Indian Journal of Geo-Marine Sciences
Vol. 40 (3), June 2011, pp 460-470

Sunflower oil cake as a replacement for fish meal in feeds of Tiger Shrimp, *Penaeus monodon* reared in tanks and in net cages

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Received 05 January; revised 13 April

Replacement of fish meal in marine shrimp feeds is assuming greater importance due to increasing economic and ecological considerations. This study evaluated sunflower cake as a replacement for fish meal in tiger shrimp *Penaeus monodon* juveniles reared both in tanks and in net cages at 26-33 ppt salinity. Five practical diets were prepared by incorporating sunflower cake at 0, 2.5, 5, 7.5 and 10% level by replacing fish meal. The essential amino acid index (EAAI) of test diets ranged from 0.91 to 0.88. Growth and digestibility study was conducted for 45 d in 500 L experimental tanks shrimp weighing 0.51±0.02 g with three replications for each treatment. There were significant ($P<0.05$) reductions in daily growth coefficients and protein utilization efficiency in shrimp fed with diets having above 2.5% sunflower cake in experimental tanks. The lysine and methionine digestibility (92.4±0.11 and 93.4±0.01%, respectively) was significantly ($P<0.05$) higher at 2.5% level inclusion than at 10% level (81.67±0.12 and 83.36±0.17%, respectively). The free amino acid profiles of shrimp muscle at 4 h post feeding indicated significantly ($P<0.05$) lower levels for isoleucine, leucine, lysine and valine in shrimp fed with higher level of sunflower cake. The decreased levels of these essential amino acids could have hampered protein synthesis and in turn growth. With three dietary treatments (0, 5 and 10% sunflower cake diets) a 10-week growth trial was also conducted in net cages with tiger shrimp juveniles weighing 0.2 g. Significantly ($P<0.05$) lower daily growth coefficients (%/d) were observed in shrimp fed with 10% sunflower cake diet (1.642±0.017) compared to control (1.774±0.024). The results indicate that sunflower cake can be incorporated up to 5% by replacing 20% of fish meal in marine shrimp *P. monodon* practical feeds without compromising growth.

[**Keywords:** Fish meal replacement, Shrimp feed, Plant protein sources, Sunflower cake, Amino acids, Digestibility, Free amino acid pool]

Introduction

Shrimp farming has assumed greater importance in developing countries in Asia-Pacific as a foreign exchange earner. Much of the recent increase in global aquaculture production has been brought about through the adoption of intensive farming practices using formulated feeds. Commercial shrimp feed formulations commonly include between 25 and 50% fish meal representing the primary and most expensive protein ingredient[1]. One factor considered in reducing shrimp production costs and increase producers' profitability is the use of feeds with low levels of fish meal and high levels of less expensive, high quality plant protein sources by optimizing the feed formulations[2]. With the 5% increase of demand for aquafeeds per annum, the most limiting factor for aquaculture growth will be the fish meal availability[3,4] due to its relatively static production at 6.2 million t[5] in the last two decades, and competition from other feed industry sectors such as pig, poultry and pet food industries. This over-dependence on any one ingredient presented considerable risk associated with supply, price and quality fluctuations and issues relating to sustainability[6]. As a strategy to reduce risk, and escape from fish meal trap and lower the cost of production the identification, development and use of alternatives to fishmeal in shrimp feeds remains a high priority nutrition research. The availability of sound data base on growth, digestibility and nutrient utilization of soybean meal in practical marine shrimp feeds for both tank and pond reared conditions helped in optimization of this ingredient in commercial shrimp feed formulations[1,7-11]. Efforts are being made to develop similar data base on nutrient utilization for other plant protein sources in practical shrimp feed formulations like ground nut cake, coconut cake, gingely cake in Indian white shrimp, *Penaeus indicus*[12], lupin kernel meal in tiger shrimp, *Penaeus monodon*[13-15], feed pea in *P. monodon*[16,17], cotton seed meal in *Litopenaeus vannamei*[18]. And these research efforts helped in 35% reduction in fishmeal usage in shrimp feeds during the period 1995-2007[19].

Sunflower (*Helianthus annuus*) is a widely cultivated oil seed and its cake/meal is the residue of oil extracted from seeds. The production of sunflower oil cake is around 16.6 and 1.1 million metric tones in the world and in India, respectively[20]. It is a widely used protein source in livestock, poultry and pig rations. It is a good source of methionine and arginine[21]. The maximum inclusion levels of sunflower meal reported for rainbow trout was 11%[22], seabream 12%[23], tilapia 14%[24], 20%[25] and atlantic salmon 20%[26]. No studies, however, have yet examined sunflower cake as a substitute for fish meal in shrimp feeds. The objective of the present study is to optimize the sunflower cake inclusion as a replacement of fish meal in shrimp diets by conducting growth and digestibility trial with the juveniles of tiger shrimp, *Penaeus monodon* Fabricius (Crustacea:Decapoda:Penaeidae) both in tanks and in net cages. In addition to the amino acid digestibility, the changes in free amino acids (FAA) profiles in shrimp muscle were measured as a tool to understand the availability of precursors for protein synthesis.

Materials and Methods

Experimental diets and diet preparation

In order to evaluate whether fish meal in the tiger shrimp diet could be replaced partially by sunflower cake and to determine the maximum inclusion levels, a growth and digestibility trial was conducted in the juveniles of *P. monodon*. Control diet used in this study was a typical practical shrimp feed with 25% fishmeal, having 40% crude protein. Sunflower cake was serially included at 0 (control), 2.5, 5, 7.5 and 10% in test diets by replacing fishmeal at a substitution rate of 0, 10, 20, 30 and 40% in the practical shrimp feed formulation and are serially named as diet 1, 2, 3, 4 and 5, respectively. Chromic oxide 0.5% was added as an external indicator in all the test diets to measure the digestibility parameters[27].

The coarse ingredients were powdered in a micropulveriser and passed through 250 - µm mesh screen. All the ingredients were weighed as for the formula and prepared as per the protocol[28]. Briefly, the dry ingredients were mixed manually for 5 minutes and then transferred to a domestic mixer and mixed for another 10-min. Fish oil and lecithin were gradually added to the homogenized mash. Water was then added (50 ml/100 g mash) to the diet mix and kneaded into a dough by hand. It was steamed for 5 minutes at atmospheric pressure and pelleted in an indigenous experimental pellitizer with a 2-mm diameter die. The pellets were dried in a forced-air oven at 60°C for 12 hours. The pellets were packed in plastic bags after cooling to room temperature and stored in desiccator until use.

Growth and digestibility feeding trial

Post larvae (PL15) of *P. monodon* were procured from the local private hatchery, Chennai, India. These PL were reared in net cages in the lagoon of Muttukadu Experimental Station of Central Institute of Brackishwater Aquaculture, Chennai to get the juveniles for feeding trial by feeding with standard CIBA shrimp feed. The shrimp were acclimated to wet laboratory conditions for 48 hours in 1000-L fibre glass tanks. During this period, the shrimp were fed with a standard CIBA- shrimp diet[28]. Initial samples (25 shrimp) were collected for biochemical analysis. The experiment was conducted in an indoor wet laboratory with the provision for sand filtered UV treated sea water and continuous aeration. At the beginning of the experiment, 225 healthy shrimp juveniles with initial body weight of 0.51 ± 0.02 g (mean weight ± SE) were distributed randomly into fifteen oval 500 L fiberglass tanks (three tanks/diet, 15 shrimp/tank) in a completely randomized design. The shrimp were fed *adlibitum* three times daily (08:30, 12:30, and 18:00) for 45 days. The daily feed ration was adjusted according to feed consumption and shrimp size (with 5–10% body weight). Fortnightly sampling was done to adjust the feed rations. During the whole feeding period, the shrimp were fed in a static condition with 80% daily water exchange before the first feeding. Aeration was provided through diffuse air stone. Aeration was stopped to prevent disintegration of feed pellets and leaching of nutrients from feed and faeces. The uneaten feed pellets and particles were collected by siphoning from the tank 2 h after feeding, which were dried and weighed for feed intake calculation. Faeces were gently siphoned from the tanks on to a bolting silk cloth after 3 h of feeding from 2nd week of experiment and lasted for 15 days until enough samples were collected for chemical analysis. Faecal samples were rinsed with distilled water, dried and then homogenised for analysis. The shrimp were subjected to a natural photoperiod (12h light: 12h dark). During the experimental period, the water temperature, salinity, pH, dissolved oxygen and total ammonia-nitrogen were ranged between 27-29°C, 31-33‰, 7.5-7.8, 5.5-7.5 mg/L and <0.01 ppm, respectively.

At the end of the 45-day experiment, the shrimp were counted and weighed to determine survival, daily growth coefficient (DGC), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent protein utilization (APU) after fasting for overnight. A range of approaches have been used to describe growth rate in shrimp and it was suggested that DGC (%/d) was the most appropriate for a very large part of growth curves of *P. monodon*, *P. vannamei* and *P. stylirostris* in grow-out studies[15,29]. Three animals from each replicate were taken for biochemical analysis. After the feeding trial, five shrimp in the intermoult stage were maintained in the original tank and were fed with same diet for three more days. On 4th day shrimp muscle was collected for free amino acid profiles at 4 h of post feeding.

DGC, PER, APU and Apparent digestibility coefficient of nutrients were calculated based on the following equations.

$$DGC = \left[100 \times \frac{(\text{Final Wt}^{0.333} - \text{Initial Wt}^{0.333})}{\text{No. of days}}\right]$$

$$PER = \left[\frac{\text{Weight gain (g)}}{\text{CP intake (g)}}\right]$$

$$APU = \left[\frac{\text{Protein gain (g)}}{\text{Protein intake (g)}}\right] \times 100$$

$$\% \text{ ADC} = 100 \times \left[1 - \left(\frac{\% \text{ } Cr_2O_3 \text{ in the diet}}{\% \text{ } Cr_2O_3 \text{ in the faeces}} \text{ X } \frac{\% \text{ Nutrient in the faeces}}{\% \text{ Nutrient in the diet}}\right)\right]$$

Yard trial in net cages (Hapa)

Three test diets (0, 5 and 10% level sunflower cake diets) were also evaluated in net enclosures (hapa) in the lagoon of CIBA Muttukadu Experimental station to optimize the level of inclusion of sunflower cake in the natural environment. Rectangular nylon cages of 3×2×1m dimension with 2 mm mesh size were tied with the help of casuarinas poles[30]. The mesh size of bottom and top is 1.5 mm and a zip is provided on the top for feeding and sampling. Juveniles of *P. monodon* weighing 0.2 g were stocked at the rate of 16/m^2. The feeding trial was conducted for 10 weeks with three replicates. To prevent clogging, the cages were cleaned everyday by using brush on all four sides by keeping the hapa tied intact. Initially feed was given at the rate of 10% of the biomass, which was divided into two meals 60% at evening 40% in the morning. Once in 20 days sampling was done for adjusting the feeding rate. At the end of the experiment, the shrimp were counted and weighed to determine survival and daily growth coefficient (DGC). During the experimental period, the water temperature, salinity, pH, dissolved oxygen and total ammonia-nitrogen ranged from 28-30°C, 26-28‰, 7.8-8.1, 5.2-6.8 mg/L and 0.1-0.3 ppm, respectively.

Laboratory analysis

The proximate composition of diets, faeces and experimental animals was analysed by standard AOAC method[31]. The water stability of pellets was determined at 2 h to arrive at the dry matter and protein intake[32] to measure FCR and PER. Chromium content in the diets and faeces was analysed[33] to calculate the amino acid digestibility. The total amino acids of feeds, faeces and shrimp were analysed[34,35] after sealed tube hydrolysis with 6N HCl for 22 h at 110° C. After the hydrolysis, the acid was evaporated in vacuum oven and the sample was kept in NaOH desiccator to remove traces of acid. The residue was brought into 1 ml of Sodium Citrate-Perchloric acid sample diluent (pH 2.20) and it was filtered through 0.2 µm membrane filter. The free amino acid pool in the tail muscle was measured using the method outlined[36]. Briefly, 200 mg samples, taken from the tail muscle, were homogenized in 4 ml absolute ethanol. The homogenate was centrifuged to pellet the precipitated proteins. Duplicate samples of the supernatant were then taken to measure the free pool amino acid composition. A subsample of 100 µl was dried and reconstituted in 100 µl sample diluent (pH 2.20) and filterd. Amino acids were analysed using Shimadzu HPLC model LC-10A (Shimadzu Corp., Japan). Separation of amino acids was done in a column (Shimpack ISC-07/S1504 Na) packed with a strongly acidic Na^+ type cation exchange resin (Styrene-divinyl benzene copolymer with sulfinic group) under gradient elusion at a flow rate of 0.3 ml/minute by using two buffers, (A) sodium citrate-perchloric acid (pH 3.2); (B) boric acid sodium citrate-sodium hydroxide (pH 10.0). The amino acids were detected and quantified using a fluorescent detector (FLD-6A) after post column derivitization with O-pthaladehyde and 2-mercaptoethanol. Amino acid standard solution (Sigma-Aldrich Inc., USA) for fluorescent detection was used as external standard for every ten-sample injections one standard run was carried out. Tryptophan being labile to the acid hydrolysis, this was measured by alkali hydrolysis by Spectrophotometric method[37] at 500 nm.

Statistical analysis

The experiments were conducted under completely randomized experimental design[38] and the one-way analysis of variance (ANOVA) was carried out by using SPSS statistical software package version 17.0.

Results

The proximate and amino acid composition of test diets is presented in Table 1. With the increase of sunflower cake the crude protein and ash contents of the diets decreased and crude fibre content increased. The essential amino acid index (EAAI) of the test diets was calculated based on the essential amino acid requirements of *P. monodon* and it was ranged from 0.91 to 0.88. The growth response and protein utilization efficiency are shown in Table 2. Shrimp fed diets containing 0 and 2.5% sunflower cake exhibited similar growth rate throughout the experimental period. The growth rate expressed as DGC (%/d) of shrimp fed diets 1 and 2 are significantly ($P<0.05$) higher than those of shrimp fed

Table 1—Ingredient, proximate and amino acid composition (% as fed basis) of test diets

	Diets with sunflower cake inclusion (%) levels (diet Nos)					
	0 (1)	2.5 (2)	5.0 (3)	7.5 (4)	10 (5)	
Ingredient composition						
Fish meal	25	22.5	20	17.5	15	
Sunflower cake	0	2.5	5	7.5	10	
Common ingredients*	75	75	75	75	75	
Proximate composition						
Moisture	9.86	9.33	9.64	9.34	9.18	
Crude Protein	40.95	40.18	39.40	38.62	37.84	
Ether Extract	8.06	8.05	8.04	8.02	8.01	
Crude Fibre	3.63	4.18	4.72	5.27	5.81	
Total Ash	15.62	15.1	14.63	14.06	13.59	
Nitrogen free extract	21.88	23.17	23.58	24.69	25.57	
Amino acid composition						EAA Requirement (% Protein)
Alanine	1.89	1.85	1.79	1.73	1.71	
Arginine	2.50	2.46	2.38	2.13	1.68	5.3
Aspartic acid	4.38	4.34	4.26	4.24	4.10	
Cysteine	0.49	0.39	0.37	0.36	0.33	
Glutamic acid	7.41	7.38	7.33	7.33	7.35	
Glycine	2.29	2.30	2.28	2.24	2.17	
Histidine	0.91	0.90	0.89	0.85	0.84	2.2
Isoleucine	1.42	1.43	1.40	1.34	1.32	2.7
Leucine	3.08	3.02	2.81	2.59	2.48	4.3
Lysine	2.60	2.48	2.03	1.70	1.38	5.2
Methionine	0.82	0.83	0.74	0.64	0.55	2.4
Phenylalanine	1.64	1.51	1.47	1.45	1.43	3.7
Proline	2.40	2.40	2.03	2.02	1.93	
Serine	1.55	1.54	1.49	1.48	1.38	
Threonine	1.45	1.40	1.28	1.23	1.14	3.5
Tyrosine	1.04	1.02	0.99	0.97	0.95	
Valine	1.76	1.65	1.61	1.58	1.53	3.7
Tryptophan	0.32	0.30	0.23	0.18	0.16	0.5
EAAI£	0.91	0.91	0.91	0.90	0.88	

*Common ingredients: Acetes, 12; Squilla, 12; Squid, 5; Soybean cake, 20; Wheat, 19.4; Fish oil, 2; Lecithin, 1; Vitamin and Mineral mixture, 2; Stable Vitamin C, 0.1; Binder, 1; Chromic Oxide, 0.5

£EAAI: Essential Amino Acid Index: Calculated based on shrimp amino acid requirements (% Protein)[67-70] and aa/AA ratios are set at 0.01 minimum and 1 maximum[71].

Table 2—Effect of incorporation of sunflower cake on growth coefficient, survival and protein utilization in tiger shrimp *Penaeus monodon* reared in tanks and in net cages

Parameter	Diets with sunflower cake inclusion (%) levels (diet Nos)				
	0 (1)	2.5 (2)	5.0 (3)	7.5 (4)	10 (5)
DGC* (%/d)	0.82^{c}±0.01	0.81^{c}±0.01	0.69^{b}±0.04	0.61^{ab}±0.04	0.55^{a}±0.01
Survival (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	93.3 ± 6.67	93.3 ± 6.67
FCR@	1.79^{a} ± 0.04	1.91^{a} ± 0.08	2.19^{b} ± 0.20	2.37^{bc}± 0.05	2.54^{c} ± 0.03
PER#	1.50^{b}±0.03	1.44^{b}±0.06	1.31^{a}±0.05	1.23^{a}±0.03	1.17^{a}±0.01
APU$ (%)	30.89^{b}±0.59	31.41^{b}±1.38	27.03^{a}±1.16	26.01^{a}±0.54	24.33^{a}±0.29

Net cages

Parameter	Diets with sunflower cake inclusion (%) levels (diet Nos)		
	0 (1)	5.0 (3)	10 (5)
DGC* (%/d)	1.77^{b} ± 0.024	1.76^{ab} ± 0.056	1.64^{a} ± 0.017
Survival (%)	85.33 ± 3.18	86.67 ± 4.26	83.67 ± 0.88
FCR@	1.44^{a} ± 0.041	1.48^{a} ± 0.038	1.72^{b} ± 0.04
PER#	1.70^{b} ± 0.048	1.65^{b} ± 0.042	1.42^{a} ± 0.033
APU$ (%)	35.06^{b} ± 0.993	34.15^{b} ± 0.863	29.42^{a} ± 0.692

Mean values bearing different superscripts in a row differ significantly (P<0.05)
* daily growth coefficient (DGC)
@ feed conversion ratio (FCR)
# protein efficiency ratio (PER)
$ apparent protein utilization (APU)

diets 3, 4 and 5. Survival rates in all test diets were above 93% and it was not significantly different among treatments. The dry matter loss (7.87-11.73%) and protein loss (8.56-11.45%) due to leaching at 2 h were measured to calculate the correct nutrient intake. Feed conversion ratio (FCR), protein efficiency ratio (PER) and the apparent protein utilization (APU) were similar for diets 1 and 2.

The sunflower cake incorporation significantly (P< 0.05) influenced the crude protein and amino acid digestibility in *P. monodon* (Fig. 1). All the amino acid digestibilities were significantly (P<0.05) lower in diet 4 and 5. Significant (P<0.05) higher levels of amino acid digestibilities of arginine, histidine and methionine were observed in diet 2 compared to diet 4 and 5. Alanine was having the lowest digestibility (78.60 ± 0.67%) across all the test diets. The average essential amino acid digestibility was highest (90.08 ± 0.46%) in diet 2 and was lowest in diet 5 (82.87 ± 0.4%). Whole body proximate composition of shrimp expressed on dry matter basis is presented in Table 3. The crude protein, ether extract, total ash % of shrimp fed with test diets were not significantly different. The predominant amino acids in whole shrimp (% dry basis) are glutamic acid (10.34-11.25), aspartic acid (6.12-6.59) and

Table 3—Proximate and amino acid composition of whole shrimp, *P. monodon* fed with varying levels sunflower cake

	Diets with sunflower cake inclusion (%) levels (diet Nos)				
	0 (1)	2.5 (2)	5.0 (3)	7.5 (4)	10 (5)
Proximate composition (% dry basis)					
Crude Protein	72.12	72.58	71.98	71.38	70.04
Ether Extract	10.11	9.03	10.12	10.22	11.13
Crude Fibre	2.72	2.85	3.11	2.88	3.05
Total Ash	13.24	14.26	13.58	14.55	14.85
Nitrogen free extract	1.81	1.28	1.21	0.97	0.93
Amino acid composition (% dry basis)					
Alanine	3.62	3.54	3.65	3.58	3.51
Arginine	5.02	4.98	5.12	4.87	5.06
Aspartic acid	6.38	6.57	6.12	6.48	6.59
Cysteine	0.81	0.88	0.86	0.77	0.79
Glutamic acid	11.12	11.07	10.34	11.25	10.49
Glycine	5.44	5.25	5.16	5.31	5.26
Histidine	1.24	1.18	1.26	1.21	1.34
Isoleucine	2.58	2.41	2.65	2.54	2.87
Leucine	5.75	5.79	5.87	6.03	5.98
Lysine	4.44	4.29	4.31	4.57	4.39
Methionine	1.51	1.52	1.63	1.47	1.58
Phenylalanine	2.98	2.97	3.04	3.11	2.87
Proline	4.66	4.62	4.58	4.31	4.55
Serine	2.66	2.72	2.68	2.45	2.79
Threonine	2.78	2.59	2.63	2.51	2.6
Tyrosine	2.51	2.45	2.52	2.61	2.58
Valine	3.11	3.37	3.11	3.21	3.08
Tryptophan	0.55	0.57	0.61	0.54	0.58

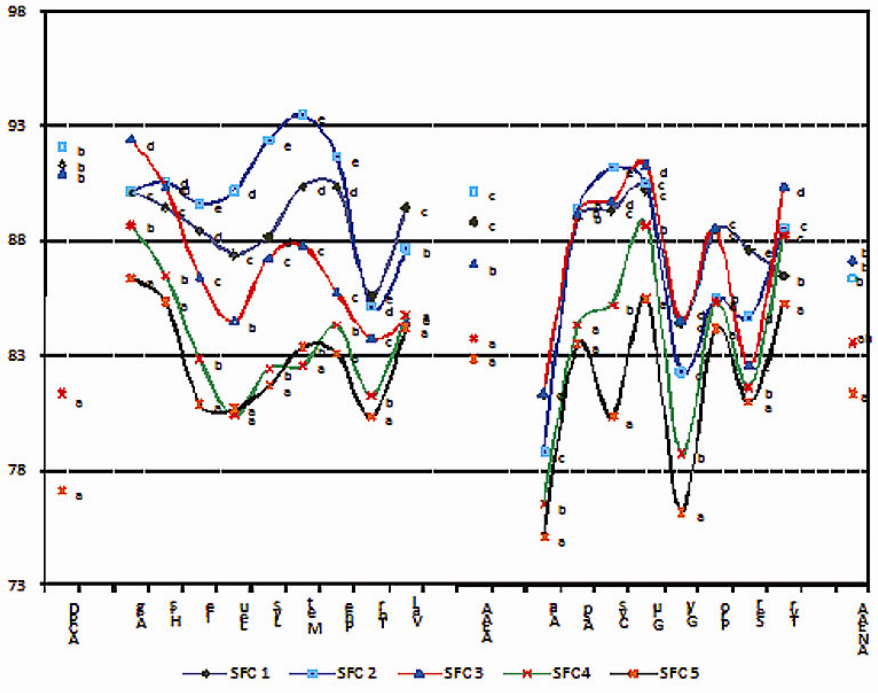

Fig. 1—Mean values bearing different superscripts differ significantly (P<0.05)

leucine (5.75-6.03) and the amino acids were also not different significantly (P>0.05) between the treatments.

The free amino acid (FAA) profiles of shrimp muscle were measured at 4 h of post-feeding is presented in Table 4. In all the diet groups the most abundant essential FAAs were arginine, leucine, lysine and valine and non-essential FAA were glycine, alanine and proline. Most of the non-essential FAAs in the shrimp muscle were not significantly different between the treatments, whereas the diet had significant (P<0.05) effect on the individual essential FAA concentrations. Significantly (P<0.05) lower levels of essential FAA were observed for isoleucine, leucine, lysine and valine in diets 3 to 5 fed animals.

The trials in net cages were conducted to test the replacement of fish meal with sunflower cake in natural condition. There was no significant difference in DGC, PER and APU in shrimp fed between control and 5% sunflower cake incorporated diet whereas significantly (P<0.05) lower protein assimilation efficiency interms of PER and APU was observed in shrimp fed with 10% sunflower cake compared to control and 5% sunflower incorporated diet. No significant differences were observed in survival (%) between the treatments (Table 2).

Discussion

Feedstuffs' containing at least 20% crude protein are considered to be protein supplements[39] and sunflower cake with 30% crude protein and was rich in methionine and arginine[21] has been evaluated in practical shrimp feeds by including at varying levels. For optimization of this cake inclusion, it was evaluated in *P. monodon* juveniles both in tanks and net cages. Similar type of tank and pond study was conducted to develop low cost feed for *P. monodon*[40]. At the conclusion of the 45 d growth trial, survival was good (>93%) and the growth of the shrimp was typical for shrimp offered a high quality practical diet under research conditions. The high survival during the feeding trial indicated the good health condition of the shrimp and confirmed the absence of any nutrient deficiency. Additionally, there were no indications of the feed being rejected even at 10% inclusion. In the present study with the increase of sunflower cake beyond 2.5% level there was decreased growth rate in tanks, increased FCR and reduced protein utilization efficiency in terms of PER and APU. Similar results of decreased performance with the incorporation of sunflower meal above these levels resulted in decreased performance in fish such as seabream 12%[23], tilapia 14%[24], 20%[25] and atlantic salmon 20%[26].

Decreased crude protein and amino acid digestibilities were observed in shrimp fed diets having sunflower cake above 2.5% (Fig. 1). This may due to the high dietary fibre levels, non-starch polysaccharides, cellulose and non-cellulose fibre fractions and lignin in sunflower cake compared to soybean cake[41]. Similar results of decreased digestibilities were observed with the increased fibre levels (Table 1) as in *P. japonicus*[42]; *P. monodon*[28]. Significantly (P< 0.05) higher amino acid digestibility values were observed for arginine, glutamic acid, and histidine in the present study. Significantly (P<0.05) lower amino acid digestibility values were observed for alanine, glycine and threonine. Higher AAAD values were earlier reported for arginine[43], lysine and histidine[44] and glutamic acid[27] in shrimp. Lysine is often one of the most limiting amino acid in ingredients used for production of commercial shrimp feeds, especially when fish meal is replaced by plant protein sources[45]. More than 10% lower digestibility of lysine was observed in diet 5 (81.65±0.66%) when compared to diet 2 (92.39±0.10%) in our study which may be one of the reason for low growth performance at higher sunflower cake inclusion. Lysine is a substrate for the synthesis of carnitine, which is required for the transport of long chain fatty acids from cytosol into mitochondria for oxidation[46]. Alanine was poorly digested (78.60±0.67%) compared to other amino acids regardless of diet. This low apparent digestibility may be explained by the secretion of the chitinous peritrophic membrane surrounding the faecal material[7]. Alanine is found at high levels in chitin and the secreted alanine would lower the apparent digestibility value. Alanine is also found in high concentrations in body fluids of crustaceans. This indicates that it may be a metabolic product which could be secreted into the faecal material. A similar relationship has been suggested for glycine (81.20±0.88%) in shrimp[7].

No significant (P>0.05) differences were observed in proximate and amino acid profiles of the shrimp fed with varying levels of sunflower cake. Glutamic acid, aspartic acid, leucine and arginine were the predominant amino acids in tiger shrimp, *P. monodon*. Similar amino acid profiles were reported in earlier studies with shrimp, *P. monodon*[47]; *Fenneropenaeus indicus*[48].

Apparent nutrient digestibility is a common index of quality of dietary ingredients comprising commercial production feeds for marine penaeid shrimp, it can only approximate overall bioavailability. But the overall retention efficiency depends on the availability of nutrients for protein synthesis at tissue level for growth. Direct determination of the extent of nutrient deposition is difficult and time-consuming, requiring the use of radioisotopic labeling techniques. Another means of characterizing nutrient availability to shrimp is change in tissue nutrient levels and has been previously used to evaluate post feeding time-course

Table 4—Effect of incorporation of sunflower cake on free amino acid (FAA) levels (μmol/g tissue) in shrimp muscle *Penaeus monodon* at 4 h post feeding in tanks

	Free amino acids (μmol/g tissue) in shrimp muscle at 4 h post fed				
AAs	1	2	3	4	5
Ala	34.33^{a}±3.87	36.75^{ab}±2.27	41.25^{abc}±2.74	45.39^{bc}±3.31	47.35^{c}±3.37
Arg	44.55^{a}±0.89	44.06^{a}±1.86	45.19^{a}±1.67	42.01^{a}±1.43	41.31^{a}±2.01
Asp	4.05^{a}±0.60	4.55^{ab}±0.28	4.62^{ab}±0.31	5.09^{ab}±0.47	5.77^{b}±0.50
Glu	14.25^{a}±0.72	14.58^{a}±0.80	15.67^{a}±1.00	16.43^{a}±0.84	14.88^{a}±0.66
Gly	72.23^{a}±2.48	77.26^{a}±3.13	70.34^{a}±2.63	67.85^{a}±2.29	68.74^{a}±3.84
His	3.43^{a}±0.47	3.52^{a}±0.22	3.32^{a}±0.24	3.32^{a}±0.28	2.64^{a}±0.21
Ile	4.98^{b}±0.73	5.28^{b}±0.28	4.26^{ab}±0.25	3.60^{a}±0.18	3.69^{a}±0.16
Leu	11.58^{b}±0.58	10.22^{b}±0.56	10.34^{b}±0.66	7.68^{a}±0.39	8.02^{a}±0.36
Lys	10.29^{b}±0.95	9.27^{b}±0.77	10.53^{b}±1.18	9.89^{b}±0.79	6.58^{a}±0.75
Met	4.43^{a}±0.65	4.51^{a}±0.28	4.53^{a}±0.30	4.58^{a}±0.42	4.43^{a}±0.39
Phe	2.51^{a}±0.37	2.66^{a}±0.17	2.77^{a}±0.18	2.44^{a}±0.22	2.33^{a}±0.20
Pro	13.37^{a}±1.97	12.63^{a}±0.78	14.58^{a}±0.97	12.59^{a}±1.15	13.42^{a}±1.17
Ser	5.48^{ab}±0.81	4.65^{a}±0.29	4.95^{ab}±0.33	6.32^{ab}±0.58	6.65^{b}±0.58
Thr	9.05^{a}±0.92	9.13^{a}±0.91	9.22^{a}±1.41	9.16^{a}±1.30	9.15^{a}±1.31
Tyr	2.24^{a}±0.11	2.15^{a}±0.12	3.13^{b}±0.20	3.14^{b}±0.16	3.21^{b}±0.14
Val	10.26^{c}±0.20	11.23^{c}±0.46	8.80^{b}±0.33	7.88^{b}±0.27	6.53^{a}±0.36

Mean values bearing different superscripts in a row differ significantly (P<0.05)

change in tissue FAA[36,49,50]. While reviewing the plant protein utilization in fish feeds it has been suggested[51] to study amino acid data along with changes in free amino acid profiles. The FAA profile of *P. monodon* of the present study (Table 4) is similar to that of *P. kerathurus*[52], *P. esculentus*[53] and *L. vannamei*[36,50]. The present study demonstrated that although there were some variations in the free pool concentration of individual amino acids, the total level of essential and non-essential amino acids in the shrimp muscle free pool remained stable in all diets groups. This suggests that intracellular amino acid pools are not determined by passive movements of amino acids, but rather are regulated by active trans-membrane transport[36]. Arginine, the most predominant essential FAA in the shrimp muscle was the most stable in all dietary treatments due to its prime metabolic role as a precursor to the important phosphagen phosphoarginine, in crustacean muscle[54,55]. Significantly ($P<0.05$) lower concentrations of isoleucine, leucine, lysine and valine in shrimp muscle fed diet 4 and 5 compared to other diets. Similar results of decreased essential free amino acids were also observed in *L. vannamei* fed with unbalanced diet[36]. Leucine, isoleucine plus valine account for 18–20% AA in plant and animal proteins. As an activator of the target of protein kinase, rapamycin, leucine is considered as a functional amino acid to stimulate muscle protein synthesis, inhibit proteolysis and regulates gene expression[56]. Reduced growth rates in shrimp fed with higher levels of sunflower cake (Table 2) may be due to significantly lower crude protein and amino acid digestibilities (Fig. 1) and also due to the deficiency or asynchronous availability of branched chain essential amino acids (Table 4). This could have led to decreased protein synthesis and increased protein turn over[36].

Studies evaluating the ability of shrimp to utilize diets with low levels of animal protein sources have been commonly carried out in clear water systems, where the length of the culture period is limited and environmental conditions greatly differ from those found in commercial ponds[57]. Although these studies have provided valuable information regarding shrimp capacity to utilize these ingredients under controlled conditions, the practical application of data from these studies is limited unless it is validated under natural conditions[57-60]. Pond experiments are expensive and are usually limited in number of treatments or replicates. To over-come this, net cages have been used as an acceptable compromise with replicates[61]. In this way, the experiments are executed in a realistic pond environment while keeping the testing on a small scale. In the present study, three diets with 0, 5 and 10% sunflower cake incorporated were tested in net cages with three replicates. The results indicate that shrimp can use up to 5% sunflower cake incorporated diets by replacing fish meal. There was a better performance in cages compared to experimental tanks (Table 2). Besides the nutritional component of the feed, part of the success of the replacement of animal proteins by plant protein sources is supported on shrimp capacity to utilize natural productivity. Different authors have indicated that the enhanced growth and performance of animals reared under natural culture conditions was due to their ability to obtain additional nutrients from natural food organisms present in natural brackishwater ecosystems[58,59,62,63] as *P. monodon* is one of the most omnivorous of shrimp species, and has been shown to consume plant and detrital material[64,65]. And also the improved digestibility enzyme profiles of shrimp reared in natural conditions especially cellulase and proteases and this in turn improved the nutrient digestibility of the feed and also improved growth rates[60,66].

Conclusion

Results from this study provide information regarding the potential utilization of plant protein sources like sunflower oil cake in place of fish meal both in tanks and in net cages by *P. monodon*. Apart from growth and digestibility studies, the changes in free amino acids at tissue level have indicated that higher levels of sunflower cake hampered protein synthesis and in turn growth rates. Based on this study, maximum inclusion level of sunflower cake could be 5% in tiger shrimp diets at 20% replacement of fish meal and the feed cost can be reduced by more than one rupee/kg.

Acknowledgements

Authors express their sincere thanks to ICAR funding under AP Cess Scheme and to Dr. A. G. Ponniah, Director, CIBA, Chennai for his encouragement and intellectual support to carry out this work.

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