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Silk cotton cake as an alternate protein source in the diet of tiger shrimp, *Penaeus monodon* Fabricius 1798, and its effects on growth, nitrogen utilisation and metabolism

V. RAJARAM, J. SYAMA DAYAL, K. AMBASANKAR AND S. AHAMAD ALI
Central Institute of Brackishwater Aquaculture, 75, Santhome High Road
Raja Annamalai Puram, Chennai - 600 028, Tamil Nadu, India
e mail: syamdayal@rediffmail.com

ABSTRACT

Silk cotton cake (SCC) was incorporated in the practical diets of tiger shrimp, *Penaeus monodon* at 5 different levels (0, 2.5, 5.0, 7.5 and 10.0%) by replacing fish meal. Growth cum digestibility study was conducted for 45 d in 500 l experimental tanks using shrimps weighing 2.09 ± 0.06 g with three replications for each treatment. There was significant ($p<0.05$) reduction in specific growth rate (SGR) and daily growth coefficient (DGC) in shrimps fed diets having more than 2.5% SCC compared to other treatments. Apparent crude protein, lysine and methionine digestibilities decreased ($p<0.05$) in shrimp fed with more than 2.5% SCC incorporation. The free amino acid profiles of shrimp muscle at 4 h post-feeding indicated significantly ($p<0.05$) lower levels for lysine and methionine in shrimp fed with higher levels of SCC. Post-prandial ammonia nitrogen excretion (PPANE) on hourly basis revealed that incorporation of SCC has greatly influenced the N excretion. SCC incorporation has not affected the haemolymph protein concentration up to 7.5% and the haemocyanin content up to 5% inclusion. The experimental results indicate that SCC can be incorporated up to 2.5% in the diet of black tiger shrimp *P. monodon*.

Keywords: Fishmeal replacement, Nitrogen metabolism. Plant protein sources, Shrimp nutrition, Silk cotton cake

Introduction

Commercial shrimp feed formulations generally include 25% fish meal and it is the primary and expensive protein ingredient. One important factor considered in reducing shrimp production costs and to increase producers' profitability is the use of feeds with low levels of fish meal and high levels of less expensive plant protein sources by optimising the feed formulations (Davis *et al.*, 2008). Gatlin *et al.* (2007) reviewed the use of sustainable plant products in aquafeeds and stressed on need for comprehensive nutritional studies on these feed resources. The availability of sound database on growth, digestibility and nutrient utilisation of soybean meal in practical marine shrimp feeds for both tank and pond reared conditions helped in optimisation of this ingredient in commercial shrimp feed formulations (Akiyama *et al.,* 1989, Lim and Dominy, 1990; Piedad- Pascual *et al.,* 1990; Akiyama, 1991; Lemos *et al.,* 2000; Amaya *et al.,* 2007). Efforts were made to develop similar database on nutrient utilisation for other plant protein sources in practical shrimp feed formulations like groundnut cake, coconut cake, and gingely cake in Indian white shrimp, *Fenneropenaeus indicus* (Ali, 1992); lupin kernel meal in tiger shrimp, *Penaeus monodon* (Fabricius) (Sudaryono *et al.,* 1995; Smith *et al.,* 2007); feed pea in *P. monodon* (Smith *et al.,* 1999; Bautista –Teruel *et al.,* 2003) and cotton seed meal in *P. monodon* (Lim, 1996). All these research efforts helped in 35% reduction in fishmeal usage in shrimp feed formulations during the period 1995-2007 (Tacon and Metian, 2008). More recently in our studies, sunflower cake (Dayal *et al.,* 2010) and palm kernel cake (Rajaram *et al.,* 2010) were incorporated in practical shrimp feeds to optimise their inclusion levels in practical feed formulations of *P. monodon*.

Kapok or silk cotton tree (*Ceiba pentandra*) is native to the American tropics, but has subsequently been naturalised throughout the tropics, including India. The silk cotton cake (SCC) contains about 25-30% crude protein and not much has been documented about its value as a source of protein in shrimp diets. However, it has been incorporated as a protein source for broiler rations (Kategile *et al.,* 1978; Siriwardene and Manamperi, 1979; Kadirvel *et al.,* 1986). SCC is rich in arginine which is one of the limiting amino acid in shrimp diets due to its high dietary requirement in *P. monodon* (Chen *et al.,* 1992; Millamena *et al.,* 1998).

The objective of the present study was to optimise the SCC incorporation in shrimp diets by conducting growth, digestibility and metabolism trials in juveniles of

P. monodon. The quantification of nitrogen (N) intake and ammonia N excretion were measured to study N metabolism with the incorporation of this ingredient. In addition, the changes in free amino acids (FAA) profile in shrimp muscle were estimated as a tool to understand the availability of precursors for protein synthesis.

Materials and methods

Experimental diets and diet preparation

Control diet used in this study was a typical practical shrimp feed with 25% fishmeal, having 40% crude protein (Table 1). Silk cotton cake was serially included at 0 (control), 2.5, 5, 7.5 and 10% in test feeds by replacing fishmeal at a substitution rate of 0, 10, 20, 30 and 40% in the practical shrimp feed formulation. Chromic oxide (0.5%) was added as an inert marker in all the test diets to measure the digestibility parameters in the shrimp diets used for tank study.

The dietary coarse ingredients were powdered in a micropulveriser and passed through 250 µm mesh. All the ingredients were weighed according to the percent ingredient composition as shown in Table 1 and prepared as described in Dayal *et al.* (2003). Briefly, the dry ingredients were mixed along with micronutrients like minerals and heat stable vitamins manually for 5 min and then transferred to a domestic mixer for another 10 min mixing. Fish oil and lecithin were gradually added to the homogenised mash. Water was then added (50 ml per 100 g mash) to the diet mix and hand kneaded into dough. The dough was steamed for 5 min at atmospheric pressure and pelleted in an experimental pelletiser with a 2 mm die. The pellets were dried in a forced-air oven at 60 ^{0}C for 12 h. The pellets were packed in plastic bags after cooling to room temperature and stored in desiccator until use. These pellets were fed as it is, as the initial size of the shrimps were around 2 g.

Growth, digestibility and metabolism trial

Post-larvae (PL15) of tiger shrimp, *P. monodon* were procured from the local private hatchery, Chennai. These PL were reared in hapas in the lagoon of Muttukadu Experimental Station of Central Institute of Brackishwater Aquaculture (CIBA), Chennai, to get juveniles for feeding trial. The juveniles were acclimated to wet laboratory conditions for 48 h in 1000 l fibre glass tanks. The experiment was conducted in the indoor Nutrition Wet Laboratory of CIBA, Chennai. Briefly, 225 juveniles with initial body weight of 2.09 ± 0.06 g (mean weight ± SE) were distributed randomly in15 oval fiberglass tanks (500 l; three tanks per diet, 15 shrimps per tank) in a completely randomised design. The shrimps were fed in slight excess 3 times daily (08:30, 12:30, and 18:00) for 45 days. Aeration was stopped during feeding times and while removing the uneaten feed particles and faecal strands. The uneaten feed pellets and particles were collected by siphoning from the tank after 2 h of feeding, which were dried and weighed for feed intake calculation. Faeces, remaining intact, were gently siphoned from the tanks to a bolting silk cloth after 2 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 ground for analysis. The shrimps were exposed to a natural photoperiod regime. The dissolved oxygen concentration in the water was measured by Winkler's method. Total ammonia was determined colorimetrically by the phenolhypochlorite method as per Solorzano (1969). During the experimental period, the water temperature, salinity, pH, dissolved oxygen and total ammonia-nitrogen ranged from 27-29 ^{0}C, 31-33 ppt, 7.5-7.8, 5.5-7.5 mg l^{-1} and <0.01 ppm, respectively.

At the end of the 45 day experiment, the shrimps were counted and weighed to determine survival, daily growth coefficient (DGC) and feed conversion ratio (FCR) subsequent to starving for 24 h. Growth rate was calculated and expressed as average as daily growth coefficient, DGC (% d^{-1}) (Cho, 1992; Bureau *et al.,* 2000). Both initial and final shrimp muscle samples were collected for proximate and amino acid analysis.

For measuring the metabolic parameters 30 shrimps (weighing 5.47±0.04 g) were maintained for 15 days in 1000 l FRP tank for each dietary treatment. These acclimatised intermoult stage animals were used for measuring the ammonia nitrogen excretion, muscle free amino acids and haemolymph parameters. At 4 h post-feeding, haemolymph was collected to measure total protein and haemocyanin, and muscle for free amino acid profiles. The post-prandial ammonia nitrogen excretion (PPANE) was measured (Nelson and Kropp, 1985) on hourly basis for five hours.

The following equations were used for calculating specific growth rate (SGR), daily growth coefficient (DGC), feed conversion ratio (FCR), apparent protein utilisation (APU), protein efficiency ratio (PER) and apparent digestibility coefficient (ADC):

$$SGR = \frac{\text{In (Final Wt) - In (Initial Wt)}}{\text{No. of days}}$$

$$DGC = 100 \times \frac{\text{(Final Wt}^{1/3} - \text{Initial wt}^{1/3})}{\text{No. of days}}$$

$$FCR = \frac{\text{Wet weight gain (g)}}{\text{Feed intake (g)}}$$

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

$$PER = \frac{\text{Weight gain (g)}}{\text{CP intake (g)}}$$

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

Laboratory analyses

The proximate composition of diets, faeces and experimental animals were analysed following standard AOAC (1998) methods. Chromium content in the diets and faeces was analysed to calculate the crude protein and amino acid digestibility (Furukawa and Tsukahara, 1966). The water stability of pellets was determined by as described by Cruz-Suárez *et al.* (2001) at 2 h to arrive at the dry matter and protein intake to measure FCR and PER. The total amino acids of feeds, faeces and shrimp were analysed after sealed tube hydrolysis with 6N HCl for 22 h at 110 ^{0}C (Spackman *et al.,* 1958; Finlayson, 1964). After hydrolysis, the acid was evaporated in vacuum oven and the sample was kept in NaOH desiccator to remove traces of acid. The residue is brought in to 1 ml of sodium citrate-perchloric acid sample diluent (pH 2.20) and filtered through 0.2 µm membrane filter. The free amino acid pool in the tail muscle was measured using the method outlined by Mente *et al.* (2002). 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 per min. The amino acids were detected and quantified using a fluorescent detector (FLD-6A) after post-column derivitisation with O-pthaladehyde. Amino acid standard solution (Sigma-Aldrich Inc., USA) for fluorescent detection was used as external standard. Tryptophan being labile to the acid hydrolysis, this was measured by alkali hydrolysis following Spectrophotometric method (Sastry and Tammuru, 1985) at 500 nm.

Ammonia was estimated as per Strickland and Parsons (1972) method. Haemocyanin concentration was calculated using an extinction coefficient (Nickerson and Van Holde, 1971). The concentration of haemolymph protein was determined by Lowry's method (Lowry *et al.,* 1951) using bovine serum albumin as a standard. Haemolymph ammonia was estimated using Sigma kit (Tulli *et al.,* 2007).

Statistical analyses

The feeding trials were conducted under completely randomised experimental design. Statistical analysis was carried out using SPSS -17 version of statistical software package for one way ANOVA after transforming the percent values to arcsine. The significance levels of treatments were compared using Duncan's multiple range test.

Results

Silk cotton cake contains 29.29% crude protein and was rich in arginine 3.04% (Table 1). The proximate and amino acid composition of the test diets are presented in Table 1. With the increase of silk cotton 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 feeds was calculated based on the essential amino acid requirements of tiger shrimp, *P. monodon* and it ranged between 0.92-0.91. To calculate the correct nutrient intake, the hydro-stability of test diets was measured at 2 h.The leaching loss of dry matter and protein increased from 8.85 and 7.73% in diet 1 to 10.98 to 10.43%, in diet 5, respectively. The growth response and feed utilisation efficiency are shown in Table 2. Shrimp fed diets containing 0 and 2.5% SCC exhibited similar growth patterns throughout the experimental period. The growth pattern expressed as DGC (% d^{-1}) of shrimp fed diets 1 and 2 are significantly ($p<0.05$) higher than those of shrimp fed diets 3, 4 and 5. Survival rates in all test diets were above 88% and it was not significantly different among treatments. Feed utilisation efficiency in terms of FCR was not significantly different up to 2.5% level of inclusion and the apparent protein utilisation (APU) and protein efficiency ratio (PER) decreased significantly with increasing level of SCC inclusion (Table 2). The SCC incorporation significantly ($p<0.05$) influenced the amino acid digestibility in shrimp (Table 2). Digestibility of all the amino acids were significantly ($p<0.05$) lower in diets 5 and 4.

Whole body composition of shrimp expressed on dry matter basis is presented in Table 3. The crude protein, ether extract and total ash % of shrimp fed test diets were non-significant. Similar pattern of whole shrimp amino acid profiles were recorded in all treatments.

The PPANE on hourly basis revealed that incorporation of SCC has greatly influenced the N excretion. The peak PPANE was noticed during 3 h irrespective of the test diets, but SCC4 and SCC5 showed significantly high ($p>0.05$) PPANE during 3rd and 4th h (Fig. 1). N excreted per unit of N intake, shows that SCC 4 and SCC 5 were significantly higher ($p>0.05$) compared to control (Fig. 2). Haemolymph protein and haemocyanin were significantly ($p<0.05$) lower at 10% level of incorporation (112.34 ± 5.94; 87.64 ± 5.32 mg ml^{-1}, respectively) compared to 2.5% inclusion (131.85 ± 4.26;

Table 1. Percent ingredient composition, proximate and amino acid composition of silk cotton cake (SCC) and test diets (% fed)

		Diets				
	SCC	SCC1	SCC 2	SCC 3	SCC 4	SCC 5
Ingredient composition						
Fish meal		25	22.5	20	17.5	15
Silk cotton cake (SCC)		0	2.5	5	7.5	10
Common ingredients*		75	75	75	75	75
Proximate composition						
Moisture	6.85	7.12	8.04	8.55	7.62	9.18
Crude protein	29.29	40.02	39.32	38.53	37.74	36.95
Ether extract	5.11	7.71	7.39	7.31	7.24	7.16
Crude fibre	22.73	3.47	3.95	4.51	5.06	5.62
Total ash	6.83	13.26	13.00	12.70	12.39	12.08
Nitrogen free extract	29.19	28.42	28.30	28.40	29.95	29.01
Amino acid composition						
Alanine	1.21	1.91	1.91	1.88	1.86	1.85
Arginine	3.04	2.62	2.63	2.66	2.65	2.67
Aspartic acid	2.22	4.39	4.33	4.29	4.26	4.15
Cysteine	0.51	0.65	0.65	0.67	0.66	0.64
Glutamic acid	6.02	8.21	8.16	8.11	8.06	8.01
Glycine	1.26	2.45	2.39	2.34	2.28	2.23
Histidine	0.49	0.93	0.92	0.91	0.90	0.90
Isoleucine	0.93	1.49	1.48	1.44	1.45	1.43
Leucine	1.75	3.13	3.09	3.06	3.02	3.04
Lysine	1.19	2.52	2.49	2.45	2.42	2.41
Methionine	0.34	1.04	1.02	1.01	0.99	0.97
Phenylalanine	1.33	1.62	1.61	1.61	1.60	1.60
Proline	1.15	2.63	2.59	2.56	2.52	2.48
Serine	1.55	1.54	1.55	1.56	1.54	1.53
Threonine	0.79	1.31	1.30	1.28	1.27	1.26
Tyrosine	0.65	1.01	1.00	0.99	0.98	0.99
Valine	1.42	1.69	1.68	1.68	1.67	1.66
Tryptophan	0.20	0.29	0.29	0.31	0.30	0.33
£EAAI	0.80	0.92	0.92	0.92	0.92	0.91

*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

#Vitamin and mineral mix (Sudaryono *et al.*, 1995): vitamin mix (mg kg^{-1} diet) (vitamin A, 10 000 IU; vitamin D, 1500 IU; vitamin E, 60; vitamin K, 1.5; niacin, 200; riboflavin, 37.5; calcium pantothenate, 125; vitamin B12, 20; Thiamine, 15; pyridoxine, 15; folic acid, 5.5; biotin, 750; choline chloride, 100; stable Vitamin C 1000) and mineral mix (mg kg^{-1} diet) (cobalt, 0.2; iodine, 0.6; copper, 5.0; iron, 14.0; manganese, 55.0; zinc, 24.0) and 1% dicalcium phosphate.

£EAAI: Essential Amino Acid Index: Calculated based on shrimp amino acid requirements (% Protein) and aa/AA ratios are set at 0.01 minimum and 1 maximum (Penaflorida, 1989).

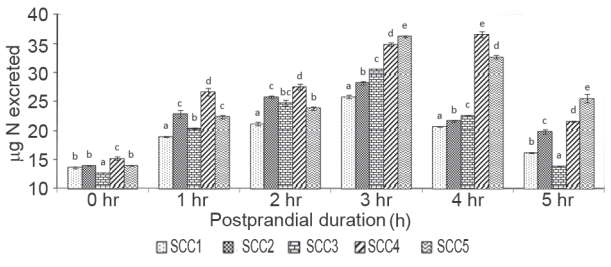

Fig. 1. Post-prandial N excretion pattern of *P. monodon* fed with SCC incorporated diets

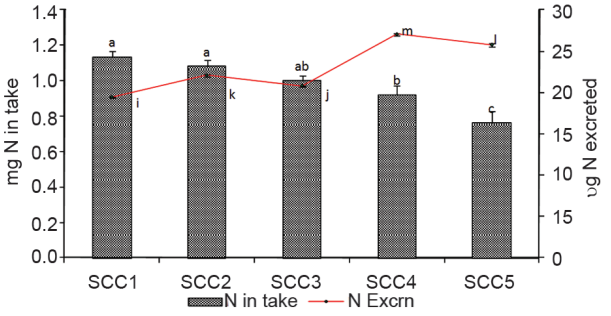

Fig. 2. N intake *vs* N excretion in *P. monodon* fed with SCC incorporated diets

Table 2. Effect of incorporation of SCC on SGR, DGC, FCR, survival and digestibility in tiger shrimp, *P. monodon* in 45 d feeding trial in tanks

Parameters	Diets				
	SCC1	SCC 2	SCC 3	SCC 4	SCC 5
SGR	$2.32^{d}\pm0.02$	$2.26^{d}\pm0.02$	$2.05^{c}\pm0.01$	$1.73^{b}\pm0.01$	$1.44^{a}\pm0.03$
DGC (% d^{-1})	$1.17^{d}\pm0.03$	$1.15^{d}\pm0.01$	$1.04^{c}\pm0.01$	$0.84^{b}\pm0.02$	$0.68^{a}\pm0.02$
Survival (%)	$93.33^{a}\pm3.85$	$88.89^{a}\pm2.22$	$88.89^{a}\pm2.22$	$91.11^{a}\pm2.22$	$91.11^{a}\pm4.45$
FCR	$1.91^{a}\pm0.11$	$2.02^{ab}\pm0.03$	$2.13^{bc}\pm0.02$	$2.22^{cd}\pm0.02$	$2.37^{d}\pm0.06$
PER	$1.42^{b}\pm0.08$	$1.36^{ab}\pm0.02$	$1.33^{ab}\pm0.01$	$1.31^{ab}\pm0.01$	$1.25^{a}\pm0.03$
APU (%)	$29.29^{b}\pm1.63$	$26.85^{ab}\pm0.40$	$26.99^{ab}\pm0.26$	$27.49^{ab}\pm0.22$	$25.40^{a}\pm0.67$
Digestibility (%)					
ACPD	$83.20^{c}\pm0.62$	$84.01^{c}\pm3.09$	$74.23^{b}\pm1.94$	$72.31^{b}\pm0.99$	$65.79^{a}\pm0.41$
AAD (%)					
Ala	$84.37^{c}\pm0.21$	$82.46^{d}\pm0.31$	$79.84^{c}\pm0.19$	$73.49^{b}\pm0.40$	$69.75^{a}\pm0.15$
Arg	$91.64^{d}\pm0.15$	$84.62^{c}\pm0.15$	$81.32^{b}\pm0.08$	$80.31^{a}\pm0.42$	$80.14^{a}\pm0.40$
Asp	$89.52^{c}\pm0.29$	$87.63^{d}\pm0.40$	$84.24^{c}\pm0.17$	$80.37^{b}\pm0.09$	$78.45^{a}\pm0.25$
Cys	$91.05^{d}\pm0.38$	$90.37^{d}\pm0.21$	$85.74^{c}\pm0.16$	$81.75^{b}\pm0.24$	$79.75^{a}\pm0.21$
Glu	$90.42^{d}\pm0.09$	$89.65^{c}\pm0.32$	$88.91^{c}\pm0.28$	$85.46^{b}\pm0.22$	$80.44^{a}\pm0.21$
Gly	$81.59^{d}\pm0.26$	$82.41^{e}\pm0.17$	$79.43^{c}\pm0.36$	$75.41^{b}\pm0.21$	$70.07^{a}\pm0.22$
His	$86.36^{d}\pm0.08$	$87.06^{d}\pm0.09$	$84.37^{c}\pm0.31$	$80.79^{b}\pm0.43$	$78.96^{a}\pm0.28$
Ile	$88.97^{d}\pm0.17$	$88.12^{c}\pm0.25$	$84.36^{b}\pm0.17$	$80.37^{a}\pm0.27$	$80.10^{a}\pm0.26$
Leu	$91.32^{e}\pm0.15$	$90.24^{d}\pm0.20$	$84.35^{c}\pm0.17$	$80.37^{b}\pm0.15$	$78.98^{a}\pm0.21$
Lys	$92.34^{d}\pm0.27$	$88.34^{c}\pm0.26$	$86.47^{b}\pm0.20$	$85.67^{b}\pm0.38$	$82.34^{a}\pm0.26$
Met	$90.32^{d}\pm0.32$	$91.31^{e}\pm0.36$	$87.23^{c}\pm0.31$	$82.34^{b}\pm0.24$	$80.34^{a}\pm0.22$
Phe	$86.37^{d}\pm0.09$	$85.93^{d}\pm0.09$	$84.37^{c}\pm0.16$	$82.39^{b}\pm0.33$	$78.23^{a}\pm0.22$
Pro	$84.96^{d}\pm0.22$	$85.29^{d}\pm0.25$	$82.47^{c}\pm0.25$	$80.74^{b}\pm0.15$	$74.28^{a}\pm0.28$
Ser	$83.29^{d}\pm0.34$	$82.94^{d}\pm0.33$	$80.27^{c}\pm0.25$	$76.84^{b}\pm0.31$	$72.87^{a}\pm0.15$
Thr	$88.94^{c}\pm0.32$	$89.67^{d}\pm0.18$	$84.33^{b}\pm0.08$	$84.10^{b}\pm0.25$	$78.69^{a}\pm0.20$
Tyr	$85.13^{c}\pm0.17$	$82.97^{d}\pm0.17$	$80.18^{c}\pm0.17$	$78.67^{b}\pm0.08$	$73.55^{a}\pm0.25$
Val	$89.97^{e}\pm0.16$	$84.67^{d}\pm0.08$	$80.32^{c}\pm0.33$	$78.01^{b}\pm0.17$	$77.09^{a}\pm0.32$

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

122.65 ± 4.40 mg ml^{-1}, respectively). Ammonia levels in haemolymph were higher in diet 5 fed shrimp compared to diet 2 fed shrimp (2.96 ± 0.15 and 2.21 ± 0.14 mg l^{-1}, respectively) (Fig. 3).

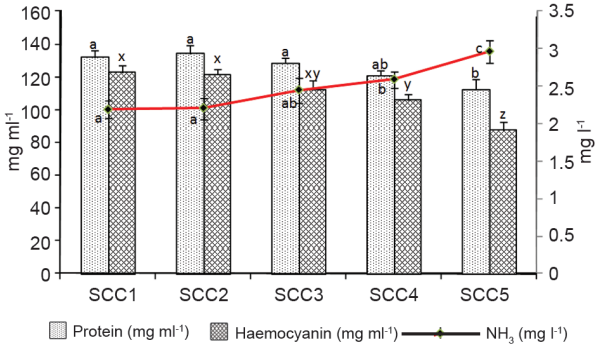

Fig. 3. Effect of SCC inclusion on haemolymph protein, haemocyanin and ammonia in *P. monodon*

The FAA profiles of shrimp muscle were measured at 4 h post-feeding. In all the diet groups, the most abundant essential FAAs in the tail muscle were arginine, leucine, lysine, threonine and valine. Glycine, alanine, glutamate and proline were the predominant non-essential FAAs irrespective of the diet in shrimp muscle at 4 h post-feeding. Significantly ($p<0.05$) lower levels of essential FAA *viz*., isoleucine, leucine, lysine, methionine and valine were recorded in shrimps fed diets 3, 4 and 5 (Table 4).

Discussion

Feedstuffs containing at least 20% crude protein are considered to be protein supplements (Tacon and Barg, 1998). Kapok seed meal with 29.29% crude protein and EAAI of 0.8, seems to be useful ingredient (Table 1). Mente *et al.* (2002) reported that WA diet/ingredient can be assumed to be of good quality when the EAAI is 0.90 or greater, to be useful when it is approximately 0.80, and to be inadequate when it is below 0.70. On termination of the 45 d growth trial, survival was good (>88%) and the growth of the shrimps was typical for shrimp offered a high quality practical diet under research conditions. Additionally, there were no indications of the feed being rejected even at 10% inclusion. A range of approaches have

Table 3. Proximate composition (% dry matter basis) of whole shrimp, *P. monodon* fed with varying levels of SCC

Proximate composition	Diets				
	SCC1	SCC 2	SCC 3	SCC 4	SCC 5
Crude protein	72.31	70.89	71.51	72.17	71.74
Ether extract	10.01	9.64	9.78	9.25	8.94
Crude fibre	2.94	2.34	2.78	2.11	2.57
Total ash	13.54	14.98	14.25	14.79	14.81
Nitrogen free extract	1.2	2.15	1.68	1.68	1.94

Table 4. Effect of incorporation of SCC on free amino acid (μmol g tissue $^{-1}$) in *P. monodon* at 4 h post-feeding in tanks

FAA	SCC1	SCC 2	SCC 3	SCC 4	SCC 5
Ala	31.48^{ab}±0.43	32.43^{b}±0.46	30.17^{a}±0.57	32.51^{b}±0.16	30.58^{a}±0.27
Arg	44.22^{b}±3.47	44.77^{b}±0.04	42.12^{b}±0.57	40.75^{b}±0.01	33.94^{a}±2.27
Asp	4.22^{a}±0.09	4.41^{a}±0.14	4.31^{a}±0.06	4.53^{a}±0.01	4.99^{a}±0.13
Glu	16.00^{b}±0.32	16.31^{b}±0.12	14.53^{a}±0.13	14.04^{a}±0.05	14.27^{a}±0.37
Gly	77.93^{b}±1.39	77.59^{a}±0.06	83.31^{bc}±1.55	84.63^{c}±2.88	86.39^{c}±0.60
His	3.00^{a}±0.09	3.42^{b}±0.12	3.06^{a}±0.08	3.09^{a}±0.01	2.98^{a}±0.04
Ile	4.86^{b}±0.87	4.96^{b}±0.10	4.31^{a}±0.05	4.18^{a}±0.04	4.29^{a}±0.20
Leu	13.86^{ab}±0.03	14.36^{b}±0.24	14.28^{b}±0.29	12.61^{ab}±1.04	11.74^{a}±1.14
Lys	7.31^{c}±0.70	7.77^{c}±0.05	6.82^{bc}±0.19	5.79^{ab}±0.08	5.32^{a}±0.56
Met	4.48^{bc}±0.73	4.77^{d}±0.09	3.91^{bc}±0.05	3.34^{ab}±0.04	2.49^{a}±0.53
Phe	7.31^{c}±0.70	2.33^{a}±0.04	2.29^{a}±0.03	2.22^{a}±0.01	5.32^{b}±0.57
Pro	13.94^{a}±0.14	13.42^{a}±0.54	14.26^{a}±0.40	14.15^{a}±0.01	14.36^{a}±0.22
Ser	5.75^{b}±0.07	5.92^{b}±0.06	5.33^{a}±0.07	5.83^{b}±0.08	5.63^{b}±0.15
Thr	8.93^{abc}±0.07	9.03^{bc}±0.04	9.36^{c}±0.29	8.84^{ab}±0.14	8.47^{a}±0.07
Tyr	2.33^{b}±0.04	2.33^{b}±0.06	2.42^{b}±0.05	2.36^{b}±0.01	2.03^{a}±0.02
Val	9.79^{b}±0.14	9.90^{b}±0.14	9.15^{a}±0.17	9.06^{a}±0.04	8.86^{a}±0.08

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

been used to describe growth rate in reports of *P. Monodon.* Growth data expressed in the present study in terms of DGC (% d^{-1}) is the most appropriate for a very large part of growth curves of *P. monodon, Litopenaeus vannamei* and *P. stylirostris* (Cho, 1992; Bureau *et al.,* 2000; Smith *et al.,* 2007) along with SGR in grow-out studies. In the present study, as the level of silk cotton cake increased beyond 2.5% there was a decreased performance in growth parameters in terms of SGR and DGC (Table 2). Similar results of decreased performance with the incorporation of plant protein sources were reported in *L. vannamei* (Lim and Dominy, 1990), in *P. monodon* (Bautista-Teruel *et al.,* 2003; Dayal *et al.,* 2010; Rajaram *et al.,* 2010), in *Litopenaeus schmitti* (Alvarez *et al.,* 2007) and in *L. vannamei* (Huai *et al.,* 2009). Kategile *et al.* (1978; 1986) noticed growth depression in broilers at higher levels of incorporation and this growth depression was attributed the cyclopropenoid fatty acids of the SCC (Narahari and Rajini, 2003).

Mente *et al.* (2002) and Sorensen *et al.* (2002) suggested the need for determination of amino acid digestibility along with EAAI value in order to optimise the inclusion of SCC in practical feeds. As the level of silk cotton cake increased beyond 2.5%, there was a reduction trend in amino acid digestibility (Table 2). Similar results of decreased digestibilities were observed with the increased fibre levels in *L. vannamei* (Akiyama *et al.,* 1989); in *P. japonicus* (Koshio *et al.,* 1993); in *P. setiferus* (Brunson *et al.,* 1997) and in *P. monodon* (Dayal *et al.,* 2003, 2010). More than 10% reduction in digestibility of methionine was observed in diet 5 (80.34±0.22%) when compared to diet 2 (91.31±0.36%) in our study, which would have resulted in lower growth performances at higher silk cotton cake incorporation.

No significant differences were observed in proximate profiles of the shrimp fed with varying levels of silk cotton cake (Table 3). The individual essential amino acid/total amino ratios of experimental diets and shrimp indicated no significant differences in the amino acid ratios. Glutamic acid, aspartic acid, leucine and arginine were the predominant amino acids in *P. monodon*. Similar amino acid profiles were reported in earlier studies with

P. monodon (Sarac *et al.,* 1994; Dayal *et al.,* 2010; Rajaram *et al.,* 2010); *Marsopenaeus japonicus* (Alam *et al.,* 2004); *F. indicus* (Dayal *et al.,* 2005).

PPANE estimation is a valuable technique to assess the substrate under catabolism. Schmitt and Santos (1998) reported that ammonia-N effluxes of shrimp reveals that an increased deamination of amino acids caused by food ingestion. PPANE is greatly influenced by the nutritional state of the animal, physiological condition and the environmental factors. Needham (1957) was first to study the factors affecting ammonia excretion in the crab *Carcinus maenas* and found that the quantity of ammonia excreted was affected by temperature, salinity, diet, injury and moulting. In the present study, except nutritional state all other parameters were kept in control. So, the variation in PPANE can be solely attributed to the effects of incorporation of SCC. Irrespective of all test diets, peak PPANE was recorded at 3rd h post feeding (Fig. 1). The post-feeding period in crustaceans was normally characterised by an enhanced ammonia-N excretion rate (Regnault, 1987). N excreted per unit of N intake shows the quality of the protein being consumed in terms of digestibility and assimilation efficiency. In the present study, SCC4 and SCC5 shows very high N excretion per unit of N intake when compared to control and 2.5% SCC feeds (Fig. 2). This may be due to the increased protein turnover resulting in higher ammonia excretion. The protein quality of diet in terms of chemical composition as well as both protein and amino acid digestibilities have influenced ammonia efflux rates in the present study. Similarly protein quality has significantly influenced the ammonia efflux rates in *P. japonicus* (Chen and Chen, 1997), *M. lar* (Nelson and Kropp, 1985) and *P. esculentus* (Hewitt and Irving, 1990).

Similarly, presence of higher haemolymph protein and haemocyanin in control and 2.5% SCC fed shrimps indicate their higher nutritional status (Fig. 3). Haemocyanin is the main protein in the haemolymph and is implied in several functions like oxygen transport, enzymatic activities, osmoregulation or buffering (Paul and Pirow, 1997). Haemolymph haemocyanin and protein levels are affected by nutritional state in *H. gammarus* (Hagerman, 1983), *C. maenas* (Busselen, 1970) and common shrimp *C. crangon* (Djangmah, 1970). In a previous research with. *L. vannamei,* it was observed that haemocyanin changes with the nutritional status of shrimp showing high or low values according to direct changes in dietary protein levels (Rosas *et al.,* 2001; 2002).

Apparent nutrient digestibility is a common index of quality of dietary ingredients and 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 labelling techniques. Another means of characterising nutrient availability to shrimp is change in tissue nutrient levels and has been previously used to evaluate post-feeding time-course change in tissue free amino acids (FAA) in shrimp (Deshimaru, 1976a, 1976b, Mente *et al.,* 2002; Fox *et al.,* 2009). The concentration of FAA in most crustaceans is higher than that in vertebrate tissues (Claybrook, 1983), possibly for osmoregulatory reasons (Awapara, 1962; Claybrook, 1983) and are precursors for protein synthesis and substrates for oxidation. The present study demonstrated that although there was some variation in the free pool concentration of individual amino acids, the total level of essential and non-essential amino acids in the tail-muscle free pool remained stable in all diet 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 (Mente *et al.,* 2002). Arginine, the most predominant essential free amino acid in the shrimp muscle is most stable in all the dietary treatments due to its prime metabolic role as a precursor to the important phosphagen phosphoarginine, in crustacean muscle (Beis and Newsholme, 1975; Hird *et al.,* 1986).

Significantly ($p<0.05$) lower concentrations of isoleucine, leucine, lysine and valine in shrimp muscle fed diet 4 and 5 were recorded, compared to other diets. Leucine, isoleucine plus valine account for 18–20% AA in plant and animal proteins. As an activator of the target of rapamycin (a protein kinase), leucine is considered as a functional AA to stimulate muscle protein synthesis, inhibit proteolysis and regulates gene expression (Nakashima *et al.,* 2007). Similar trend of decreased essential FAA profiles were reported in shrimp fed with feeds having more than 5% sunflower cake (Dayal *et al.,* 2010) and 3% palm kernel cake (Rajaram *et al.,* 2010) in *P. monodon.* The decreased amino acid digestibility on one hand and increased protein turnover on the other hand would have resulted in decreased essential free amino acids required for protein synthesis and this in-turn would have resulted in lower growth rates in our study. Natural stable isotope study with different combinations of fish meal and soy protein isolates revealed that there was a biased nutritional contribution from fish meal (Gamboa-Delgado and Le Vay, 2009) in *L. vannamei.*

The present study concludes that silk cotton cake can be included at the maximum level of 2.5% in the diets of black tiger shrimp (*P. monodon*) without compromising growth and FCR. Biochemical findings also emphasised that beyond 2.5% inclusion levels, there was a change in the individual free amino acids at tissue level which hinders

protein synthesis and in turn growth rates. More over, increased catabolism of dietary amino acids lead to high N excretion per unit of N intake. However, there is a need for quantification of anti-nutritional factors (ANFs) and the methods of amelioration of these ANFs present in non-conventional feed resources such as silk cotton cake, to improve their inclusion and nutrient utilisation in aquafeeds.

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