

Influence of Dietary Supplementation of Carotenoid (Diacetate of Lutein-Mesozeaxanthin) On Growth Performance, Biochemical Body Composition in Freshwater Prawn, *Macrobrachium rosenbergii*

Abstract

The aim of this study was to evaluate effects of one of the carotenoids (diacetate of lutein-mesozeaxanthin) on growth performance, proximate composition of body and water quality in freshwater prawn, *M. rosenbergii* in fiber reinforced plastic tanks (120 l capacity) for 60 days in a recirculatory aquaculture system. Uniform sized PL of *M. rosenbergii* with an average range of weight (0.32 to 0.37g) was used for the study. The study was carried out in triplicate groups and prawns were stocked at the rate of 50 numbers per tank. Three test diets namely T₁, T₂ and T₃ with 35% protein content were formulated. Diet T₁ had 60ppm, T₂ had 120ppm and T₃ had 180 ppm Diacetate of lutein-mesozeaxanthin and diet without diacetate of lutein-mesozeaxanthin supplementation served as control (T₀). Growth parameters and survival factors were analyzed at the end of feeding trial. After 60 days, weight gain (1.238±0.07), Specific Growth Rate (2.53±0.05), Feed Efficiency Rate (0.440±0.00), Protein Efficiency Rate (1.259±0.00), Survival Rate (80.00±12.02), Percentage of Mean Weight Gain (356.77±0.65), Daily Growth Rate (5.94±0.21), Daily Growth Index (0.77±0.08), Growth Coefficient (1.630±0.05) were higher in prawns fed the Diacetate of lutein-mesozeaxanthin added diets compared to control and lower feed conversion ratio (2.26±0.01) was observed in T₃. The best results of *M. rosenbergii* in terms of growth factors were recorded in treatment T₃ followed by T₂, T₁, T₀, and significant differences (P<0.01) were observed among treatments according to growth parameters, but there was no significant difference (P>0.05) between them with regard to survival rate. There were no significant differences in water quality and proximate composition among different treatments. These findings demonstrated the dietary carotenoid (diacetate of lutein-mesozeaxanthin) can be used for enhancing of growth in *M. rosenbergii*, but no effect on survival, water quality and proximate composition.

Keywords: Carotenoid (Diacetate of lutein-mesozeaxanthin), Growth performance, Biochemical composition, *M. rosenbergii*

Introduction

Macrobrachium rosenbergii [1] the most commercially cultured palaemonid in the world. Because of its fast growth, large size, moderate disease tolerance and export market value, the species has been widely used in aquaculture and has thus been introduced throughout the tropical and temperate regions of the world. It is now cultured at least in 43 countries across five continents, with Asia contributing more than 98% of global production [2]. Global production of this prawn exceeded 200000 metric tons (mt) in 2008 [3] with potential scope for growth. In Asia, the major producers are China, India, Viet Nam, Thailand and Bangladesh. In India too, a spurt in freshwater prawn farming has been seen in recent years. Considering its high export market, the giant freshwater prawn enjoys immense potential for culture in India. Demand for this species in both domestic and international markets are increasing, hence programming and planning for increase in production of *M. rosenbergii* is necessary.

Use of carotenoids can influence growth and survival of fish and shellfishes, but the effects of carotenoids on growth and survival of aquatic organisms have been controversial, because several studies reporting a positive influence on growth, whereas others did not find any effect [4]. The Diacetate of lutein-mesozeaxanthin is a paste like compound, it contains the acetylated forms of lutein and meso-zeaxanthin which is one of the Carotenoids and can be used for different purposes in aquatic animals as well as crustaceans especially in prawn culture. However, it was not used as a feed additive for growth purpose so far and this study was done for first time. But similar carotenoids were used for different objectives in aquaculture production such as fish and shellfish culture. Pre-juvenile (0.115g) white shrimp (*L. vannamei*) fed dietary supplementation of Hi-Zeaxanthin or Zeaxanthin short chain like diacetate during 7 weeks, the average weight and survival rate was significantly higher than control group [5]. White shrimp (*L. Vannamei*) were fed with three different treatments (synthetic

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astaxanthin, lutein and astaxanthin derives from marigold extract), results have shown higher growth, lower FCR and higher survival on marigold treatments as compared to other treatments and control group [6,7] reported a higher survival rate and growth in *P. Japonicus* fed with astaxanthin-supplemented diets than that of β -carotene or algal meal [8] have studied that the Atlantic Salmon fed with two xanthophylls carotenoids (astaxanthin and lutein), had no significant effect on growth performance such as FCR, SGR, WG and condition factor. Eduardo et al. [9] have noted that supplementation of experimental diets with (*Tagetes erecta*) extract (marigold oleoresin) on *L. vannamei* did not significantly alter the proximate composition of the practical diet because very small amounts were required to attain the desired concentrations in the feeds (less than 0.5% of the total ingredients). The same results were reported by Arredondon & Flores et al. [10,11]. This study was conducted to determine whether diacetate of lutein-mesozeaxanthin would affect on growth performance, biochemical composition and water quality in freshwater prawn, *M. rosenbergii*. Carotenoids are pigments naturally occurring in a number of fruits and vegetables. They are synthesized by all photosynthetic organisms and many non-photosynthetic bacteria and fungi. There are two main classes of naturally occurring carotenoids: (1) carotenes such as β -carotene and α -carotene, which are hydrocarbons, are either linear or cyclized at one or both ends of the molecule, and (2) xanthophylls, the oxygenated derivatives of carotenes. All xanthophylls produced by higher plants, such as violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, and lutein, are also synthesized by green algae [12]. Xanthophylls (oxygenated carotenoids) are used as additives for poultry (e.g. chicken), crustaceans (e.g. shrimp) and fish (e.g. salmon) feeds to provide bright colours in egg yolks, skin, and fatty tissues due to its pigmenting properties [13-16]. Among the xanthophylls, lutein, and zeaxanthin are two of the most abundant oxygenated carotenoids found in the diet [17]. Chemically, lutein and zeaxanthin (Figure 1) contain two cyclic end groups (α , β and α -ionone ring) and the basic C_{40} isoprenoid structure common to all carotenoids. Structurally, lutein and zeaxanthin have identical chemical formulas and are isomers, but they are not stereoisomers. The chemical formula of lutein and zeaxanthin is $C_{40}H_{56}O_2$ and the molecular weight is 568.88. The main difference between them is in the location of a double bond in one of the end rings (Figure 1) [18]. Reported that the minute structural differences are responsible for variations in the biological activities of these compounds (carotenoids) [19]. Indicated that multiple conjugate double bonds exist in carotenoids, which confer specific biological characteristics on the family of carotenoids. Furthermore, the orientation of carotenoids depends on the molecular structure. While lutein is present as a single stereoisomer, zeaxanthin occurs as a mixture of three isomers [20], two of which are referred to as zeaxanthin and meso-zeaxanthin, respectively [21]. Meso-zeaxanthin is a unique member of the xanthophylls family of carotenoids and along with lutein [(3R, 30R, 60R)-b, e-carotene-3, 30-diol] (L) and zeaxanthin [(3R,30R)-b,b-carotene-3,30diol] (Z) are members of the xanthophylls class of carotenoids [22-24]. The presence of meso-zeaxanthin was reported in shrimp carapace, fish skin, and turtle fat, where all three isomers of zeaxanthin were found [25]. The Diacetate of lutein-mesozeaxanthin is a paste like compound. It contains the acetylated forms of lutein

and meso-zeaxanthin. Diacetate, a salt or ester containing two acetate groups ($CH_3COO^- + CH_3COO^-$), whereas acetate, a salt or ester of acetic acid (CH_3COOH), on the other hand, acetate are usually seen derivatives of acetic acid and acetate is also the ion formed when acetic acid losses its acidic hydrogen: $CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$

Material and Methods

Carotenoids (Diacetate of lutein-mesozeaxanthin)

The study was conducted in fiber reinforced plastic tanks (120 l capacity) in indoor recirculatory system at the fish farm of the College of Fisheries, Mangalore to evaluate the effect of diacetate of lutein-mesozeaxanthin on growth factors, proximate composition of protein and water quality in rearing *M. rosenbergii*. The study was carried out in triplicate tanks for each treatment for a period of 60 days. The complete randomized design (CRD) was followed.

Proximate composition of the feed ingredients

All the feed ingredients were analyzed for proximate composition prior to formulation of the test diets employing standard methods (AOAC, 1975). Moisture content was estimated by heating samples at $105^\circ C$ for 30 min and then cooling and weighing to a constant weight. Crude protein was analyzed using Kjeltex system (Tecater 1002 Distilling Unit), fat content by Soxtech System (Tecater 1043 Extraction Unit), and fibre content by using Fibretech System (Tecater 1017 Hot Extractor). Carbohydrate content was calculated as nitrogen free extract (NFE) by the difference method as given below.

$$NFE = 100 - (\% \text{ moisture} + \% \text{ crude protein} + \% \text{ crude fat} + \% \text{ crude fibre} + \% \text{ ash})$$

The ash content was determined by first drying the sample and then heating it in a muffle furnace at $550 \pm 10^\circ C$ for 6h. The proximate composition of the ingredient used in the experimental diets and proximate composition of experimental diets are given in Tables 1 & 2.

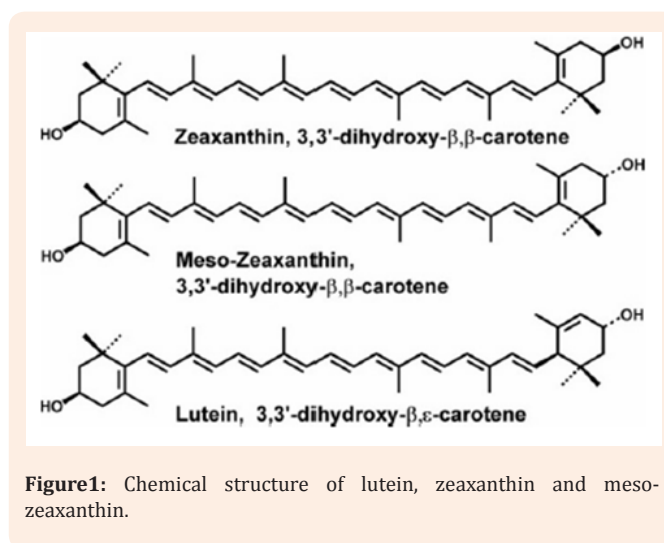


Figure1: Chemical structure of lutein, zeaxanthin and meso-zeaxanthin.

Table 1: Proximate composition of the ingredient (% on dry weight basis) used in the experimental diets.

| Ingredients | Dry Matter (%) | Crude Protein (%) | Crude Fat (%) | Crude Fibre (%) | Ash (%) | NFE (%) | Energy Kcal /Kg |
|-------------------|----------------|-------------------|---------------|-----------------|---------|---------|-----------------|
| Fish meal | 94.86 | 62.28 | 9.53 | 0.72 | 18.82 | 3.51 | 4523.90 |
| Rice bran | 93.17 | 6.90 | 4.98 | 28.62 | 15.60 | 37.07 | 2337.32 |
| Shrimp meal | 91.89 | 62.07 | 3.08 | 1.69 | 17.10 | 7.95 | 4082.44 |
| Groundnut oilcake | 92.99 | 34.28 | 6.89 | 3.02 | 10.80 | 38.00 | 4087.34 |
| Wheat flour | 93.50 | 11.23 | 1.73 | 1.42 | 1.28 | 77.84 | 3905.10 |
| Soya flour | 91.66 | 39.67 | 19.62 | 6.50 | 5.24 | 20.63 | 4891.00 |

Table 2: Proximate composition of experimental diets (% on dry weight basis).

| Parameters | Dietary Treatments | | | |
|-------------------|--------------------|----------|-----------|-----------|
| | Control | 60 mg/kg | 120 mg/kg | 180 mg/kg |
| Dry matter (%) | 93.50 | 92.76 | 93.15 | 92.85 |
| Crude protein (%) | 35.09 | 34.95 | 35.20 | 34.87 |
| Crude fat (%) | 7.52 | 7.65 | 7.40 | 7.76 |
| Crude fibre (%) | 26.28 | 27.05 | 26.15 | 27.45 |
| Ash (%) | 15.05 | 16.10 | 15.70 | 15.65 |
| NFE (%) | 9.56 | 7.01 | 8.70 | 6.12 |
| Energy Kcal /Kg | 3054.32 | 2956.70 | 3014.80 | 2926.96 |

*Means of three replicates

Diet preparation

The ingredients used in the formulation of different experimental diets were fishmeal, rice bran, groundnut oil cake, wheat flour, soya beans meal, shrimp meal and vitamin and mineral premix. The basal diet was supplemented with diacetate of lutein-mesozeaxanthin, ground and sieved to get particles of uniform size. Three test diets namely T₁, T₂ and T₃ having 35% protein were formulated using the square method [26]. Diet T₁ had 60mg/kg diacetate of lutein-mesozeaxanthin, T₂ had 120mg/kg diacetate of lutein-mesozeaxanthin and T₃ had 180mg/kg diacetate of lutein-mesozeaxanthin, and the diet without diacetate of lutein-mesozeaxanthin supplementation served as a control (T₀). The required quantities of ingredients were weighed accurately, mixed and hand needed to required consistency with just sufficient quantity of water (1:0.8) to get smooth dough. The dough so obtained was cooked under steam in a pressure cooker at 105° C for 20 to 30min. The cooked feed was cooled to room temperature rapidly by spreading in an enamel tray. Then required dose of marigold oleoresin and vitamin-mineral premix were added, mixed and blended. The dough was extruded through a pelletize. Pellets of size 1.2mm were dried in a hot air oven at 60° C till the moisture content was reduced to less than 10%. Diets were packed separately in high density polythene bags, labelled and stored in a wooden shelf at room temperature for further use.

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Experimental animals, stocking and feeding

The post larvae (PL-20) produced in the Prawn Hatchery at the College of Fisheries, Mangalore was reared in cement tanks and juveniles were used for experiments. The juveniles were acclimatized to the experimental condition by feeding with dry pelleted basal diet. Uniform sized PL of *M. rosenbergii* with an average range of weight (0.85 to 0.89g) was stocked at the rate of 50 numbers per tank. The experiment was carried out for a period of 60 days with an exchange of water once in two days in the tank. Fecal matter and uneaten food was removed daily in the morning hours. Prawns were fed at the rate of 5% of their body weight till the end of the experiment. The feed was broadcasted over the surface of water twice daily in the morning and evening. After each sampling the quantity of feed given was re-adjusted based on the increased weight of prawn.

Water quality

Water samples were collected for determining its quality every fortnight. Water quality parameters were maintained within the normal range throughout the experimental period. Water samples collected on each sampling day were analyzed for pH, temperature, dissolved oxygen, free carbon dioxide, $\text{NH}_3\text{-N}$ and total alkalinity. Digital pocket pH meter (Hanna) was used to record pH. Atmospheric temperature and water temperature were recorded by using thermometer. Dissolved oxygen was estimated by Winkler's method. Total alkalinity, NH_3 and free carbon dioxide were determined by following standard methods [27].

Prawn sampling

The prawns were sampled every fortnight to assess the growth. Length was measured from tip of rostrum to tip of telson by using a fibreglass measuring scale fixed on wooden frame. Weight was measured on electronic balance (Essae, India).

Biochemical composition

Proximate composition of prawn muscle was estimated soon after completion of the experiment. All the prawns were peeled and deveined. Whole meat of the prawn was dried at 60° C for 48hrs to obtain the dry matter. The dry matter was powdered in mortar and used for further analysis. The samples were analyzed for crude protein, crude fat, total ash and carbohydrate (NFE) employing standard methods as explained earlier.

Statistical analysis

Mean growth of prawn achieved in response to different formulated test diets were estimated statistically by using one way analysis of variance (ANOVA) followed by Duncan's multiple range test were done by using SPSS software (16.0 version).

Results

Water quality parameters

The results of water quality parameters such as temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and ammonia-nitrogen are presented in Table 3. Temperature of water ranged from 28.5° C to 29.5° C and mean value of water temperature had not significantly fluctuated during experiment. The pH recorded during the study period, ranged from 7.8

to 9.1 but mean value of pH water did not vary significantly in duration of feeding trial. The average values of dissolved oxygen were 7.77±0.24 to 7.89±0.18 in different treatments and did not find significantly difference among them, as well as other parameters of water quality namely; free carbon dioxide, total alkalinity and total ammonia-nitrogen were in normal range and significant differences among treatment groups and control was not observed.

Growth studies

The increase in weight of prawn in treatments and control during experimental period are given in Table 4. It was observed that the best growth of prawn, *M. rosenbergii* in terms of weight was recorded in treatment T_3 followed by the treatment T_2 , T_1 and T_0 . The average final weight recorded after 60 days of culture in treatment T_3 was 1.585±0.08g, followed by 1.518±0.05g in T_2 , 1.420±0.08g in T_1 and 1.225±0.07 g in T_0 . Specific Growth Rate (% / day) of prawn was highest in T_3 (2.53±0.05), followed by T_2 (2.45±0.12), T_1 (2.39±0.06) and T_0 (2.09±0.07). The best results of WG (1.238±0.07), PER (1.259±0.00), FCR (2.26±0.01), SR (80.00±12.02%), FER (0.440±0.00), PDG (0.020±0.06g), PMVG (356.77±0.65%), DGR (5.94±0.21), DGI (0.77±0.08) and GC (2.543%) was obtained in T_3 followed by T_2 , T_1 .

Growth analysis: The growth parameters were calculated by using the following formula:

- Mean weight gain (g) = W_f (g) - W_i (g) [28]
- Feed Conversion Rate (FCR) = total feed consumed (g) / (initial number of fishes / final number of fishes) [29]
- Per day growth (g) = mean weight gain (g) / number of days [28]
- Survival (%) = number of fishes survival at the end of the experiment / number of fishes stocked at the start of the experiment [30]
- Percentage of mean weight gain (PMWG) = $\frac{(W_f \text{ (g)} - W_i \text{ (g)})}{W_i \text{ (g)}} \times 100$ [31]
- Specific growth rate (SGR) = $\frac{\{\ln W_f \text{ (g)} - \ln W_i \text{ (g)}\}}{t}$
- Condition factor (CF) = $W \times 100 / L^3$ [30]
- Daily growth rate (DGR) = $\frac{\{100 \times (\text{final weight (g)} - \text{initial weight (g)})\}}{\text{days} \times \text{initial weight (g)}}$; [29]
- Daily growth index (DGI) = $\{100 \times (W_f^{1/3} - W_i^{1/3}) / \text{days}\}$ [29]
- Growth coefficient (GC) = $\{100 \times (W_f^{1/3} - W_i^{1/3}) / \Sigma\theta\}$ [29], where W_f = Final weight, W_i = Initial weight, $\Sigma\theta$ = sum of average daily temperature in °C.

Biochemical composition

The results of biochemical composition of prawn muscle recorded at the end of the experiment are shown in Table 5. Maximum moisture content was recorded in T_0 (80.86±0.56%) and minimum in T_3 (78.46±0.54 %). The values were 78.59±0.42 % in T_1 and 78.80±0.49 % in T_2 . Protein content of prawn muscle was maximum in T_2 (22.95±0.27%) followed by T_0 (21.80±0.24%), T_1 (22.73±0.18 %) and T_3 (22.42±0.16%). Crude fat was maximum in T_0 (1.8±0.11%) and minimum in T_3 (1.3±0.15%). Intermediate

values of 1.7 ± 0.20 % in T_2 and 1.5 ± 0.19 % in T_1 . Ash levels were 5.8 ± 0.21 % in T_2 , 5.3 ± 0.18 % in T_0 , 5.2 ± 0.17 % in T_3 and 4.9 ± 0.13 % in T_1 . The highest NFE was recorded in T_1 (1.6 ± 0.09 %), followed by T_2 (1.5 ± 0.08 %), T_3 (1.4 ± 0.04 %) and T_0 (1.3 ± 0.06 %).

On the basis of these results there were no significant differences among treatments in all parameters of biochemical composition of prawn muscles.

Table 3: Water quality parameters recorded in different experimental tanks.

| Water parameter | Dietary Treatments | | | |
|--------------------|--------------------|------------|------------|------------|
| | Control | 60mg/kg | 120 mg/kg | 180 mg/kg |
| W.T ¹ | 29.18±0.18 | 29.22±0.17 | 29.10±0.14 | 29.25±0.15 |
| pH | 8.56±0.11 | 8.56±0.11 | 8.51±0.11 | 8.44±0.13 |
| D.O ² | 7.83±0.19 | 7.77±0.24 | 7.89±0.18 | 7.85±0.19 |
| F.C.D ³ | 0.83±0.11 | 0.78±0.09 | 0.85±0.07 | 0.90±0.10 |
| T.A ⁴ | 78.19±1.69 | 78.05±1.44 | 77.66±1.65 | 77.87±1.51 |
| T.A.N ⁵ | 0.04±0.01 | 0.04±0.00 | 0.04±0.01 | 0.04±0.00 |

1. Water temperature
2. Dissolved oxygen
3. Free carbon dioxide
4. Total alkalinity
5. Total ammonia-nitrogen.

Table 4: Growth parameters of *M. rosenbergii* fed with different marigold oleoresin levels.

| Growth Indices | Dietary Treatments | | | |
|------------------------------------|-------------------------|--------------------------|----------------------------|---------------------------|
| | Control | T ₁ (60mg/kg) | T ₂ (120 mg/kg) | T ₃ (180mg/kg) |
| Initial weight (g) | 0.348±0.00 | 0.347±0.00 | 0.348±0.00 | 0.347±0.00 |
| Final weight (g) | 1.225±0.07 | 1.420±0.08 | 1.518±0.05 | 1.585±0.08 |
| Mean Weight Gain (g) | 0.877±0.06 ^a | 1.073±0.07 ^{ab} | 1.170±0.07 ^b | 1.238±0.07 ^b |
| Specific Growth Rate (%) | 2.09±0.07 ^a | 2.39±0.06 ^{ab} | 2.45±0.12 ^b | 2.53±0.05 ^b |
| Feed Conversion Ratio | 2.73±0.16 ^a | 2.46±0.14 ^a | 2.32±0.16 ^a | 2.26±0.01 ^a |
| Protein Efficiency Rate | 1.043±0.05 ^a | 1.159±0.06 ^a | 1.226±0.08 ^a | 1.259±0.00 ^a |
| Survival Rate (%) | 69.32 ^a | 74.00 ^a | 77.32 ^a | 80.00 ^a |
| Feed Efficiency Rate | 0.365±0.02 ^a | 0.405±0.02 ^a | 0.429±0.02 ^a | 0.440±0.00 ^a |
| Per Day Growth(g) | 0.0146 ^a | 0.0178 ^{ab} | 0.0195 ^b | 0.020 ^b |
| Percentage of Mean Weight Gain (%) | 252.01 ^a | 309.22 ^{ab} | 336.2 ^{ab} | 356.77 ^b |
| Condition Factor | 1.91 ^a | 2.07 ^a | 1.94 ^a | 1.78 ^a |
| Daily Growth Rate (g) | 4.20 ^a | 5.15 ^{ab} | 5.60 ^b | 5.94 ^b |
| Daily Growth Index | 0.61 ^a | 0.70 ^{ab} | 0.74 ^b | 0.77 ^b |
| Growth Coefficient (%) | 1.288 ^a | 1.482 ^{ab} | 1.570 ^b | 1.630 ^b |

Table 5: Proximate composition of prawn meat taken from different treatments (% on dry weight basis).

| Parameter | Dietary Treatments | | | |
|-------------------|--------------------------|---------------------------|----------------------------|-----------------------------|
| | T ₀ (Control) | T ₁ (60 mg/kg) | T ₂ (120 mg/kg) | T ₃ (180 /mg/kg) |
| Moisture (%) | 80.86±0.56 | 78.59±0.42 | 78.80±0.49 | 78.46±0.54 |
| Dry matter (%) | 19.14±0.65 | 21.41±0.52 | 21.20±0.48 | 21.54±0.44 |
| Crude protein (%) | 21.80±0.24 | 22.73±0.18 | 22.95±0.27 | 22.42±0.16 |
| Crude fat (%) | 1.8±0.11 | 1.5±0.19 | 1.7±0.20 | 1.3±0.15 |
| Ash (%) | 5.3±0.18 | 4.9±0.13 | 5.8±0.21 | 5.2±0.17 |
| NFE (%) | 1.3±0.06 | 1.6±0.09 | 1.5±0.08 | 1.4±0.04 |

*Means of three replicates. Moisture and dry matter on wet weight basis

Discussion

Effect of marigold oleoresin on water quality

The quality of water is very important in the culture of crustaceans, since use of feeds is known to have an influence on water quality, there by affecting the species cultured. Maintenance of good water quality is essential for both survival and optimal growth of fish and shellfish. Important water quality parameters such as temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and ammonia-nitrogen were measured weekly throughout the experiment and no adverse effect of diacetate of lutein-mesozeaxanthin on water quality was observed and the recorded values could be considered suitable for optimum growth of *M. rosenbergii* which is similar to results obtained by Ravishankar [32], [1, 33-35].

Effect of diacetate of lutein-mesozeaxanthin on growth of *M. rosenbergii*

Carotenoids can influence on growth and survival of fish and shellfishes, but the effects of carotenoids on growth and survival rate of aquatic organisms have been controversial, because several studies reporting a positive influence whereas others did not find any effect [4,36,37] have been reported that the carotenoids could enhance nutrient utilization and might ultimately improve growth, play an important role in the intermediary metabolism of aquatic animals. Pre-juvenile (0.115g) white shrimp (*L. vannamei*) fed dietary supplementation of Hi-Zeaxanthin or Zeaxanthin short chain like diacetate during 7 weeks, the average weight and survival rate was significantly higher than control group [5]. Eduardo Aguirre-Hinojosa et al. [9] have indicated that juvenile of *L. vannamei* fed diets supplemented with xanthophylls (75% Zeaxanthin, 15% lutein) improved survival of shrimps fed treatment diets compared to those fed the control diet but there were no significant differences in growth between experimental groups fed different diets. White shrimp (*L. Vannamei*) fed with three different treatments (synthetic astaxanthin, lutein and astaxanthin derives from marigold extract) have shown higher growth, lower FCR and higher survival on marigold treatments as

compared to other treatments and control group [6]. Survival rate of juvenile (2.5g) white shrimp (*L. vannamei*) fed supplemented diet of Hi-Zeaxanthin for 30 days was significantly higher as compared to the control group [5]. White shrimp (*L. vannamei*) post larvae (PL 17) fed for 11 days with H1-Zeaxanthin had a noticeable improvement in their survival rate [9]. In present study, diacetate of lutein-mesozeaxanthin as a one of the carotenoids could enhance growth of *M. rosenbergii*. However Olsen RE et al. [8] have reported that the Atlantic salmon fed with two xanthophylls carotenoids (astaxanthin and lutein), had no significant effect or growth performance such as FCR, SGR, WG and condition factor. Similarly, [38-40] found no effect of synthetic and natural carotenoids on growth in salmonids.

Effect of marigold oleoresin on proximate composition of prawn meat

Carcass composition is known to be influenced by many factors such as age, sex, maturity and feeding conditions. Among these factors, the type and nature of feed ingested are considered to be the most important [41-43]. In the present study, *M. rosenbergii* fed different levels of diacetate of lutein-mesozeaxanthin did not affect significantly on biochemical composition of prawn muscle. Similar results were also reported by other workers. Eduardo Aguirre-Hinojosa et al. [9] have noted that supplementation of experimental diets with *Tagetes erecta* extract (marigold oleoresin) on *Litopenaeus vannamei* did not significantly alter the proximate composition of the practical diet because very small amounts were required to attain the desired concentrations in the feeds (less than 0.5% of the total ingredients). The same results were reported by Arredondo-Figueroa et al. [10] & Flores et al. [11]. There were no significant differences in proximate composition (crude protein, crude fat, moisture and ash) of rainbow trout with initial weight of 135g after 60 days of feeding with supplementation of astaxanthin [44]. Dietary carotenoid sources (1.8% marigold flowers, 5% red pepper and 70 mg kg⁻¹ astaxanthin) on rainbow trout weighing 120.57g for 60 days did not significantly affect fatty acid composition of the fish filets [45-47].

Conclusion

The present study showed that the giant freshwater prawn, *M. rosenbergii* fed a diet containing 60mg/kg, 120mg/kg and 180mg/kg of diacetate of lutein-mesozeaxanthin for 60 days indicated increased growth of *M. rosenbergii* but did not affect significantly on biochemical composition of prawn muscle in this species. Further work needs to be carried out for more investigation on effect of diacetate of lutein-mesozeaxanthin on growth and proximate composition in other finfish and shellfish.

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