR = Final weight - Initial weight / Initial weight x 100

SGR = Ln[Final weight] – Ln[Initial weight] / No. of days x 100

PER = Wet weight gain/ Dry weight of protein consumed

FCR = Dry weight of feed consumed/ Wet weight gain.

Apparent dry matter digestibility (ADMD) and apparent protein digestibility (APD) were calculated using the formula,

Apparent digestibility coefficient (ADC) = 100 - 100 (% Cr_2O_3 in feed / % Cr_2O_3 in faeces) x (% nutrient in faeces/ % nutrient in feed)

***Chemical analyses**:* Feed ingredients, experimental feeds and faeces were analyzed for their proximate compositions following AOAC (1990). Chromic oxide (Cr_2O_3) was estimated following Furukawa and Tsukahara (1966). Seawater was analyzed according to the standard methods of Strickland and Parsons (1972) for temperature, pH, and salinity and dissolved oxygen (DO).

***Data analyses**:* Analysis of variance of the data was done following Snedecor and Cochran (1973) using SPSS software. To estimate the optimum levels of protein and GE, second degree polynomials were fitted.

Results and Discussion

The results of the feeding trial are presented in Table 4, where responses were assessed in terms of biomass gain. $shrimp^{-1}$, RGR, SGR, PER, FCR, ADMD and APD. Statistically significant differences were observed in the final biomass per shrimp ($p < 0.05$), PER ($p < 0.01$), ADMD and APD ($p < 0.05$). Total mortality of shrimps in one of the replicates fed with diet 6 distorted the symmetry in data and all the corresponding missing values were estimated by standard missing plot technique statistically.

Table 4. Average values of initial and final biomass, biomass gain, RGR, SGR, PER, ADMD and APD fed with test diets

Diet	Initial biomass $shrimp^{-1}$ (g)	Final biomass $shrimp^{-1}$ (g)	Biomass gain $shrimp^{-1}$ (g)	RGR	SGR	PER	FCR	ADMD	APD	Survival (%) ***
1	0.39	0.64	0.25	64.10	0.89	2.60	4.40	83.11	57.03	80
2	0.41	0.87	0.47	112.20	1.64	3.16	2.56	83.33	54.17	87
3	0.38	0.72	0.34	89.47	1.20	2.54	3.05	84.00	58.35	73
4	0.46	0.84	0.38	82.61	1.36	1.27	3.52	89.00	74.58	85
5	0.43	0.92	0.47	113.95	1.75	1.44	2.52	92.89	74.20	87
6	0.48	0.91	0.43	89.58	1.54	1.42	2.86	79.11	71.11	84
F test	NS	*	NS	NS	NS	**	NS	*	*	NS

NS - Not significant
* Significant at 5% level
** Significant at 1% level
*** Arcsine transformed for statistical analysis

The highest final biomass was observed in shrimps receiving diets 5 and 2. Even though diet 5 recorded 0.92 g final biomass gain. $shrimp^{-1}$, when absolute growth rate (AGR) was calculated following Hopkins (1992), an average daily gain of 0.017 g was observed in the diets 5 and 2. Similarly, the biomass gain was the highest in the aforementioned diets without any statistically significant difference. The highest RGR of 114% was found in shrimps fed with diet 5 followed by 112.2% in shrimps fed with diet 2. The PER was the lowest (1.25) in shrimps fed with diet 5 and maximum (3.16) in shrimps fed with diet 2 ($p < 0.01$). The FCR also indicated a similar trend without statistical significance. The highest ADMD coefficient of 92.9% and APD coefficient of 74.2% were recorded with diet 5. The highest APD coefficient of 74.6% was obtained with diet 4 and the lowest in diet 2, which was devoid of shrimp meal.

Diet 1 was devoid of fishmeal whereas diet 2 was devoid of shrimp meal. Diet 3 was devoid of both fishmeal and shrimp meal and diets 4, 5 and 6 contained all the major feed ingredients. Certain ingredients, especially the protein sources, were not included in diets 1, 2 and 3 to obtain protein levels ranging from 20% to 50% and energy levels ranging from 413.69 kcal g^{-1} to 470.83 kcal g^{-1}. This was because protein requirement for *Penaeus indicus* was reported as 42 - 43% by Colvin (1976) and as 35 – 37.5% by Gopal and Raj (1990). Bhaskar and Ali (1984) and Udayakumara and Ponniah (1988) reported that early postlarvae and juvenile *P. indicus* require 40% caesin in purified diets for optimum growth. Moreover, in a comparative evaluation of four purified proteins in *P. indicus*, Ali (1994) reported a requirement of 25% with albumin and 29% with caesin. Thus the experimental diet design in this study covered these range of protein levels (Table 3).

In terms of energy and protein interrelationships, AQUACOP (1976) estimated an optimum requirement of 330 kcal. 100 g^{-1} energy and 40% protein for *P. monodon*. In *P. merguiensis*, Sedgwick (1979) reported that the optimum protein levels are in the range of 34 - 42% with an energy content of 290 - 440 kcal. $100g^{-1}$. Later, Bautista (1986) opined that a twofold increase in body weight could be achieved with diets containing 40-50% protein, 5-10% lipid and 20% carbohydrate with energy values of 285-370 kcal.$100g^{-1}$ in *P. monodon* juveniles (0.60 - 0.80 g). Hajra *et al.* (1988) observed 46% protein and 412 kcal. $100g^{-1}$ GE to be the most appropriate dietary combination for *P. monodon* juveniles (0.5 g) reared in near freshwater conditions (3.5 - 4.5 ‰). Shiau and Chou (1991) reported 36% protein and 330 kcal. $100g^{-1}$ GE combination to be the best for *P. monodon* juveniles (0.82 g) reared in seawater (32 - 34 ‰). The only report assessing the optimum energy level in *P. indicus* is that of Ali (1990) who found 414.75 kcal 100g^{-1} as the optimum in a purified diet containing 40% protein (caesin). In the present study, the GE ranged from 407.68 kcal. 100g^{-1} to 469.76 kcal. 100g^{-1}. This includes a lower energy level of 414.75 kcal. 100g^{-1} reported by Ali (1990) with 40% protein and a higher level of 472 kcal. 100g^{-1} recorded with a protein optimum of 42.8% by Colvin (1976) in *P. indicus*.

In quantifying the nutrient requirements in fish, Zeitoun *et al.* (1976) pointed out the advantages of polynomial regression analyses. With the present data set, an attempt was made to fit second degree polynomials by regressing the final biomass of shrimps with protein and energy concentrations in the experimental diets.

Second degree polynomials of the form $y = a + bx + cx^2$ were fitted for subjectively deriving the optimum protein level and optimum energy level from the data obtained where, y = dependent variable (growth), x = independent variable (protein or energy) and a, b and c are constants. The estimated values for protein were $r^2 = 0.51$, $\hat{a} = -0.0724$, $\hat{b} = 0.0859$, $\hat{c} = -0.0015$ and SE ($\hat{c}$) = 0.000658. The optimum protein level was obtained by the equation $-\hat{b}/2\hat{c}$, which was 37.14%. Similarly for optimum energy level, the estimated values were ($r^2 = 0.527$), $\hat{a} = -37.6804$, $\hat{b} = 0.178462$, $\hat{c} = -0.000207$ and SE ($\hat{c}$) = 0.000144. The optimum energy level derived ($-\hat{b}/2\hat{c}$) was 430.95 kcal. 100 g^{-1}. Using the estimated optimum level of protein and GE, the P/E obtained was 86.18 mg protein. kcal^{-1}. The equation obtained for protein was $y = -0.7274 + 0.0859x - 0.0015x^2$ ($r^2 = 0.51$) indicating a maximum growth at 37.14% protein which corresponds to the optimum protein reported by Gopal and Raj (1990) for this species. Similarly, for energy, the equation obtained was $y = -37.6804 + 0.178462x - 0.000207x^2$ ($r^2 = 0.527$) indicating a maximum growth at 430.95 kcal. 100g^{-1} energy. Being an empirical fit to the growth response of living organisms, the polynomial approach has the advantage of being continuous and is believed to be more accurate than other methods (Zeitoun *et al.,* 1976).

Similar performance in terms of growth of *F. indicus* during the 28 day feeding period was observed with diets 2 and 5. In diet 5, the protein level was 47.65% and GE was 451.80 kcal. 100 g^{-1}. However, in diet 2, the protein level was 31.99% and energy level was 451.63 kcal. 100 g^{-1}. It was also observed that the non-protein energy yielding constituents *viz.,* the ether extract (EE) or crude fat and nitrogen free extractives (NFE) or soluble carbohydrates in diets 2 and 5 were 57.80% and 28.22% respectively (Table 3). Thus this study confirms the hypothesis of Sedgwick (1979) that a complementary reduction in the requirement of protein occurs for shrimps when adequate non-protein energy is available. Shiau and Chou (1991), in their experiments with *P. monodon,* (average weight: 0.81$\pm$0.10 g), reported an energy requirement of 330 kcal. 100 g^{-1} with 36% protein and 320 kcal.100 g^{-1} for 40% protein, which amounts to a protein sparing of 4% with an increment of 10 kcal.100 g^{-1} calculated GE. Similar performance of diets 2 and 5 in *F. indicus* appears to be due to the propensity of the early juveniles of this species to utilize higher amounts of carbohydrates (Ali, 1996). With an increase of non-protein energy constituents, the soluble carbohydrates-NFE (28.22%) and lipids-EE (57.80%), the growth and other nutritional indices with feets containing 32% to 48% protein were found to be similar in the shrimp. The PER was found to be significantly higher ($p < 0.01$) in the shrimps fed with diet 2 and significantly lower with diet 5. Implications here are that (1) diet 2 would have been adequately balanced in terms of aminoacids, and (2) a good quality shrimp diet can be formulated avoiding shrimp meal. An inverse relationship between PER and dietary protein as reported by Colvin (1976) was evident in the present study, which reiterates that dietary protein in excessive quantities may be either unassimilated or used as an expensive source of energy (Sedgwick, 1979). A high APD coefficient in the case of diets 4 and 5 could have been due to the excessive catabolism of protein to meet the energy demands. The FCR depicts the same trend with diets 2 and

5 registering similar feed: gain ratios, and protein and energy densities below and above the optimum leading to elevated FCRs.

Digestibility of dry matter and protein were two other nutritional responses recorded and perused. The data was juxtaposed with survival percentage for the sake of comparison, even though the survival data did not record any significant differences. However, plummeting of survival rate to a low of 73% in shrimps fed with diet 3 may be *a priori* due to the aminoacid imbalance, because the feed was devoid of both shrimp and fish meal. The PER obtained with this diet is also indicative of the above.

Ali (1990) in *P. indicus* reported an optimum requirement of 40% protein and 414.75 kcal. 100 g^{-1} GE when fed with purified diets and the present estimate of 37.14% protein and 430.95 kcal. 100 g^{-1} GE by feeding a diet made of natural feed ingredients indicates a marginally lower requirement of protein (Gopal and Raj, 1990) and slightly higher requirement of energy. Ali (1996), using a series of purified diets for *P. indicus* (initial weight: 10 mg) with a fixed lipid level of 7% and varying protein and carbohydrate levels, observed increasing trends in live weight gain, FCR and apparent carbohydrate digestibility without an optimum. A protein level of 21.9% and 53.4% carbohydrates with a GE of 399.4 kcal. 100 g^{-1} registered maximum weight gain, lowest FCR and highest carbohydrate digestibility, even though survival rates dropped with diets containing more than 45% carbohydrates. However, a protein level as low as 21.9% may be due to the feeding of purified proteins (Ali, 1994). The estimated protein requirement of 37% in the present study could be due to the natural sources of protein used in the experimental diets, which strengthens the finding of Gopal and Raj (1990) who observed 37.5% as protein optimum. Shiau and Chou (1991), applying the same technique in *P. monodon,* reported optimum levels of 320 kcal. 100 g^{-1} in 40% protein diet and 330 kcal. 100 g^{-1} in 36% protein diet, which is in agreement with the report by Bautista (1986) in the same species. However, the capability *F. indicus* to derive large quantum of energy from non-protein energy constituents, as established by Ali (1996), was obvious in this investigation also, where, diet 2 with 58% of non-protein energy constituents performed nutritionally at par with diet 5. The applicability of this result is that, unlike purified diets tested by Ali (1996), all the feed ingredients used for the diet were natural and location specific. Thus, the results are tangible enough for utilizing the diets evolved in this study for nursery rearing of postlarvae and it may also be cost-effective.

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