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Evaluation of Some Animal and Plant Protein Sources in the Diet of the Shrimp *Penaeus indicus*

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Abstract

Five animal protein sources, clam meat powder (*Sunneta scripta*), fishmeal, man-tis shrimp (*Oratosquilla nepa*), shrimp waste and silkworm pupa; three plant protein sources, coconut cake, gingely cake, groundnut cake; and the single cell protein *Spirulina* were evaluated by formulating isonitrogenous diets for the shrimp *Penaeus indicus*, through the measurement of true digestibility of protein, protein efficiency ratio (PER), net protein utilization (NPU), biological value (BV), growth and feed:gain ratio (FGR). Among the animal protein sources tested, fishmeal, clam meat powder, shrimp waste and mantis shrimp produced high PER, NPU and BV in decreasing or-der ($P<0.01$). Digestibility of protein from clam meat powder was significantly ($P<0.01$) higher than the protein from shrimp waste, followed by that of mantis shrimp and silkworm pupa. Silkworm pupa was a poor protein source for *P. indicus* with low di-gestibility, NPU, PER and BV. In the second group, groundnut cake and *Spirulina* produced significantly ($P<0.01$) higher growth than coconut and gingely cakes. How-ever, the NPU and BV of *Spirulina* were significantly higher than those of groundnut cake. Coconut cake and gingely cake showed low growth and BV, even though their protein had high digestibility. Comparison of the animal and the plant protein sources revealed that the animal protein sources were superior to the plant protein sources. In general, animal proteins produced higher growth, PER, NPU and BV over the plant proteins including *Spirulina*, but the digestibilities of the former were lower than those of the latter.

Introduction

In the pursuit of sustained production of compounded feeds to meet the growing demand of the aquaculture industry, there is constant search for feed ingredients which are cheap and, at the same time, available in fairly large quantities. Since aquatic animals require relatively high protein feeds and since the protein component

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in the feed is the most expensive, a rational approach for identifying and selecting protein sources is essential to make the cost of the feed economical. In this context, data on the evaluation of feed ingredients for the cultivable species of fish and shellfish will have immense practical utility. Thus, some animal and plant protein sources of low cost and available in sustainable quantities, were systematically evaluated for the Indian white shrimp *Penaeus indicus* which is one of the suitable candidate species for culture.

Materials and Methods

Five protein sources, clam meat powder (*Sunneta scripta*), fishmeal (commercial), mantis shrimp (*Oratosquilla nepa*), shrimp waste meal and silkworm pupa; three plant protein sources - coconut cake, gingely cake, and groundnut cake - and the single cell protein *Spirulina platensis* were selected for evaluation in the diet of the shrimp *P. indicus*. The chemical composition of these protein sources is given in Table 1.

Table 1. Composition of the animal and plant protein sources and *Spirulina*.

Material	% on dry basis						
	Total nitrogen	Protein nitrogen	Crude protein	Lipid	Carbo-hydrate	Crude fiber	Ash
Clam meat	7.69	6.35	48.10	13.55	16.69	-	7.62
Fishmeal	10.30	6.93	64.40	4.70	0.97	-	19.26
Mantis shrimp	7.05	5.23	44.06	7.55	1.27	8.2	23.63
Shrimp waste	5.63	4.34	35.20	6.60	0.97	14.2	23.95
Silkworm pupa	9.95	6.25	62.17	10.64	1.86	-	19.03
Coconut cake	4.15	3.80	25.96	11.20	22.19	11.9	8.88
Gingely cake	4.90	4.27	34.03	10.80	24.76	-	12.52
Groundnut cake	7.74	6.16	48.42	7.56	28.18	6.5	6.03
Spirulina	9.74	8.05	60.89	9.00	6.63	-	13.00

Approximately isonitrogenous diets were formulated, having each one of the materials as the sole protein source. The diet with coconut cake also contained fishmeal in order to make it isonitrogenous with other diets since coconut cake contained only 25% crude protein. The diets were balanced by adding fish oil, tapioca powder (source of starch), and a vitamin and mineral mixture.

Chromium oxide (Cr_2O_3) was included at 1% as an inert marker for the determination of digestibility. Polyvinyl alcohol was used as the binder. A zero-protein diet was also formulated as a control. The composition of these diets is presented in Table 2. The dry ingredients were powdered to pass through a 250-micron sieve and mixed together. The binder, polyvinyl alcohol, was melted in water (40 ml for 100 g diet) and added to the diet mixture. After homogenizing, the mixture was steamed for 10 min., extruded through a 3-mm die hand pelletizer and the pellets dried in a hot air oven at 60°C for 24 hours. The dry pellets were crushed into 1-mm granules for feeding the shrimp.

Table 2. Composition of experimental diets formulated with the selected protein sources.

Ingredient	Diet (%)									
	A	B	C	D	E	F	G	H	I	J
Clam meat powder	51.0	-	-	-	-	-	-	-	-	-
Fishmeal	-	47.0	-	-	-	16.0	-	-	-	-
Mantis shrimp	-	-	68.5	-	-	-	-	-	-	-
Shrimp waste meal	-	-	-	86.0	-	-	-	-	-	-
Silkworm pupa	-	-	-	-	50.0	-	-	-	-	-
Coconut cake	-	-	-	-	-	72.5	-	-	-	-
Gingely cake	-	-	-	-	-	-	88.5	-	-	-
Groundnut cake	-	-	-	-	-	-	-	62.0	-	-
Spirulina	-	-	-	-	-	-	-	-	50.0	-
Fish oil (sardine)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Tapioca powder	40.5	44.5	23.5	5.5	41.5	3.0	3.0	29.5	41.5	92.5
Vitamin and mineral mixture*	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Chromium oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	-
Polyvinyl alcohol	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0

*Vitamin and mineral mixture: 100 g of the diet contained the following vitamins and minerals: Vitamin A (acetate) 5,000 I.U., thiamine mononitrate 4 mg, riboflavin 6 mg, nicotinamide 50 mg, pyridoxine hydrochloride 3 mg, calcium pantothenate 10 mg, cyanocobalmine 2 mg, ascorbic acid 100 mg, calciferol 400 I.U., vitamin E 20 mg, biotin 0.1 mg, calcium phosphate 0.416 g, ferrous sulphate 24.24 mg, magnesium phosphate 96 mg and manganese hypophosphite 1.2 mg.

For feeding experiments, early juveniles of *P. indicus*, with an average length and weight of about 26 mm and 75 mg, respectively, from a single brood reared in the hatchery, were stocked in 10-l circular tanks. There were eight animals in each tank and three replicates for each treatment in random block design. A weighed quantity (about 20% of body weight) of diet was distributed twice a day so that the feed was available to the animals through most of the day. Leftover feed was recovered, dried and subtracted from the feed dispersed for computing the feed consumed. Three-fourths of the water was exchanged with fresh water every day. Feces were collected with a pipette three hours after the morning feeding, washed gently with distilled water, dried in the oven at 60°C and accumulated for analysis for digestibility studies. The duration of the feeding experiment was 30 days. A group of eight animals in triplicate were fed simultaneously with the zero-protein diet. The body nitrogen of the animals fed with this diet was used in calculating the net protein utilization (NPU).

The nitrogen in diets, feces and animals was determined by the Kjeldahl method and the chromium oxide was estimated by the method of McGinnis and Kasting (1964). The parameters for evaluation of the diets were calculated as follows:

$$\text{Feed:gain ratio} = \frac{\text{feed distributed in dry weight}}{\text{increase in live weight}}$$

$$\text{True digestibility coefficient} = 100 \times \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in feces}} \times \frac{\%\ \text{corrected protein in feces}}{\%\ \text{protein in diet}}$$

The fecal nitrogen due to metabolic activity, called the metabolic fecal nitrogen (MFN) of 326.4 mg per 100 g dry diet consumed, as determined by Ahamad Ali (1988) for this shrimp, was subtracted from the fecal nitrogen of the test group animals to obtain corrected fecal nitrogen for determining the true digestibility of protein.

$$\text{Protein efficiency ratio} = \frac{\text{increase in live weight}}{\text{protein consumed in dry weight}}$$

$$\text{Net protein utilization} = \frac{\text{body nitrogen of test group animals} - \text{body nitrogen of animals fed zero-protein diet}}{\text{Nitrogen consumed}}$$

$$\text{Biological value} = \frac{\text{net protein utilization}}{\text{true digestibility of protein}} \times 100$$

The salinity, temperature, dissolved oxygen and pH of the water used in the feeding experiment were maintained at 18.13±1.0 ppt, 28.5±0.5°C, 3.69 ml l^{-1} and 8.12±0.1, respectively, during the experiment.

The experimental data were subjected to analysis of variance (ANOVA) to test the significance among the treatments with respect to growth, feed:gain ratio (FGR), digestibility coefficient, protein efficiency ratio (PER), NPU and biological value (BV). The method of least significant difference (also known as critical difference) was applied for comparison between the treatments, following the method of Snedecor and Cochran (1973).

Results

The results of the feeding trials with the experimental diets are shown in Table 3 and the growth curves of shrimps fed with different diets during the experimental period are depicted in Fig. 1.

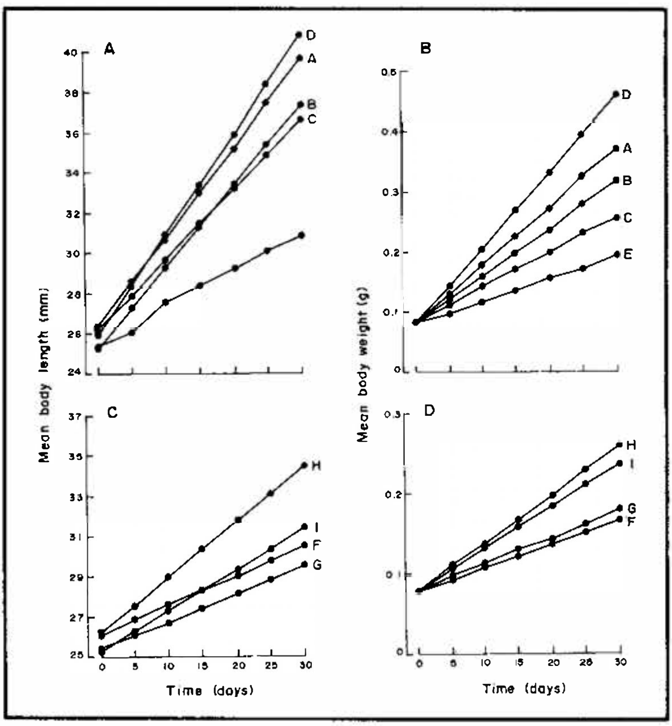

Fig. 1. Growth curves of *P. indicus* fed with diets formulated with animal and plant proteins and *Spirulina*. (a) and (c) body length and (b) and (d) body weight. Curves are: A-clam meat powder; B-fishmeal; C-mantis shrimp; D-shrimp waste; E-silkworm pupa; F-coconut cake; G-gingely cake; H-groundnut cake; and I-*Spirulina*.

Table 3. Results of feeding experiments on *P. indicus* with formulated diets. Values are expressed as mean ± standard deviation except in case of survival.

Diet	Increase in length (mm)	Increase in weight (g)	Feed:gain ratio	True digestibility of protein	Protein efficiency ratio (PER)	NPU	BV	Survival %
A	13.8 ± 0.9^{ab}	0.26 ± 0.03^{bc}	1.83 ± 0.27^{a}	81.2 ± 1.6^{a}	1.77 ± 0.25^{a}	60.8 ± 1.4^{a}	74.8 ± 0.2^{b}	66.6
B	12.5 ± 0.8^{bc}	0.23 ± 0.02^{c}	2.15 ± 0.10^{a}	70.1 ± 0.4^{b}	1.46 ± 0.06^{a}	65.0 ± 5.6^{a}	92.3 ± 2.3^{a}	83.3
C	11.2 ± 0.8^{bc}	0.17 ± 0.02^{cd}	1.95 ± 0.15^{a}	66.9 ± 6.8^{b}	1.52 ± 0.23^{a}	33.7 ± 1.9^{bc}	50.4 ± 4.9^{d}	62.5
D	15.0 ± 0.7^{a}	0.38 ± 0.01^{a}	3.33 ± 0.62^{c}	77.2 ± 6.0^{a}	0.96 ± 0.18^{b}	47.8 ± 5.9^{cd}	61.9 ± 5.8^{c}	79.2
E	5.0 ± 0.5^{d}	0.12 ± 0.01^{d}	2.85 ± 0.11^{b}	61.0 ± 1.6^{d}	0.88 ± 0.04^{b}	27.1 ± 8.1^{d}	44.4 ± 7.9^{e}	75.0
LSD*	2.29	0.06	0.71	5.4	0.4	17.8	6.7	-
	($P<0.01$)	($P<0.01$)	($P<0.05$)	($P<0.05$)	($P<0.05$)	($P<0.05$)	($P<0.01$)	-
F	4.2 ± 0.3^{b}	0.10 ± 0.01^{b}	2.35 ± 0.23^{a}	88.4 ± 3.54	1.29 ± 0.13^{a}	23.3 ± 2.7^{b}	26.3 ± 4.2^{c}	87.5
G	4.1 ± 0.9^{b}	0.09 ± 0.01^{b}	2.98 ± 0.46^{a}	85.0 ± 1.7	0.78 ± 0.20^{b}	31.7 ± 4.5^{a}	37.3 ± 4.9^{b}	83.3
H	8.2 ± 1.5^{a}	0.15 ± 0.02^{a}	4.99 ± 0.16^{b}	86.1 ± 2.0	0.63 ± 0.05^{b}	20.6 ± 1.3^{b}	23.9 ± 1.1^{cd}	66.6
I	6.0 ± 0.8^{b}	0.18 ± 0.01^{a}	4.02 ± 0.41^{c}	77.4 ± 1.5	0.74 ± 0.14^{b}	39.3 ± 6.2^{a}	50.8 ± 2.5^{a}	37.5
LSD*	2.18	0.04	0.96	N.S. at 5%	0.31	7.26	4.2	-
	($P<0.05$)	($P<0.01$)	($P<0.01$)		($P<0.05$)	($P<0.01$)	($P<0.01$)	-

*LSD = Least significant difference; N.S. = not significant.
Values with same superscript in vertical rows do not differ significantly.

Animal Protein Sources

Among the animal proteins tested, diet D prepared with shrimp waste showed significantly ($P<0.01$) the highest growth. This was followed by diet A with clam meat powder, diet B with fishmeal, diet C with mantis shrimp and diet E with silkworm pupa. The protein of clam meat powder had highest digestibility ($P<0.05$) followed by protein of shrimp waste. The digestibilities of the proteins of fishmeal and mantis shrimp were comparable and lower than those of clam meat powder and shrimp waste. The protein of silkworm pupa showed the lowest digestibility among the five animal materi-

Table 4. Analytical data on diets, feces and animals.

Diet*	Nitrogen % in diet	Nitrogen % in feces	Corrected fecal nitrogen (%)	Chromium oxide % in diet	Chromium oxide % in feces	Average nitrogen intake (g)	Body nitrogen after feeding with diet (%)
A	5.06	4.28	3.17	0.97	3.28	0.02	11.26
B	5.12	4.81	3.85	1.00	2.25	0.02	11.57
C	5.15	4.35	3.77	0.93	1.79	0.03	11.29
D	5.20	2.42	1.84	1.05	1.77	0.02	11.16
E	5.08	4.88	4.20	0.86	1.42	0.02	10.64
F	5.32	2.02	1.31	1.00	2.18	0.01	10.43
G	5.32	2.46	1.81	0.93	1.84	0.01	10.62
H	4.80	2.42	2.23	1.00	2.92	0.03	10.86
I	5.03	5.08	2.63	0.84	1.64	0.02	10.82
J	0.31	-	-	-	-	-	10.16

*Letters refer to diets in Table 2.

als tested. Though the diet with shrimp waste gave higher growth, PER was low ($P<0.05$) and FGR was high compared to other diets. The PER and FGR of the diets with clam meat powder, fishmeal and mantis shrimp were comparable, but the PER and FGR of the diet with silkworm pupa were inferior to those of the other diets. The nitrogen content of shrimps fed with different diets is shown in Table 4. The animals fed with the diets having fishmeal and clam meat powder showed high nitrogen in their bodies. The NPU and BV recorded by these diets were also significantly ($P<0.01$) higher than the diets with shrimp waste, mantis shrimp and silkworm pupa in decreasing order.

Plant Proteins and Spirulina

Among the plant proteins, the diet prepared with groundnut cake produced better growth. The growth obtained by the diets having coconut cake and gingely cake was identical and was lower than that for the diet with groundnut cake. The growth of shrimp fed with the *Spirulina* diet was higher than for those receiving the groundnut cake diet. However, survival of the shrimp fed with *Spirulina* diet was notably poor.

The digestibility of protein of all the plant protein sources and *Spirulina* was similar. But the NPU and BV of *Spirulina* were distinctly higher ($P<0.01$) than those of plant proteins. The NPU and BV of gingely cake were slightly higher than those of coconut cake, but the values for both coconut and gingely cakes were higher than the NPU and BV of groundnut cake. The FGR recorded by groundnut cake and *Spirulina* was high, and their PER was low compared to coconut cake and gingely cake. Among them coconut cake registered the lowest FGR and the highest PER.

Discussion

Even though shrimp waste showed superior growth in *P. indicus*, clam meat powder had high digestibility of its protein and showed good PER, NPU and BV. Fresh clam meat is conventionally used for feeding shrimp. Many species of clams were found to give good growth in shrimps (Kanazawa et al. 1970; Forster and Beard 1973, 1974). However, inferior results with clam meat were also reported in certain species of shrimps (Colvin 1976; Ahamad Ali 1982). Results obtained in the present study with *P. indicus* are comparable with those for dry clam meat powder by Ahamad Ali (1982) with the same shrimp.

Fishmeal is extensively used in shrimp diets with varying results: a high assimilation of 90.5 in caridean shrimp *Palaemon serratus* (Forster and Gabbott 1971) and 89.0 in *Penaeus japonicus* (Nose 1964) to a low of 69.4 in *P. indicus* (Colvin 1976), while Akiyama et al. (1989) recorded a digestibility coefficient of 80.7 in *P. vannamei* for fishmeal protein. Even though the digestibility of fishmeal (70.1) in *P. indicus* in the present study is lower than that found in other shrimps, its protein showed high NPU and BV indicating its superiority as a protein source for this shrimp. Computing the essential amino acid index of some protein sources, Peñaflorida (1989) concluded that local fishmeals were good and comparable to Norwegian white fishmeal for *P. monodon*, which is in agreement with the present findings. Contrary to these results, poor performance of fishmeal was reported for *P. monodon* (Deshimaru and Shigueno 1972; Pascual and Destajo 1978) and *P. indicus* (Colvin 1976; Ahamad Ali 1982). Such results were attributed to the differences in fishmeals and their amino acid profiles. Fishmeals were reported to be deficient in the essential amino acids tyrosine and phenylalanine.

Mantis shrimp protein showed low digestibility (66.9) but its PER (1.52) and BV (50.4) were good indicating that mantis shrimp has good quality protein. The low digestibility of mantis shrimp protein might be due to its chitinous shell content. The protein extracted from it was tested for the shrimp *P. indicus* by Ahamad Ali (1982) and found to be comparable to that of clam meat powder. Mantis shrimp was also used as one of the ingredients in compounded feeds for shrimp (Alikunhi et al. 1980; Ahamad Ali and Sivadas 1983; Ahamad Ali and Mohamed 1985). The results of evaluation of mantis shrimp in the present study confirmed these findings.

The digestibility of shrimp waste (77.2) in *P. indicus* is slightly better than its digestibility in *P. vannamei* (74.6) as determined by Akiyama et al. (1989) but marginally lower than its assimilation recorded in *Palaemon serratus* (87.0) and *Pandalus plotyceros* (82.0) by Forster and Gabbott (1971). Shrimp waste showed good NPU and BV in the present study. It has been identified as one of the important by-products from the shrimp processing industry. Its protein is rich in essential amino acids (Forster 1975; Peñaflorida 1989) and the oil extracted from shrimp head contains polyunsaturated fatty acids essential for shrimps (Joseph and Meyers 1975; Joseph and Williams 1975). The utilization of shrimp waste in shrimp diets has been studied by different workers (Pascual and Destajo 1978; Venkataramaiah et al. 1978; Ahamad Ali 1982; Ahamad Ali and Mohamed 1985; Akiyama et al. 1989; Peñaflorida 1989; Lim and Dominy 1990) for different species with varying results. The differential performance of shrimp waste is due to differences in its quality, from different species of shrimps and using different methods of processing. During this investigation it was observed that the residual meat of shrimp in the waste was different in different consignments from the same processing unit. Nevertheless, shrimp waste is a potential feed ingredient in shrimp feeds, having high biological value of its protein. Its flavor seems to be an attractant in shrimp diets (Pascual and Destajo 1978) and the shell (source of chitin) of shrimp waste is found to have a growth-promoting effect in *P. indicus* (Vaitheswaran and Ahamad Ali 1986) besides being a good protein source.

Silkworm pupa produced poor growth in *P. indicus* and its protein showed very low NPU (27.0) and moderate BV (44.3). However, the FGR of the diet (2.85) was reasonable, showing that the diet was not ingested well and whatever was ingested was converted fairly well into growth. The low digestibility (60.9) of its protein might be due to the high percentage of non-protein nitrogen in the material which also might have resulted in low NPU. Silkworm pupa is utilized in poultry feeds, but its utility in shrimp feeds appears to be low. The lowest intake of nitrogen by the shrimp was from the silkworm pupa diet (Table 4) among the animal proteins tested.

Digestibility of protein of all the plant proteins and *Spirulina* was almost similar. The PER of coconut cake (1.29) was, however, high but its NPU (23.3) and BV (26.3) were low, which might be due to the deficiency of lysine in it. Coconut cake is used in a limited way in shrimp diets (Balazs and Ross 1976; SEAFDEC 1978). However, it cannot be used as a major protein source in shrimp diets. The true digestibility of gingely cake protein (85.0) in *P. indicus* is higher than that in land animals. Gingely cake also is deficient in lysine and has moderate NPU (31.7) and BV (37.3). Results in *P. indicus* have shown that gingely cake can be used only in limited quantities in shrimp diets, but not as the sole source of protein. Protein from groundnut cake had excellent digestibility (86.1), comparable to the assimilation of its protein in *Palaemon serratus* (89.5) as determined by Forster and Gabbott (1971). It is also comparable to the digestibility of soybean protein (89.9) in *Penaeus vannamei* reported by Akiyama et al. (1989). The amino acids, cystine, methionine and lysine, are the limiting amino acids in groundnut cake which may be responsible for the low NPU (20.5) and BV (23.9). In spite of this, groundnut cake is widely used in shrimp diets (New 1976) as it is available in large quantities. Results of the present study indicate that groundnut cake can be used in shrimp feeds only sparingly and should not be used as the main protein source.

Spirulina scored a high NPU (39.3) and BV (50.8). The digestibility of *Spirulina* protein in *P. indicus* is low (77.4) compared to its digestibility (87.0) reported in carp *Cyprinus carpio* by Atack et al. (1979). On the other hand, the NPU and BV of *Spirulina* in *P. indicus* are higher than those recorded in carp. Even though the growth of shrimp fed with *Spirulina* diet is good, survival of the animals is poor. Similar observations were made in *P. monodon* postlarvae by Lim et al. (1978). Cuzon et al. (1981) found that the lipid in *Spirulina* had a high peroxide value and attributed this to the low survival of *P. japonicus* when they were fed with this diet. The unicellular alga is emerging as a new source of protein and a potential ingredient in aquatic feeds. It may, however, be used in shrimp diets only at a limited level (up to 8%) as advocated by Cuzon et al. (1981) taking into consideration the poor survival of the shrimps fed with *Spirulina* diet.

The animal proteins have low digestibility but they have high NPU and BV, whereas plant proteins and *Spirulina* have high digestibility but low NPU and BV. The differences between the groups are mainly due to differences in protein quality especially of their essential amino acid (EAA) profiles. The animal proteins are generally rich in EAA lysine which is the limiting EAA in the plant proteins. Based on the BVs obtained in *P. indicus* in the present study, the protein sources tested can be given the following 'protein ratings': clam meat, fishmeal, mantis shrimp and shrimp waste can be regarded as 'good' (BV above 50) and silkworm pupa as 'average' (BV 30-50); coconut cake, gingely cake and groundnut cake can be rated 'average,' while *Spirulina* can be rated 'good'.

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