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Growth Performance, Survival, and Body Composition of Post Larvae of the Freshwater Prawn (*Macrobrachium rosenbergii*) Fed Graded L-Carnitine Diets

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Key words: *Macrobrachium rosenbergii*, L-carnitine, growth, feed conversion, protein efficiency

Abstract

The aim of this study was to investigate the efficacy of L-carnitine incorporated diets on growth, specific growth rate (SGR), feed conversion efficiency (FCE), protein efficiency ratio (PER), survival, and body composition of post larvae of the freshwater prawn *Macrobrachium rosenbergii*. Five diets containing 0, 0.25, 0.50, 0.75, or 1 g L-carnitine/kg diet were fed to post larvae (0.105±0.028 g, 2.21±0.13 cm) for thirteen weeks. The highest SGR (3.42±0.44%/day), FCE (40.40±1.45%), and PER (1.05±0.02) were obtained in the 0.50 treatment. The lipid level in the post larvae was inversely related to the L-carnitine level in the diet ($p<0.05$). The protein level increased with supplementation until the 0.50 level, then decreased with higher levels. Using second-order polynomial regression analysis, the optimal weight gain, SGR, and FCE were obtained with L-carnitine levels of 0.484 g/kg, 0.466 g/kg, and 0.48 g/kg, respectively. In conclusion, 0.50 g L-carnitine per kg feed is optimum for good growth in *M. rosenbergii* post larvae.

Introduction

L-carnitine (γ-trimethylammonium-β-hydroxy butyrate) is an optically active, water soluble, quaternary ammonium base, widely distributed in tissues of nearly all organisms (Yagcioglu and Aktas, 2006). It is synthesized from the essential amino acids, lysine and methionine, with the assistance of vitamin C, secondary compounds produced in the body (Harpaz, 2005), and a product derived from protein metabolism (Bremer, 1961). Its main function is to transport long-chain fatty acids from the cytoplasm to the mitochondrial matrix where they are metabolized by β-oxidation enzymes (Fritz and Yue, 1963; Bilinski and Jonas, 1970). Higher transport rates can lead to a greater supply of energy from β-oxidation that improves feed utilization (Torreele et al., 1993).

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L-carnitine stimulates the tricarboxylic acid (TCA) cycle for the production of α-ketoglutarate and acyl group flux into mitochondria, a theory that involves the production of non essential amino acids by carnitine (Santulli et al., 1990). It also helps in the transportation of acetylic groups from the mitochondria to the cytoplasm where they are utilized by the fatty acid synthetax complex. Thus, supplementation of carnitine in the diet enhances the rate of fatty acid oxidation and increases metabolic flux in the Kreb's cycle, resulting in the production of energy for metabolic processes and sparing protein for growth (Jayaprakas and Sambhu, 1996). When L-carnitine is deficient, oxidation of fatty acids is reduced and diverted into tri-acyl glycerol synthesis, particularly in the liver. Mitochondria failure can occur when there is insufficient tissue carnitine available to buffer toxic acyl-coenzyme (CoA) metabolites (McDowell, 1989).

L-carnitine in fish feed leads to excretion of high levels of TMA (tri-methylamine) and TMAO (tri-methylamine oxide) into the environment. These, in turn, act as chemostimulants that increase feed consumption and utilization by fish (Harpaz, 2005). TMA and TMAO are powerful attractants for fish and crustaceans (Harpaz, 1997). Their presence in the environment leads to intensive food searching in crustaceans (Harpaz et al., 1987), higher food consumption, and better growth (Harpaz, 1997).

Growth enhancement in the commercially cultured freshwater prawn, *Macrobrachium rosenbergii*, has been a challenging task for aquaculturists. The purpose of the present study was to determine the effects of various levels of dietary L-carnitine on growth performance, survival, and body composition of post larvae *M. rosenbergii*.

Materials and Methods

Five experimental diets, containing 0, 0.25, 0.50, 0.75, or 1 g L-carnitine per kg feed, were prepared separately by hand-kneading the ingredients, mixing them with water (1:0.8), heating the mixture at 105°C for 30 min, and adding vitamin and mineral mixtures and L-carnitine (Hi-Media Laboratory, Mumbai, India) to the cooled dough (Table 1). The well-mixed dough was pelletized using a 1-mm die. The pellets were dried at 40°C in a hot-air oven and stored in airtight plastic bottles. Diets were formulated to contain 38% crude protein and 2500 kcal digestible energy per kg diet.

The experiment was conducted at Taraporevala Marine Biological Research Station laboratory at Mumbai, India. *Macrobrachium rosenbergii* post larvae (20-day-old) were obtained from the hatchery of the Central Institute of Fisheries Education in Mumbai, India. Upon arrival, they were acclimated to laboratory conditions for two weeks in a plastic pool (1.21 m diameter, 0.61 m height; 710-l capacity), during which time they were fed pelleted feed to satiation. After acclimatization, 15 experimental glass aquaria (0.45 x 0.3 x 0.3 m; 40.5-l capacity) were stocked with 20 post larvae each. Each day, 25% of the water was removed from the plastic tank and glass aquaria to remove uneaten food and excreta and to replenish that volume with fresh water. Diets were tested in three replicates and fed to post larvae at 5% of their body weight, twice a day (9:00 and 17:00) for thirteen weeks.

Temperature (28-29.5°C), pH (7.1-7.3), dissolved oxygen (6.0-6.4 mg/l), and total alkalinity (63-65 mg/l) were recorded on alternate days (APHA, 1985). The body length and weight of the post larvae were measured at 15-day intervals to assess growth performance and calculate specific growth rate. Post larvae were weighed on an electronic weighing balance (Contech, minimum 0.001 g). Growth parameters were calculated as: specific growth rate (SGR) = $(\ln W_2 - \ln W_1)/T \times 100$, where W_2 is final weight, W_1 is initial weight, and T is duration in days; feed conversion efficiency (FCE) = wet wt gain/dry wt of feed x 100; protein efficiency ratio (PER) = increment in body wt/protein intake; weight gain = final wt - initial wt; and length gain = final length - initial length.

At the end of experiment, 15 post larvae from each treatment were analyzed for final body composition. Proximate compositions of the diets and post larvae were analyzed for

Table 1. Formulation and proximate composition of the experimental diets.

	Diet (L-carnitine content)				
	0	*0.25*	*0.50*	*0.75*	*1.0*
Ingredient (%)					
Fishmeal	45	45	45	45	45
Soy bean meal	10	10	10	10	10
Fish oil	2	2	2	2	2
Soya oil	2.5	2.5	2.5	2.5	2.5
Vitamin premix[a]	2.5	2.5	2.5	2.5	2.5
Mineral premix[b]	2.5	2.5	2.5	2.5	2.5
Carboxy methyl cellulose	1.5	1.5	1.5	1.5	1.5
Wheat flour	34	33.75	33.50	33.25	33
L-carnitine	0.0	0.25	0.50	0.75	1
Proximate composition (%)					
Protein	38.37	38.41	38.34	38.32	38.49
Lipid	9.21	9.19	9.29	9.30	9.34
Ash	12.6	12.11	12.16	12.9	12.2
Moisture	8.6	8.12	8.19	8.6	8.14
Digestible energy (kcal/kg)[c]	2490	2510	2510	2480	2510

[a] per kilogram feed: 5500 IU vitamin A; 1000 IU vitamin D_3; 50 IU vitamin E; 10 IU vitamin K_3; 550 mg choline chloride; 100 mg niacin; 20 mg riboflavin; 20 mg thiamin; 50 mg pantothenic acid; 0.1 mg biotin; 5 mg folacin; 20 mg cyanocobalmin (B_{12}); 100 mg vitamin C; 100 mg inositol.

[b] per kg feed: 257 mg NaCl; 3855 mg $MgSO_4$; 6425 mg $Na_2H_2PO_4$; 8224 mg KH_2PO_4; 13,540 mg $Ca(H_2PO_4)_2$; 642.5 mg $FeC_6H_5O_7$; 90.7 mg $ZnSO_4$; 41.6 mg $MnSO_4$; 7.97 mg $CuSO_4$; 0.26 mg $CoCl_2$; 0.77 mg KIO_3.

[c] Digestible energy (kcal/kg) was calculated as per De Silva and Anderson (1995).

crude protein (N Kjeldahl x 6.25), crude lipid (solvent extraction with petroleum ether, B.P. 40-60°C for 10-12 h), and ash (incineration in a muffle furnace at 650°C for 4-6 h) by methods described in AOAC (1990).

Growth data were subjected to two-way analysis of variance (Snedecor and Cochran, 1967) and multiple range test (Duncan, 1995). Second-order polynomial regression analysis ($Y= a + bX + cX^2$) was used to determine the optimum dietary L-carnitine (Sancheti and Kapoor, 1981). Differences were considered significant when $p<0.05$.

Results

The L-carnitine diets produced better growth than the control diet (Table 2). There were significant differences in weight gain among all treatments. The weight gains from the start of the experiment until day 15 ranged 70-130%; from day 16 to day 30, 82.35-152.17%; from day 31 to 45, 48.78-57.14%; from day 46 to 60, 31.48-59.74%; from day 61 to 75, 23.17-43.33% and from day 76 to 91, 8.80-22.48%. From day 0 to 30, the weight gain was highest in the 0.50 L-carnitine diet, followed by the 0.25, 0.75, 1.0, and 0 diets. After day 30, the weight gain gradually decreased in all treatments.

Table 2. Effects of dietary L-carnitine on growth, survival, feed efficiency, and body composition of *Macrobrachium rosenbergii* post larvae (means±SD; n = 3).

	Diet (L-carnitine content)				
	0	*0.25*	*0.50*	*0.75*	*1.0*
Initial wt (g)	0.105±0.028	0.105±0.028	0.105±0.028	0.105±0.028	0.105±0.028
Final wt (g)	0.96±0.14	1.89±0.11	2.29±0.24	1.68±0.13	1.31±0.07
Wt gain (g)	0.86±0.12^{a}	1.79±0.14^{b}	2.19±0.28^{c}	1.58±0.12^{d}	1.21±0.09^{e}
Wt gain (%)	760	1690	2090	1480	1110
Daily wt increment (g/day)	0.009±0.001^{a}	0.020±0.002^{b}	0.024±0.001^{c}	0.017±0.001^{d}	0.013±0.002^{e}
Specific growth rate (%/day)	2.46±0.38^{a}	3.21±0.33^{b}	3.42±0.44^{c}	3.08±0.22^{d}	2.80±0.17^{e}
Initial length (cm)	2.21±0.13	2.21±0.13	2.21±0.13	2.21±0.13	2.21±0.13
Final length (cm)	4.93±0.35	6.00±0.24	6.40±0.38	5.81±0.18	5.42±0.87
Length gain (cm)	2.72±0.22^{a}	3.79±0.41^{b}	4.19±1.01^{c}	3.6±0.23^{d}	3.21±0.18^{e}
Feed conversion efficiency (%)	33.45±2.32^{a}	38.06±2.32^{b}	40.40±1.45^{c}	36.27±3.05^{d}	35.87±2.56^{d}
Protein efficiency ratio	0.87±0.06^{a}	0.99±0.04^{b}	1.05±0.02^{c}	0.92±0.07^{d}	0.93±0.01^{d}
Survival (%)	86.32±4.26^{a}	84.26± 2.04^{a}	86.66±1.44^{a}	84.71±3.53^{a}	85±4.08^{a}
Proximate composition (%)					
Moisture	76.93±1.56^{a}	76.14±1.56^{a}	74.81±2.53^{c}	75.79±3.12^{a}	77.35±0.88^{a}
Protein	15.42±1.58^{a}	16.67±1.08^{b}	18.48±0.73^{c}	17.51±0.25^{d}	16.21±0.64^{b}
Lipid	2.84±0.14^{a}	2.56±0.28^{b}	2.23±0.12^{c}	1.92±0.22^{d}	1.79±0.18^{e}
Ash	3.32±0.32^{a}	3.12±0.84^{a}	3.00±0.42^{a}	3.28±0.35^{a}	3.21±0.58^{a}

Values in a row with different superscripts significantly differ (p<0.05).

The 0.50 diet produced the best SGR, FCE, and PER. There were no significant differences in survival. The protein content in body tissues of the post larvae was significantly highest in the 0.50 diet. Lipid content was significantly lower in all L-carnitine treatments than in the control. The relationships between dietary L-carnitine to weight gain, SGR, and FCE are best described using a second-order polynomial regression analysis according to which the X_{max} value that corresponds with Y_{max} is defined as the optimum dietary L-carnitine level for promoting growth, SGR, and FCE; beyond this level, these factors decrease (Fig. 1). According to this analysis, the optimal weight gain, SGR and FCE were obtained with L-carnitine levels of 0.484 g/kg, 0.466 g/kg, and 0.48 g/kg, respectively.

Discussion

The diet containing 0.50 g L-carnitine per kg feed produced the highest weight and length gains while the lowest were obtained in the unsupplemented control. Similarly, 500 mg/kg L-carnitine significantly enhanced growth in white shrimp (*Penaeus indicus*; Jayaprakas and Sambhu, 1996) and tiger shrimp (*Penaeus monodon*; Groth et al., 1998). In contrast, there were no statistical differences in growth or survival of the tiger shrimp (*Penaeus monodon*; Schmeckel, 1995; Groth, 1997) or kuruma shrimp (*Marsupenaeus japonicus*; Yagcioglu and Aktas, 2006) fed diets containing different levels of L-carnitine. The lower weight gain accompanying higher levels of L-carnitine could be attributed to energy loss through excretion of excess

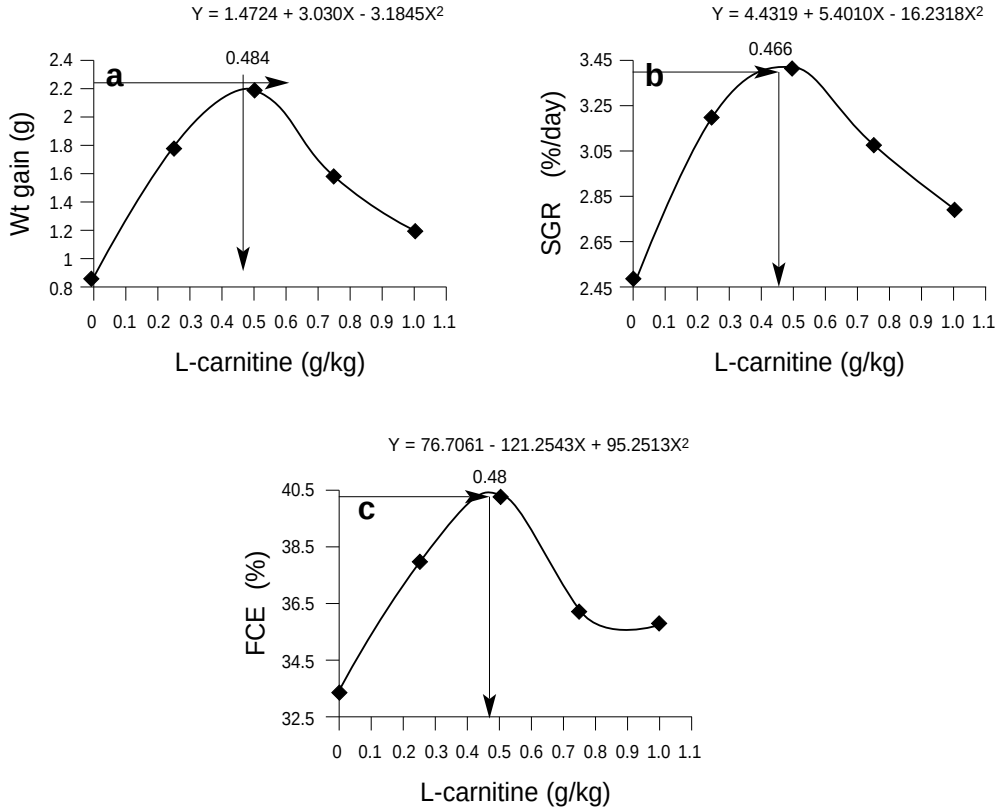

Fig. 1. Second-order polynomial fitting of (a) weight gain, (b) specific growth rate, and (c) feed conversion efficiency to levels of L-carnitine supplementation in diets for *Macrobrachium rosenbergii* post larvae.

acyl-carnitine (Keshavanath and Renuka, 1998).

SGR, FCE, and PER were best in the 0.50 L-carnitine. Similar observations were observed in white shrimp fed a diet containing 500 mg/kg L-carnitine (Jayaprakas and Sambhu, 1996). The increased growth rate seems to be due to the improved feed conversion via increased fatty acid oxidation and utilization of dietary energy (Torreele et al., 1993; Becker and Focken, 1995; Keshavanath and Renuka, 1998). The addition of carnitine to a diet facilitates the transport of long-chain fatty acids into the mitochondria, resulting in extra energy from β-oxidation (Bilinski and Jonas, 1970). Higher feed conversion efficiency may be attributed to enhanced digestion and assimilation of food resulting from carnitine administration (Jayaprakas et al., 1996).

L-carnitine plays a major role in lipid metabolism (Borum, 1987; Torreele et al., 1993; Burtle and Liu, 1994) by transferring fatty acids from the cytoplasm to the mitochondrial matrix where they are metabolized by β-oxidation. Oxidation of fat provides the highest and most cost effective energy yield per weight of dietary ingredients. L-carnitine promotes the oxidation of fat and, therefore, addition of carnitine to fish diets should enhance protein sparing action and lead to better growth (Harpaz, 2005). In the present investigation, lipid concentration in the body of

the post larvae considerably dropped with the increase in L-carnitine, similar to results in tiger shrimp (Groth et al., 1998) and white shrimp (Jayaprakas and Sambhu, 1996). L-carnitine increased the protein content in the post larvae, as it did in tiger shrimp (Groth et al., 1998) and white shrimp (Jayaprakas and Sambhu, 1996).. Muscle protein content in carnitine treated shrimps is indicative of enhanced protein synthesis (Jayaprakas and Sambhu, 1996).

In conclusion, the present study shows that 0.50 g L-carnitine per kg feed helps *M.rosenbergii* post larvae attain maximum weight gain and better SGR, FCE, and PER values. Body lipid decreased with the increase of L-carntine supplementation, indicating that L-carnitine reduces unnecessary lipid accumulation, an essential attribute of cultured species. This study is a first step towards the establishment of a better feed for *M. rosenbergii* post larvae. However, further studies with economic evaluations are suggested.

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