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Cashew nut *Anacardium occidentale* L. byproducts as an alternative protein source for post-larvae of *Macrobrachium rosenbergii* (de Man, 1879)

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Abstract

The potential of cashew nut, *Anacardium occidentale* L., by-products as partial fish meal alternative in diets for post-larvae of *Macrobrachium rosenbergii* was evaluated. The Cashew nut byproducts - testa was characterized with respect to proximate, mineral, and energy profile. The crude protein, crude fiber, fat and ash contents were, in g kg⁻¹ 190.0, 103.0, 20.1, and 20.2, respectively, with metabolizable energy of 7.12 MJ kg⁻¹. The experiment was conducted in triplicate for the period of 60 days. In a feeding trial, diets containing cashew nut testa at the rate 0, 1.52, 3.04, 6.08 and 12.16% (designated as control, T₁, T₂, T₃, and T₄) of fish meal protein replacement were tested for growth parameters and survival for the post-larvae of *M. rosenbergii*. The length gain was greater (73.97%) with T₂ diet and lower (43.48%) in post-larvae fed with T₄ diet. The superior growth performance of weight gain (384.76%) and highest specific growth rate (2.62%) was observed in post-larvae of *M. rosenbergii* fed with T₂ diet. The maximum survival was 59.17% with T₂ diet while post-larvae fed with T₁ diet showed the minimum survival of 44.17%. Among all the test diets, post-larvae fed with 3.04% cashew testa powder incorporated diet showed significantly higher weight gain, specific growth rate and survival. It can be concluded that, 3.04% of cashew testa could be incorporated in the diet of the post-larvae of *M. rosenbergii* against the fishmeal to achieve better weight gain and specific growth. But to achieve better survival percentage of the post-larvae of *M. rosenbergii* future investigations needs to be focus on the anti-nutritive factors of the cashew testa.

Keywords: Cashew testa, fish meal, *M. rosenbergii*, post-larvae, weight gain

1. Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii*, popularly known as 'scampi' has been recognized as one of the potential aquaculture candidate species for freshwater bodies of different parts of Indo-pacific region. Scampi farming has gained momentum after the set-back in shrimp farming due to disease outbreaks. *M. rosenbergii* has gained importance in freshwater farming because of its faster growth under controlled conditions, greater disease resistance, omnivorous feeding habit, efficient feed conversion and good demand in both domestic and export market.

Feed is the most expensive cost item in prawn farming system. It constitutes 40-60% of operational cost in prawn production (Mitra *et al.* 2005) [18]. The nutritional value of fish meal is high. So the best quality fish meal incorporated feeds are very expensive (Mendoza *et al.* 2001) [17]. Many researches, therefore, attempted to search alternative protein sources as replacement to fish meal to lower the cost of production as well as to overcome the problems of fish meal in aquaculture feed (AQUACOP, 1976; Belsare, 2004) [2, 4]. Plant protein concentrates are the potential ingredients to replace fish meal in aquafeeds than plant meals. Canola, soy, pea, barley, rice protein concentrates, along with wheat gluten meal, have all been tested as fishmeal replacements with varying degrees of success (Forster *et al.*, 1999; Thiesses *et al.* 2003; Barrows *et al.*, 2007; Lim *et al.*, 2008; Gaylord and Barrows, 2009) [9, 24, 5, 16, 10].

New (1976) [20] has stressed for the utilization of regionally available ingredients for development of aquaculture feed. It is desirable to formulate feed using regionally available feed ingredients to ensure cost-effective aquaculture. In view of this, present study was aimed to utilize locally (Konkan region of Maharashtra) available cashew nut byproducts as replacement to fish meal in the diet of post-larvae of *M. rosenbergii*. However, the nutritional quality of cashew nut byproduct is below that of fish meal and depends largely on their level of proteins and antinutritional factors.

In Maharashtra state, the production and productivity of cashew nut is highest in the country, as of high yielding varieties and also cultivators are adopting better management practices. Maharashtra topped cashew production with 1,83,000 MT with productivity of 1300 kg/ha (Patil and Sapatarshi, 2010) [21]. The Konkan belt, western coastal region of Maharashtra in India is renowned for its cashew, *Anacardium occidentale L.* nut production. About 1,43,000 ha area is under cultivation, out of that 62, 317 ha area is in Ratnagiri district. The average cashew production is about 1.5 tonnes per ha in Ratnagiri district (Department of Agriculture, Maharashtra, 2007).

Cashew kernel is known for its delicious, pleasant taste and for balanced nutritive profile. The by-products like cashew nut shell liquid (CNSL) which is a valuable raw material for preparation of oil paints, varnishes, water proofing agents, adhesive ingredients, pigments of gums, typewriter rolls, automobile break lining and lubricants in aircraft (Patil and Sapatarshi, 2010) [21]. During cashew processing, the cashew nut shell and cashew testa are the byproducts. Along with cashew testa, rotten cashew nut and small pieces of cashew nut are also discarded on large scale, which contains 11.25% protein and 3.2% of fat.

The cashew nut byproducts have been attempted by many researchers for manufacturing various products. By considering nutritive value of cashew byproducts, in present study an attempt has been made to determine the suitability of cashew testa powder as alternative protein source to the fish meal in the diet of post-larvae of *M. rosenbergii*.

2. Materials and Methods

2.1 Test animal

Post-larvae of *M. rosenbergii* were obtained from the freshwater prawn hatchery of Marine Biological Research Station, Ratnagiri, Maharashtra (India). They were maintained in plastic pool of 500-L capacity and were acclimatized for one week in the laboratory condition. The feed was given at the rate of 10% of body weight thrice a day with control diet (T₀). The 25% water was replaced with freshwater every day. Faeces and unutilized feed were removed from the plastic pool by daily siphoning. Aeration was provided throughout the acclimatization process to avoid stress.

2.2 Diets

2.2.1 Control diet

Feed ingredients such as fish meal, wheat flour, rice bran and groundnut oil cake were used for preparation of experimental diet containing 35% crude protein which was referred as control diet (Balaze & Ross, 1976) [3]. The ingredients proportion was estimated followed by Pearson square method (De Silva & Trevor, 1995) [6].

All the ingredients were completely dried, grounded and sieved through 100 mesh sieve to obtain the uniform size of ingredients. They were weighed according to the composition of diet and mixed together by using domestic mixer. After mixing, 40 mL/kg water was added to form dough. The dough was cooked in domestic pressure cooker for 20 minutes. After cooling, it extruded through hand pelletizer to form pellets. These pellets were sun dried and packed in air tight plastic container till the experiment continued. The feed was prepared five days before of start of actual experiment. While preparing the experimental diet on each instance, care was taken to maintain the uniformity of nutrient composition. During experiment, feed pellets were broken into smaller size

before feeding to the post larvae of *M. rosenbergii*.

2.2.2 Test diet

The cashew, *Anacardium occidentale L.* testa was procured from cashew processing factory of Ratnagiri, Maharashtra as a by-product of cashew nut processing. It consists mainly of the thin red-brown covering (testa) of the kernels, together with some germs and broken bits of kernels. Manual and mechanical methods together are employed in the processing of cashew nuts. The cashew nut testa was further sun-dried and ground to pass a 100 mesh sieve and stored in polythene sacks until used in formulations. The control diet was considered as base diet containing no cashew nut testa. While the test diets were prepared by replacing fish meal composition of control diet with cashew testa powder at the rate of 1.52% (T₁), 3.04% (T₂), 6.08% (T₃) and 12.16% (T₄). Protein levels of the test diets were maintained to 35% by adjusting the composition of groundnut oil cake, wheat flour and rice bran. Ingredients and proximate composition of control diet and test diets are depicted in Table 1.

2.3 Chemical analysis

The crude protein contents of samples of cashew testa nuts were determined using the Kjeldahl method (Table 1). The ash content was estimated after incineration of samples at 550 °C in muffle furnace for 5 hours. Nitrogen content was determined by Micro-Kjeldahl method. Crude fat content was analyzed by Soxhlet method. Ether extract (fat) and Weende crude fibre were determined according to AOAC (2005) [1] standard methods. The carbohydrate was computed by remainder method (Woods and Aurand, 1977) [26]. Calcium, phosphorus, and potassium analyses followed the procedure of Fick *et al.* 1979. The tannin content of cashew testa nut was determined using the Folin-Denis reagent as described by the Association of Official Analytical Chemists (1990). The metabolizable energy content of cashew testa nut was calculated from the proximate composition by the following equation: ME (kcal/kg) = (21.26 × % dry matter) + (47.13 × % fat) + (35.85 × % crude fibre). Gross energy in each of the diets was calculated by using conversion factors carbohydrate, 4.1; fat, 9.5 and protein, 5.65 (El-Sayed, 1994) [7]. The results are given in Table 2.

The faeces from the experimental tub were also collected from the replicate of each treatment to form sufficient quantity for the subsequent chemical analysis. Faeces were analyzed for protein and fat by using the above mentioned methods. The chemical analyses of diets are depicted in the Table 3.

2.4 Water quality parameters

During the experimental period, water quality parameters such as water temperature, pH, dissolved oxygen, total alkalinity were recorded at the interval of five days and are given in Table 4.

2.5 Experimental procedure

Following a fasting period of 24h, the post-larvae of *M. rosenbergii* were individually weighed to record the initial weight. The animals were blotted dry before being weighed to the nearest 0.01mg by using Sartorius GW6202 model electronic balance of Germany make. Post larvae with an average initial weight of 0.033 ± 0.002 g were randomly stocked in experimental tubs of 30 L capacity, filled upto 25 L.

Each tub was provided with diffuser type of air stone for aeration. Each of the four- test diets as well as the control diet were fed to four-replicate tubs at the rate of 10% of total body weight containing 30 post larvae per tub over an experimental period, using completely random design. Biomass of each tub was measured fortnightly and numbers of animals were also counted. Feeding was done thrice a day with five hours interval and ration of the each day was divided into 20, 20 and 60% in three feeding frequencies. Small semi-circular PVC pipes were kept in each tub to provide shelter to the moulted post larvae. Uneaten feed was siphoned out daily. The 25% of water of each tub was replaced once a week.

At the end of experiment, weight of individual animal was measured. The weight gain (%), specific growth rate (% day⁻¹) and survival rate (%) were also calculated.

2.6 Statistical analysis

Standard Error (SE) of the mean weight and survival percentage of the post-larvae for each replicate was calculated. Data obtained in the feeding experiments were analyzed by one-way ANOVA. Significant difference was indicated at 0.05 levels, the Least Significance Difference (LSD) test was used. ANCOVA was applied to determine the relationship between the growth and rearing period for the different diets during the experiment (Snedecor and Cochran, 1967; Zar, 2004) [23, 27].

3. Results

3.1 Growth

The length gain 73.97% was greater in post-larvae fed with T₂ diet and lower length gain (43.48%) in post-larvae fed with T₄ diet (Table 5). The one way ANOVA showed that variation in the length gain of *M. rosenbergii* fed with control and test diets were statistically significant ($P<0.05$). The LSD test showed significantly high ($P<0.05$) length gain in post-larvae fed with T₂ diet as compared to that of post-larvae fed with other diets. The relationship between length gain (Y) and rearing period (X) of *M. rosenbergii* fed with different diets was estimated with the equations $Y=-1.98+0.81X$, ($r=0.936$), $Y=-3.63+0.89X$ ($r=0.96$), $Y=-3.32+1.28X$ ($r=0.961$), $Y=-3.01+0.86X$ ($r=0.916$) and $Y=0.43+0.71X$ ($r=0.921$) for the T₀, T₁, T₂, T₃ and T₄ diets respectively. ANCOVA was applied to compare the slopes and significant difference ($P<0.05$) was observed.

The superior growth performance of weight gain (384%) was observed in post-larvae of *M. rosenbergii* fed with T₂ diet and minimum weight gain was observed 257% in post-larvae of *M. rosenbergii* fed with T₄ diet. ANOVA clearly showed that there was significant difference ($P<0.05$) among the weight gain due to diets. LSD test showed that weight gain of post-larvae fed with T₂ diet was significantly high ($P<0.05$) than weight gain of post-larvae fed with all the other diets (Table 6). At the end of the experiment, the relationship between weight gain (Y) and rearing period (X) of *M. rosenbergii* fed with different diets showed the equations were $Y=-21.25+4.45X$ ($r=0.93$), $Y=-27.73+4.56X$ ($r=0.946$), $Y=-31.73+6.40X$ ($r=0.94$), $Y=-21.81+4.76X$ ($r=0.969$) and $Y=-16.54+4.35X$ ($r=0.959$) for the T₀, T₁, T₂, T₃ and T₄ diets respectively. ANCOVA was applied to compare the slopes and significant difference ($P<0.05$) was observed.

The specific growth rate of post-larvae fed with all the test diets were fluctuated between 2.11% to 2.62%. The highest specific growth rate was 2.62% in post-larvae fed with T₂ diet while lowest was 2.11% in post-larvae fed with T₄ diet. The

ANOVA showed significant difference ($P<0.05$) in specific growth rate of post-larvae of *M. rosenbergii* fed different diets. LSD test found that weight gain of post-larvae fed with T₂ diet significantly higher ($P<0.05$) than weight gain of post-larvae fed with other diets.

3.2 Survival

The maximum survival was 59% in post-larvae fed with T₂ diet while post-larvae fed with T₁ diet showed the minimum survival of 44%. The one way ANOVA showed significant difference ($P<0.05$) in the survival of post-larvae of *M. rosenbergii* fed with different diets. LSD test found that survival of post-larvae fed with T₂ diet significantly higher ($P<0.05$) than that of post-larvae fed with other diets.

3.3 Water quality parameters

During the experimental period, water temperature ranged from 24.6 to 29.4°C, the pH was between 7.56 and 8.2, and the dissolved oxygen varied between 5.8 and 6.8 mg/L and the total alkalinity was between 186 and 218 mg/L.

4. Discussion

4.1 Growth

Results of the present study indicated that the post-larvae fed with T₂ diet (3.04% inclusion of cashew testa) showed better length gain (73.97%), weight gain (384%), specific growth rate (2.62%) and survival (59%). ANOVA showed that the rate of increase in length and weight with rearing period was significantly higher in post-larvae of *M. rosenbergii* fed with diet incorporated with 3.04% cashew testa powder.

The maximum length gain (25.83%) of the post-larvae of *M. rosenbergii* recorded by Hari and Kurup (2001) fed with a diet containing trash fish meal, shrimp head meal, groundnut oil cake, coconut oil cake, rice bran and wheat flour. By using a diet with 40% protein level, Indulkar and Belsare (2001) [14] recorded a maximum length gain (66.98%) in the post-larvae of *M. rosenbergii*. Belsare (2004) [4] found maximum length gain of 48.66% and 69.66% with mango peeling and mango seed incorporated diet respectively in the post-larvae of *M. rosenbergii*. Gitte and Indulkar (2005) [11] used pink perch meat incorporated diet and found maximum length gain (66.30%) in post-larvae of *M. rosenbergii*. The length gain recorded in the present study was found greater than that of above reports. It indicated that cashew testa incorporated diet found better as compared to the diets used by other scientists. This may be probably due to nutritive value of cashew testa which has 11.25% protein as rest of all ingredients and their compositions in the cashew testa diet and other diets were approximately equal.

The maximum weight gain of 173.03% (Hari and Kurup, 2001) [12] and 494.77% (Indulkar and Belsare, 2001) [14] recorded in the post-larvae of *M. rosenbergii*. With 1:1 and 1:2 plant: animal protein ratios maximum weight gain 102.98% and 109.16% respectively recorded by Hari and Kurup (2003) [13] in juveniles of *M. rosenbergii*. Mango peeling and mango seed incorporated diet used by Belsare (2004) [4] revealed maximum weight gain 111.18% and 286.34% respectively in the post-larvae of *M. rosenbergii*. Gitte and Indulkar (2005) [11] reported maximum weight gain (481.3%) in post-larvae of *M. rosenbergii* fed with pink perch meat incorporated diet containing 40% protein level. The weight gain (384%) recorded in the present study was superior to that recorded by all other researcher except Indulkar & Belsare (2001) [14] and Gitte & Indulkar (2005) [11].

Perhaps, Indulkar and Belsare (2001)^[14] & Gitte and Indulkar (2005)^[11] used higher amount of feeding ration during their experiment and also the protein % in diet of Gitte and Indulkar (2005)^[11] was higher (40%) than present study (35%), which may be main attributor to the higher weight gain.

Specific growth rate of 4.16% recorded by Balaz and Ross (1976)^[3] in the diet containing 1:1 plant: animal protein ratio. In the study on post-larvae of *M. rosenbergii*, Indulkar and Belsare (2001)^[14] recorded maximum specific growth rate of 11.88%. Kulkarni (2001)^[15] observed specific growth rate (3.14%), while Belsare (2004)^[4] recorded specific growth rate (1.20%) in the experiment on post-larvae of *M. rosenbergii*. Gitte and Indulkar (2005)^[11] reported specific growth rate (5.86%) in post-larvae of *M. rosenbergii* fed with diet with 40% protein level. The high specific growth rate recorded in the present study as compared to Belsare (2004)^[14]. This indicates that cashew testa powder gives better specific growth rate than mango seed kernel and mango peeling powder. But specific growth rate was less as compared to Indulkar & Belsare (2001)^[14] and Gitte & Indulkar (2005)^[11]. Indulkar & Belsare (2001)^[14] incorporated 1:1 and 1:2 plant: animal protein ratios, which would have contributed for superior result. While the reports of Gitte and Indulkar (2005)^[11] showed higher specific growth rate may be due to higher protein content in the diet and higher feeding rate than the present study.

4.2 Survival

Higher survival (80%) in post-larvae of *M. rosenbergii* fed with groundnut leaf powder incorporated diet was recorded by Vasudevappa *et al.* (1993)^[25]. Sarada Suraya Kumari and Shrotri (2000) recorded 90% survival of the post-larvae of *M. rosenbergii* at 5% inclusion level of non-conventional plant origin ingredients namely, coriander leaves, carrot and cabbage. In the experiment on post-larvae of *M. rosenbergii* Indulkar and Belsare (2001)^[14] recorded 85% survival. Hari and Kurup (2003)^[13] found 93.33% survival in post-larvae of *M. rosenbergii* fed with diet of 1:1 plant: animal protein ratio. Belsare (2004)^[4] recorded highest survival of 93.33% and 94.44% fed with diets supplemented with mango seed kernel and mango peeling respectively in post-larvae of *M. rosenbergii*. During present study, low survival was observed may be due to anti-nutritive factor such as tannin in the cashew testa nut.

4.3 Water quality parameters

The water quality parameters monitored were all within the acceptable tolerance range recommended for freshwater prawn except temperature. The difference in the survival of the post-larvae during present investigation can be attributed to water temperature.

Table 1: Ingredients composition of diets in the experiment

Ingredients g kg ⁻¹	Diets				
	T ₀	T ₁	T ₂	T ₃	T ₄
Fish meal	30.41	28.89	27.37	24.33	18.25
Cashew testa	Nil	1.52	3.04	6.08	12.16
Groundnut oil cake	30.41	31.41	34.00	39.00	47.59
Wheat flour	19.59	19.59	19.59	19.59	11.00
Rice bran	19.59	18.59	16.00	11.00	11.00

Table 2: Chemical composition of cashew testa nut

Item	Cashew testa nut (g kg ⁻¹)
Dry matter	905
Crude protein	190
Crude fibre	103
Ether extract	20.1
Ash	20.2
Calcium	5.6
Phosphorus	1.9
Potassium	1.5
Magnesium	5.8
Tannins	1.8
Metabolizable energy (MJ kg ⁻¹) ²	7.12

Table 3: Chemical analyses of diets in the experiment

Ingredients g kg ⁻¹	Diets				
	T ₀	T ₁	T ₂	T ₃	T ₄
Moisture	9.26	9.24	9.84	10.21	9.96
Crude protein	35.00	34.60	34.66	34.72	34.50
Crude lipid	7.12	7.00	6.84	6.53	6.39
Ash	6.00	6.95	7.36	7.15	7.35
Carbohydrate	42.62	42.21	41.3	41.39	41.75
Gross energy	440.132	435.05	430.14	427.90	426.80
Protein efficiency ³ ratio	79.52	79.53	80.58	81.14	80.8
Proximate Composition (% dry weight basis except in % moisture)					

Table 4: Water quality parameters during experiment

Weeks	Temperature (°C)	pH	DO (mgL ⁻¹)	Total alkalinity (mgL ⁻¹)
1	28.8 (±0.06)	7.76 (±0.05)	5.9 (±0.03)	218 (±3.21)
2	28.2 (±0.08)	7.94 (±0.06)	6.7 (±0.06)	198 (±2.56)
3	29.4 (±0.07)	8.00 (±0.04)	6.2 (±0.05)	202 (±4.6)
4	28.6 (±0.06)	7.92 (±0.06)	5.8 (±0.02)	196 (±4.8)
5	27.6 (±0.06)	8.14 (±0.06)	6.7 (±0.07)	203 (±4.9)
6	27.0 (±0.05)	7.56 (±0.04)	6.0 (±0.06)	186 (±3.8)
7	28.2 (±0.06)	7.76 (±0.07)	6.4 (±0.09)	192 (±5.7)
8	25.1 (±0.03)	8.13 (±0.02)	6.8 (±0.03)	215 (±2.13)
9	24.6 (±0.06)	8.2 (±0.04)	6.1 (±0.04)	197 (±4.3)

(Values in the parentheses are ± S.E.)

Table 5: Average initial length, final length and length gain of post-larvae of *M. rosenbergii* fed with different diets.

Sr. no.	Diets	Initial length (cm)	Final length (cm)	Length gain (%)
1	T ₀	1.46 (±0.04)	2.16 (±0.09)	47.91 (±3.91)
2	T ₁	1.49 (±0.02)	2.33 (±0.03)	56.61 (±2.61)
3	T ₂	1.54 (±0.02)	2.67 (±0.09)	73.97 (±8.08)
4	T ₃	1.52 (±0.05)	2.31 (±0.06)	53.17 (±7.83)
5	T ₄	1.52 (±0.04)	2.17 (±0.04)	43.48 (±3.0)

(Values in the parentheses are ± S.E.)

Table 6: Weight gain, specific growth rate and survival of post-larvae of *M. rosenbergii* fed with different diets.

Parameters	Diets				
	T ₀	T ₁	T ₂	T ₃	T ₄
Initial weight (g)	0.0363 (±0.001)	0.0324 (±0.001)	0.0316 (±0.003)	0.0309 (±0.002)	0.031 (±0.001)
Final weight (g)	0.1307 (±0.01)	0.1243 (±0.002)	0.1500 (±0.004)	0.1177 (±0.007)	0.1101 (±0.006)
Weight gain ¹ (%)	261.56 (±35.52)	285.29 (±10.38)	384.76 (±36.83)	281.66 (±17.55)	257.41 (±26.62)
Specific growth rate ² (% dry)	2.12 (±0.17)	2.25 (±0.04)	2.62 (±0.13)	2.23 (±0.08)	2.11 (±0.13)
Survival ³ (%)	48.33 (±2.15)	44.17 (±2.097)	59.17 (±4.38)	49.17 (±3.44)	46.67 (±3.04)

- 1 $\text{Weight gain (\%)} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$
- 2 $\text{Specific growth rate (\%)} = \frac{(\ln \text{Final weight} - \ln \text{Initial weight})}{\text{Days}} \times 100$
- 3 $\text{Survival (\%)} = \frac{\text{Final count}}{\text{Initial count}} \times 100$

5. Conclusions

With this study it can be concluded that, the fish meal in the diet of post larvae of *M. rosenbergii* can be replaced upto 3.04% by cashew testa. The growth parameters showed positive development, while the survival percentage was poor with the incorporation of cashew testa. As this was the preliminary attempt to replace animal protein (fish meal) with plant protein (Cashew testa), the future investigations should be focused on the anti-nutritive factors of the cashew testa to improve the survival of post larvae of *M. rosenbergii* and also to improve the inclusion levels of cashew testa against the fish meal.

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