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Effects of dietary supplementation of fish and vegetable oils on the growth performance and muscle compositions of the freshwater prawn *Macrobrachium rosenbergii*

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Abstract The present investigation was conducted to assess the suitability of three vegetable oils (sunflower oil, coconut oil and castor oil) as an alternative dietary lipid source for cod liver oil to culture *Macrobrachium rosenbergii* post larvae (PLs). The experimental feeds contained 40% protein with separately incorporated three vegetable oils and cod liver oil. The feeding trial was conducted on *M. rosenbergii* PL for 60 days. In the final day of the experimental period, the survival rate, weight gain, length gain, specific growth rate and protein efficiency ratio of prawns showed no significance ($P > 0.05$) between sunflower oil and cod liver oil incorporated feed fed groups. The coconut oil and castor oil showed lower performance when compared with cod liver oil. The present result showed biochemical accumulation of total protein, amino acids, carbohydrate and lipid in experimental groups. Also there is no significant difference in ash and mineral (Na^+ and K^+) contents. Among the tested diets, the recorded growth rate and biochemical constituents of sunflower oil and cod liver oil incorporated feed fed groups were similar. The present results revealed that the sunflower oil was on par with cod liver oil. Hence, the sunflower oil can be incorporated in feed formulation for *M. rosenbergii* PL culture. It can be concluded that the coconut oil and castor oil are not ideal vegetable lipid source with these concentrations which produced lower performance in survival, growth and biochemical compositions of *M. rosenbergii* PL.

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Introduction

The blue revelation of aquaculture is a major part of the food production sector in the world. The cultivable crustaceans, such as prawns, shrimps, lobsters and crabs have a vital role in aquaculture due to their nutritious delicacy for human consumption. The giant river prawn, *Macrobrachium rosenbergii* is dominating in India, and it is one of the major contributors to national economy. It represents a good source of protein, essential amino acids and polyunsaturated fatty acids, and very low in fat. Therefore, it can be used as a delicious healthy choice of food for human consumption. In the culture of this species, artificial feed constitutes a major operating cost (D'Abramo and Sheen, 1994). Animal and plant byproducts are important contributors to the growth and extension of the world aquaculture food production. They supply the chief source of proteins, essential amino acids, carbohydrate, fats, minerals and vitamins. Fishmeal, chicken waste meal and soybean meal are excellent protein sources for *M. rosenbergii* (Hasanuzzaman et al., 2009; Muralisankar and Bhavan, 2013).

Dietary lipids serve as energy source and provide essential fatty acids for freshwater prawn. They also serve as a source of sterols and phospholipids necessary for survival, growth, maintenance and proper physiological functions (Corbin et al., 1983). The *de novo* synthesis of polyunsaturated fatty acids (PUFA) from either linoleic (ω 6) or linolenic (ω 3) series is non-existent in freshwater and marine shrimps (Fenucci et al., 1981; Mukhopadhyay et al., 2003). Hence, the prawns are in need for these fatty acids from their dietary sources. Fish oils are excellent sources of *n*-6 and *n*-3 fatty acids and are better utilized by farmed species. In recent years, the production of fish oil may not be enough to cover the increasing demand for animal feed but the production of vegetable oils has increased and their prices are more stable and even less expensive than fish oil. Some vegetable oils, such as soybean and linseed oils are considerably good alternative lipid sources for salmonids, freshwater fish and prawns (Caballero et al., 2002; Kim et al., 2012). Similarly, the replacement of dietary fish oil by vegetable oils had better survival and growth of the freshwater prawn *M. rosenbergii* (Kim et al., 2012). Also, the replacement of 50–100% of fish oil by vegetable oils showed better survival, growth and health status in *Macculochella peelii*, *Tor tambroides*, *Dicentrarchus labrax*, *Diplodus puntazzo*, *Carassius auratus* and *Salmo salar* (Parameshwaran et al., 2002; Bransden et al., 2003; Richard et al., 2006; Piedecausa et al., 2007; Miller et al., 2007; Turchini et al., 2011; Kamarudin et al., 2011). The present study was conducted to assess the effects of three vegetable oils (sunflower oil, coconut oil and castor oil) on the survival, growth performance and body composition of PL of *M. rosenbergii*.

Materials and methods

Experimental animals

The PLs of *M. rosenbergii* were collected from Happy Bay Aqua Nova Hatchery, Mugaiyur, Kancheepuram, Tamilnadu, India. The PLs were acclimatized to laboratory conditions for 2 weeks before the commencement of experiments. During the acclimatization period the PLs were fed with boiled egg albumin (custard), live *Artemia* nauplii and commercially available crumple feed (Rosen fisheries, Marathakkara). The water medium was renewed on daily basis and provided mild aeration for maintaining optimum oxygen level.

Ingredients and feed preparation

The feed ingredients (green gram, soya bean meal, ground nut oil cake, tapioca flour and eggs), the vegetable oils (sunflower oil, coconut oil and castor oil), vitamin capsule and codliver oil were purchased from the local market at Coimbatore. The formulated feed basal ingredients and oils percentage are provided in Table 1. The dried basal ingredients were ground separately with electric pulvalizer and sieved with 60 μ mesh. The concerned quantity of ingredients was blended 15 min for equal mixing and steam cooked for 15 min at 90–100 °C, and allowed to cool at room temperature. The lipid source oils such as, cod liver oil, sunflower oil, coconut oil and castor oil were separately incorporated with the cooked blends. Finally, vitamin tablets, egg albumen and tapioca were incorporated with blends. The blends were again blended 15 min for thorough mixing for the binding. The final blends were mixed with water and pelletized (3 mm diameter). The pellets were dried at thermostatic oven, until reaching less than 10% moisture. The physical appearance, texture and fragrance of feed pellets were checked and kept in a plastic container. For feeding trial, three vegetable oils (sunflower oil, coconut oil and castor oil) and codliver oil incorporated feed were prepared.

Experimental procedure

M. rosenbergii post larvae ranging from 1.00 ± 0.12 cm in length and 0.15 ± 0.02 g in weight were divided into four

Table 1 Ingredients and composition of experimental diets.

Ingredients	Composition (g kg^{-1})
Soya bean meal	400
Green gram	240
Ground nut oil cake	240
Tapioca flour	60
Egg albumen	30
Vitamin mix[b]	10
Cod liver oil[a], Sunflower oil[a], Coconut oil[a] and Castor oil[a]	20
Total	1000
Proximate composition (%)	
Protein	41.74 ± 0.10
Carbohydrate	25.18 ± 0.12
Lipid	7.08 ± 0.15
Moisture	8.94 ± 0.22
Ash	11.82 ± 0.06

Each capsule contains, Total mg = 438.5 mg; Thiamine Mononitrate IP, 10 mg; Riboflavin IP, 10 mg; Pyridoxine Hydrochloride IP, 3 mg; Vitamin B_{12} (as tablets 1:100) IP, 15 mcg; Niacinamide IP, 100 mg; Calcium pantothenate IP, 50 mg; Folic acid IP, 1.5 mg; Biotin USP, 100 mcg; Ascorbic acid IP, 150 mg.

[a] Each vegetable oil was individually added to the prepared diet.

[b] Becosules capsules (manufactured by Pfizer).

groups. Three groups were fed vegetable oils (sunflower oil, coconut oil and castor oil) the fourth one was fed cod liver oil incorporated feed. Each experiment was conducted three times. The physicochemical parameters of aquarium water were maintained (temperature, 28 ± 1.34 °C; TDS, 1200.00 ± 167 mg L^{-1}; DO_2, 7.20 ± 0.54 mg L^{-1}; BOD, 30.00 ± 2.56 mg L^{-1}; COD, 125.00 ± 12.00 mg L^{-1}) throughout the experimental period. The aquarium water was renewed daily and mild aeration was provided throughout the experimental period. Also, unused feed and fecal matters were separated by the siphoning method without severe disturbance to the prawn. The prawns were fed with the formulated feeds at 10% of total body weight of the PLs for a period of 60 days.

Nutritional index analysis

At the end of the experiment the tested feed nutritional index parameters, such as survival rate (SR), weight gain (WG), length gain (LG), feeding rate and specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were assessed by the following method of Tekinay and Davis (2001).

$$\text{Survival rate (SR)} = \frac{\text{No. of live prawns}}{\text{No. of prawns introduces}} \times 100$$

$$\text{Weight gain (WG)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Length gain (LG)} = \text{Final length (cm)} - \text{Initial length (cm)}$$

$$\text{Feeding rate (FR)} = \frac{\text{Feed intake (g)}}{\text{No. of days}}$$

$$\text{Specific growth rate (SGR)} = \frac{\text{log of Final weight (g)} - \text{log of initial weight (g)}}{\text{No. of days}} \times 100$$

$$\text{Food conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain}}{\text{Protein intake}} \times 100$$

Biochemical constituent analysis

Prawn muscle tissues were subjected to analyse the biochemical composition such as, moisture, crude protein, amino acids, carbohydrate, lipid, and ash. Crude protein and nitrogen were estimated by Kjeldahl apparatus according to AOAC (1995). Total carbohydrate was estimated by the method of Roe (1955), using TCA extracted sample. Total lipid was extracted with chloroform–methanol mixture following the method of Barnes and Blackstock (1973) and estimated by the method of Folch et al. (1957). Amino acids were extracted using Sodium tungstate and H_2SO_4. The content of total amino acid was assayed by the method of Moore and Stein (1948). The pre weighed wet tissue samples were dried under 40 °C to measure the moisture content. The dried tissue sample was subjected to 600 °C under a Muffle furnace to measure the ash content following the method of APHA (2005).

Mineral analysis

Mineral contents (Na^+ and K^+) were estimated in the muscle tissues of the formulated feed fed PL by the simple flame photometric method of Jeffery et al. (1989) by using Elico CL 220 flame photometer. These values were calculated by adopting the following formula.

$$\begin{aligned}\text{Na}^+\text{(or)K}^+\text{Content(mg)} &= \text{Sample reading/Standard reading} \\ &\times \text{standard concentration/Sample concentration} \\ &\times \text{Purity of NaCl or KCl} \\ &\times \text{Molecular weight of NaCl or KCl/Dilution factor.}\end{aligned}$$

Statistical analysis

The results were expressed as Mean ± standard deviation (SD). Statistical analysis was carried out by Analysis of Variance (one way ANOVA) followed by DMRT to determine the levels of significance. All calculations were performed using: SPSS, version 16.0 for Windows (SPSS, Michigan Avenue, Chicago, IL, USA).

Results

Survival and morphometry

The survival rate and morphometric data (length and weight) of the feed fed prawns PL are shown in Table 2. The survival rate, length and weight gain showed no significant ($P > 0.05$) difference between sunflower oil and cod liver oil incorporated feed fed groups. At the same time, coconut oil and castor oil supplemented feed fed prawns attained significantly low ($P > 0.05$) level of survival and growth when compared with cod liver oil incorporated feed fed group.

Nutritional indices

The vegetable oil supplemented feed fed groups' nutritional index parameters, such as, feeding rate (FR), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) are shown in Table 2. The nutritional index parameters except FCR showed no significant ($P > 0.05$) difference between sunflower oil and cod liver oil supplemented feed fed groups. However, feed conversion ratio was significantly ($P < 0.05$) elevated in castor and coconut oil incorporated feed fed groups, while there was no significant ($P > 0.05$) difference between cod liver oil and coconut oil supplemented feed fed *M. rosenbergii*.

Proximate biochemical constituents

The formulated feed fed groups were subjected to analyze the body biochemical composition such as, crude nitrogen, protein, amino acid, carbohydrate, lipid and ash, the results are provided in Table 3. The protein, total amino acid, carbohydrate, lipid and ash contents were significantly ($P < 0.05$) elevated in sunflower oil, incorporated feed fed group, followed by cod liver oil, coconut oil and castor oil supplemented feed,

Table 2 Morphometric data and nutritional utilization parameters of *M. rosenbergii* PL fed with different oil supplemented feeds.

Parameters		Cod liver oil	Sunflower oil	Coconut oil	Castor oil
Morphometry	Length (cm)	4.50 ± 0.56^{a}	4.76 ± 0.52^{a}	3.86 ± 0.26^{b}	3.78 ± 0.42^{b}
	Weight (g)	0.80 ± 0.17^{a}	0.82 ± 0.22^{a}	0.66 ± 0.14^{b}	0.63 ± 0.16^{b}
	Survival rate (%)	83.33 ± 3.80^{a}	86.66 ± 4.10^{a}	76.66 ± 3.50^{b}	63.66 ± 2.10^{c}
Nutritional index	Weight gain (g)	0.64 ± 0.02^{ab}	0.66 ± 0.04^{a}	0.50 ± 0.05^{b}	0.47 ± 0.03^{c}
	Length gain (cm)	3.39 ± 0.20^{a}	3.65 ± 0.15^{a}	2.75 ± 0.17^{b}	2.67 ± 0.12^{b}
	Feeding rate (g d^{-1})	0.50 ± 0.016^{a}	0.53 ± 0.05^{a}	0.47 ± 0.03^{c}	0.42 ± 0.02^{d}
	Feed conversion ratio (g)	1.74 ± 0.12^{a}	1.72 ± 0.11^{b}	2.18 ± 0.14^{a}	2.24 ± 0.08^{a}
	Specific growth rate (%)	1.16 ± 0.12^{a}	1.18 ± 0.13^{a}	1.01 ± 0.11^{b}	0.98 ± 0.13^{b}
	Protein efficiency ratio (%)	0.65 ± 0.03^{ab}	0.80 ± 0.04^{a}	0.48 ± 0.01^{b}	0.47 ± 0.01^{c}

Each value is a Mean ± SD of three replicate analysis, within each row means with different superscript letters are statistically significant at $P < 0.05$ (one way ANOVA and subsequently *post hoc* multiple comparison with DMRT).

Table 3 Biochemical constituents and carcass mineral content of *M. rosenbergii* fed with different oil supplemented feeds.

Parameters		Cod liver oil	Sunflower oil	Coconut oil	Castor oil
Proximate biochemical constituents	Nitrogen (%)	10.36 ± 1.02^{a}	10.78 ± 1.13^{a}	9.07 ± 1.10^{b}	8.75 ± 1.21^{b}
	Crude protein (%)	64.75 ± 4.72^{a}	67.42 ± 3.69^{a}	56.70 ± 2.95^{b}	54.73 ± 4.67^{b}
	Amino acid (mg/g wet wt.)	86.66 ± 4.16^{ab}	89.33 ± 3.05^{a}	82.00 ± 4.00^{b}	77.33 ± 3.05^{c}
	Carbohydrate (mg/g wet wt.)	41. 90 ± 1.61^{a}	43.77 ± 2.49^{a}	40.72 ± 1.42^{a}	35.22 ± 1.07^{b}
	Lipid (mg/g wet wt.)	21.20 ± 0.92^{a}	22.63 ± 1.75^{a}	19.33 ± 2.26^{ab}	15.13 ± 3.34^{b}
	Moisture (%)	61.33 ± 2.34^{b}	60.33 ± 3.0^{b}	63.33 ± 2.54^{ab}	67.66 ± 3.82^{a}
	Ash (%)	17.00 ± 1.20^{a}	17.33 ± 1.43^{a}	16.54 ± 2.0^{a}	15.62 ± 1.42^{b}
Minerals	Sodium (mg/g)	0.27 ± 0.01^{a}	0.29 ± 0.02^{a}	0.25 ± 0.04^{a}	0.11 ± 0.03^{b}
	Potassium (mg/g)	0.62 ± 0.04^{a}	0.69 ± 0.03^{a}	0.57 ± 0.03^{a}	0.30 ± 0.05^{b}

Each value is a Mean ± SD of three replicate analysis, within each row means with different superscript letters are statistically significant at $P < 0.05$ (one way ANOVA and subsequently *post hoc* multiple comparison with DMRT).

whereas these contents are significantly lower in coconut oil and castor oil supplemented feeds, when compared to cod liver oil and sunflower oil supplemented feed fed prawns. However, the moisture content was significantly higher ($P < 0.05$) in prawns fed with coconut oil and castor oil incorporated feed.

Minerals

The sodium and potassium contents showed significantly no difference ($P > 0.05$) in sunflower oil and coconut oil incorporated feed fed groups when compared with cod liver oil. The castor oil supplemented feed fed group showed low performance when compared with other groups (Table 3).

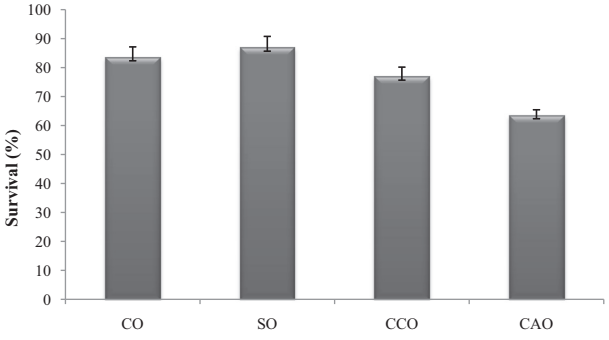

Fig. 1 Survival (%) of *M. rosenbergii* fed with different oil supplemented feeds. CO, Cod liver oil; SO, Sunflower oil; CCO, Coconut oil; CAO, Castor oil.

Discussion

Survival and growth

In the present study, three vegetable oils incorporated feeds were tested, among these oils, sunflower oil incorporated feed fed group showed a significant improvement in survival and growth performance. The present results revealed that the sunflower oil supplemented feed on par with cod liver oil (Fig. 1). These results agree with previous findings of Kim et al. (2012) and Bhavan et al. (2013). Similarly, fish oil replaced with sunflower oil, palm oil, linseed oil, rapeseed oil, coconut oil and peanut oil incorporated feed fed *M. peelii*, *Clarias gariepinus* and *D. puntazzo* attained a significant improvement in survival and growth (Turchini et al., 2011; Piedecausa et al., 2007). Also, the better survival and growth were proved in coconut oil incorporated feed fed *C. auratus* (Parameshwaran et al., 2002, corn oil incorporated feed fed mud crab, *Scylla serrata* (Holme et al., 2007) and sunflower oil supplemented feed fed *S. salar* (Miller et al., 2007). However, other trials with Atlantic salmon have shown that substitution of fish oil with sunflower oil does not significantly affect the growth (Bell et al., 1996). Hence, the present study indicates that sunflower oil incorporated feed produces better survival and growth in freshwater prawn *M. rosenbergii* PL, and it can be beneficial for the freshwater prawn culture.

Nutritional index

In the present investigation, the nutritional indices such as feeding rate, specific growth rate and protein efficiency ratio showed a significant improvement in sunflower oil supplemented feed fed prawns and the nutritional index parameters significantly were similar with the cod liver oil group. The coconut oil and castor oil supplemented feed fed groups showed low performance when compared with cod liver oil, while the feed conversion ratio was significantly low in cod liver oil and sunflower oil supplemented feed fed prawns but it showed the superior quality and better utilization of the sunflower oil supplementation. Previous studies demonstrate that significant elevations were recorded in weight, length gain, and specific growth rate of *M. rosenbergii* and *T. tambroides* fed with palm oil, sunflower oil, canola oil and linseed oil included diet (Kamarudin et al., 2011; Kim et al., 2012; Bhavan et al., 2013). Sunflower oil, linseed oil, palm oil, soya bean oil, rapeseed oil, coconut oil and peanut oil supplemented feed fed fishes, such as *S. salar*, *D. puntazzo* and *C. gariepinus* led to appreciable weight, length gain, specific growth rate, feeding rate, protein efficiency ratio, feed efficiency and protein productive value (Bransden et al., 2003; Miller et al., 2007; Piedecausa et al., 2007). The present study demonstrated that the sunflower oil could promote the feed consumption and lead to better growth and nutrient consumption of *M. rosenbergii* PL.

Biochemical constituents

In a nutritional point of view, the proximate biochemical composition of any edible organism is very crucial. The nutritive values of crustaceans depend upon their body biochemical constituents (Vijayavel and Balasubramanian, 2006). Body biochemical composition is a good indicator for physiological condition and easy to assess in cultivable organisms. In this study, the biochemical constituents such as protein, amino acids, carbohydrate, lipid and ash contents were significantly ($P < 0.05$) elevated in *M. rosenbergii* fed with cod liver oil and sunflower oil supplemented feed. Meanwhile, there were no significant differences in carbohydrate and ash contents between cod liver oil, sunflower oil and coconut oil supplemented feed fed *M. rosenbergii* PL (Fig. 2). These results clearly indicated that vegetable oils could promote the nutrient absorption, nutrient accumulation and growth of *M. rosenbergii* PL. Recently, Kim et al. (2012) and Bhavan et al. (2013) reported that sunflower oil enriched with *Artemia* supplemented feed fed *M. rosenbergii* attained better protein, lipid and ash contents when compared with fish oil. In addition, the soya bean oil, linseed oil, coconut oil and peanut oil supplemented feed fed *D. puntazzo* and *C. gariepinus* gained a significant improvement in body carcass composition (Piedecausa et al., 2007; Aderolu and Akinrem, 2009).

Minerals

Minerals play a crucial role in cultivable organisms due to their catalytic properties. Soluble minerals like Ca, P, Na, K and Cl are involved in the maintenance of acid–base balance and membrane potential (Sudhakar et al., 2009). Sodium is the main monovalent ion in extracellular fluids and constitutes 93% of the ions (bases) found in the blood stream. It has

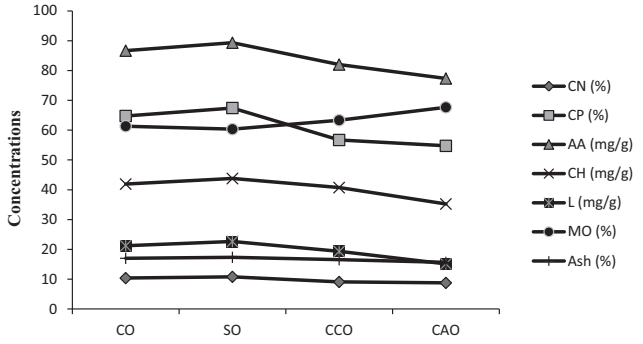

Fig. 2 Biochemical constituents of *M. rosenbergii* fed with different oil supplemented feeds. CO, Cod liver oil; SO, Sunflower oil; CCO, Coconut oil; CAO, Castor oil. CN, Crude nitrogen; CP, Crude protein; AA, Amino acid; CH, Carbohydrate, L, Lipid; MO, Moisture.

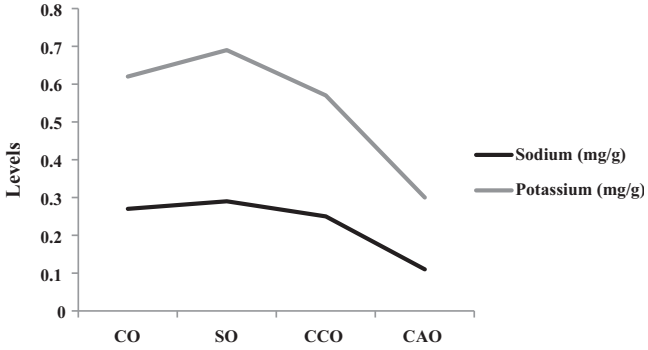

Fig. 3 Sodium (Na^+) and potassium (K^+) contents of *M. rosenbergii* fed with different oil supplemented feeds. CO, Cod liver oil; SO, Sunflower oil; CCO, Coconut oil; CAO, Castor oil.

principal role in the regulation of osmotic pressure, the maintenance of acid–base balance, muscle irritability and absorption of carbohydrate. Potassium is the major constitute in intracellular fluid, which regulates osmotic pressure, acid–base balance, and has a stimulating effect on muscle irritability. Potassium is also required for glycogen and protein synthesis, and the metabolic breakdown of glucose. In aquatic animals, increasing dietary potassium levels leads to the increase in survival, weight, feed and protein efficiency ratio in *Penaeus monodon* (Shiau and Hsieh, 2001). Kanazawa et al. (1984) reported that a dietary level of 0.9% of diet improved growth in *Mallotus japonicus*. In the present study, no significant ($P > 0.05$) difference was found in Na^+ and K^+ contents between fish oil and vegetable oils supplemented feed fed prawns (Table 3; Fig. 3). It indicates that the vegetable oils do not suppress the dietary mineral utilization, but they stimulate survival, growth and other nutritional indices of *M. rosenbergii* PL.

Conclusion

Among the three test feeds, the sunflower oil supplemented feed fed prawns show better performance than cod liver oil, coconut oil and castor oil in survival, growth indices and biochemical constituents, the results were similar with the cod liver oil supplemented feed fed group. The present result

revealed that the sunflower oil was on par with cod liver oil which can be replaced by low cost sunflower oil as an ideal dietary lipid source for sustainable nursery maintenance of *M. rosenbergii* PL.

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