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Growth Response of White Prawn, *Penaeus indicus*, to Dietary L-Carnitine

V. JAYAPRAKAS and C. SAMBHU

Department of Aquatic Biology and Fisheries
University of Kerala
Beach P.O., Trivandrum - 695 007
Kerala, India

Abstract

Juveniles of *Penaeus indicus* were fed a biosynthetic product, L-carnitine, at concentrations of 100 ppm (T1), 250 ppm (T2), 500 ppm (T3), 750 ppm (T4) and 1,000 ppm (T5) in a fish meal-based supplementary feed having 40% protein. A diet without L-carnitine supplement served as control (T0). The growth study was conducted for 120 d in nylon hapas, arranged in a brackishwater pond. All diets with L-carnitine produced significantly higher growth than the control ($P<0.01$). The diet containing 500 ppm carnitine promoted maximum growth (14.82 ± 2.86 g) compared with the control (9.07 ± 1.67 g). Feed conversion efficiency, assimilation efficiency and protein efficiency ratio were high in carnitine-fed prawns; optimum values were recorded for the 500 ppm treatment. Digestive enzyme (amylase, protease and lipase) activities were high in the mid-gut of prawns fed carnitine diets. RNA/DNA ratios in the muscle and hepatopancreas of prawns under carnitine treatments were higher than the control. Total dry matter and protein contents of the body increased in prawns fed L-carnitine. Lipid content decreased in all carnitine-fed groups with maximum value in the control (2.69%), indicating enhanced lipid catabolism, thereby sparing energy and protein for growth and anabolic processes.

Introduction

L-carnitine is a product derived from protein metabolism (Bremer 1961). Its main function is the transport of long chain fatty acids from the cytoplasm to the mitochondrial matrix where they are metabolized by beta oxidation enzymes, thus playing a vital role in the regulation of fat burning (Fritz and Yue 1963; Bremer 1983). A few investigators have assessed the role of L-carnitine on lipid metabolism and growth in farm animals and certain cultivable fishes (Snoswell and Henderson 1980; Santulli et al. 1990; Newton and Burtle 1993; Burtle and Liu 1994; Becker and Focken 1995; Keshavanath and Renuka 1995). However, no study has hitherto been conducted to evaluate the impact of L-carnitine on growth of penaeid prawns. Hence, this investigation was carried out with a view to assess the role of L-carnitine on growth, feed utilization, digestive enzyme activity, RNA/DNA ratio and proximate composition of the white prawn, *Penaeus indicus.*

Materials and Methods

The growth experiment was conducted in nylon hapas (1 m^2 area) fixed in a brackishwater culture pond (0.5 ha) adjacent to Paravoor Lake (41.8°46'N, 76°44'N-76°48'E), Kerala, India, for 120 d. Juveniles of *P. indicus* were procured from Azhikode Hatchery, and maintained in a hapa for 10 d prior to the commencement of the experiment. A standard fish meal-based prepared pelleted diet having 40% protein (Table 1) was used for carnitine incorporation. L-carnitine (Sigma Chemical Co., USA) was administered at dosages of 100 ppm (T1), 250 ppm (T2), 500 ppm (T3), 750 ppm (T4) and 1,000 ppm (T5). A control (T0) was also maintained. Thirty juveniles of uniform size (0.13 ± 0.03 g) were randomly stocked in each hapa. The treatments were randomly assigned to the hapas. Three replications were maintained for each treatment. Feeding was at 10% body weight per day during the first 30 d, reducing it to 5% thereafter. The quantity of feed was readjusted based on growth of the prawn assessed every fortnight. On each sampling, 15 prawns were collected at random to record total length and weight. Water quality parameters (temperature, pH, dissolved oxygen and salinity) were monitored biweekly (APHA 1992). On termination of the experiment, all surviving prawns were collected, their individual lengths and weights recorded, and samples analyzed for digestive enzymes (amylase, protease, lipase), RNA, DNA contents and proximate composition of the muscle. Saccharogenic assay (King 1965) was followed for estimating amylase activity. One amylase unit is the milligram of maltose liberated every 10 minutes at 30°C under assay conditions. Total protease activity was measured by the casein digestion method of Kuntiz (1947). One protease unit is the milligram of tyrosine liberated every 15 minutes at 30°C under the assay conditions. Bier's (1962) titrimetric method was used with minor modifications for estimating lipase activity. One lipase unit is the amount of 0.025 N NaOH required to neutralize the fatty acids liberated during 18 h of incubation at 30°C under the assay conditions. Total and specific activities of the digestive enzymes were estimated by King's (1965) methods as follows:

$$\text{Total activity} = \frac{\text{AU}\cdot\text{PU}^{-1}\cdot\text{LU}^{-1}}{\text{g tissue}} \qquad \text{Specific activity} = \frac{\text{AU}\cdot\text{PU}^{-1}\cdot\text{LU}^{-1}}{\text{mg protein}}$$

(AU = amylase unit; PU = protease unit; LU = lipase unit).

Table 1. Proportion of ingredients and proximate composition of the diet.

			Proximate composition of the diet	
Ingredients	Proportion (g)	Protein (%)	Parameter	Composition (%)
Ricebran	13.24	1.15	Moisture	12.31
Groundnut oilcake	36.76	18.49	Protein	40.02
Tapioca flour	13.24	0.29	Lipid	9.14
Fish meal	36.76	20.07	Carbohydrate	3.27
Total	100.00	40.00	Ash	25.11
			Fiber	4.81

DNA and RNA contents were estimated following the methods of Giles and Myres (1965) and Ceriotti (1955) and expressed as $\mu g \cdot g^{-1}$ tissue.

A separate laboratory experiment was conducted to assess feed consumption, conversion efficiency, apparent protein and lipid digestibility with the carnitine incorporated and control feed for a period of 30 d. This study was carried out in plastic troughs (50 l) employing five prawns each, and each treatment was replicated thrice. To determine digestibility, prawns were provided with weighed amounts of feed for 6 h daily. Unconsumed feed and fecal matter were collected, dried and weighed. Pooled fecal samples were used for nutrient analysis. Apparent nutrient (protein and lipid) digestibility (%) was calculated as nutrient in feed-nutrient in excreta·nutrient in feed x 100^{-1}. Proximate analyses of feed ingredients, prepared feed and prawn muscle were carried out following AOAC (1990) procedures. Crude protein was calculated from the nitrogen content determined by microkjeldahl method and the estimation of lipid was done using sulphophosphovanillin method. Glycogen was estimated using anthrone-alcohol treatment and fiber was extracted following the method recommended by Pearson (AOAC 1990). A muffle furnace was used to determine ash content.

ANOVA (Snedecor and Cochran 1968) was used for testing statistical significance of data, and the treatment means were subjected to Duncan's multiple range test (Steel and Torrie 1980).

Results

The mean water temperature ranged from 23.0 to 25°C, while water pH fluctuated between 7.42 and 7.87. Dissolved oxygen level was 5.65-6.71 $mg \cdot L^{-1}$ and salinity 21.08-28.72‰. These parameters were within the optimum range suitable for prawn growth.

Fortnightly growth recorded in terms of weight are presented in Fig. 1. Prawns receiving 500 ppm (T3) carnitine grew faster than T2 (11.61 g), T5 (10.8 g), T1 (9.81 g) and T0 (9.07 g). Specific growth rate and survival were maximum in T3 and minimum in T0 (Table 2). Feed consumption conversion efficiency, protein efficiency ratio and apparent nutrient (protein and lipid) digestibility were high in carnitine-treated prawns, maximum being in T3 (Table 3). Digestive enzyme (amylase and protease) activities increased due to carnitine administration (Table 4). Higher levels of carnitine in the diet suppressed enzyme activity. Lipase activity increased with higher levels of carnitine (Table 4). RNA/DNA ratios were high in the muscle and hepatopancreas of carnitine-treated prawns as compared to the control (Figs. 2 and 3). Protein content increased in carnitine-treated prawns, being maximum in T3. Lipid content showed a marked decrease in carnitine-fed prawns from that of the control (Table 5).

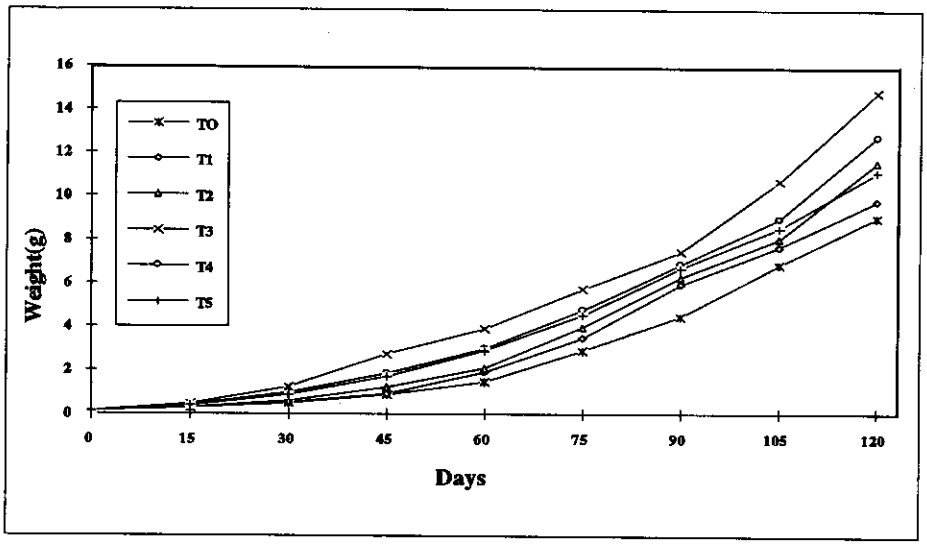

Fig. 1. Effect of L-carnitine on the growth in weight of *P. indicus*.

Discussion

It is evident from the study that carnitine promotes growth in *P. indicus* up to 500 ppm. However, higher dosages (750 and 1,000 ppm) resulted in growth retardation, though better than the control (T0). Saliny (1994) reported that 700 ppm carnitine induced maximum growth in a freshwater prawn, *Macrobrachium idella idella*. Thus, it appears that the optimum dosage of L-carnitine required for growth stimulation is species-dependent. Better growth obtained in treated *P. indicus* can be correlated with increased food consumption, conversion efficiency and nutrient digestibility (Table 3). High feed conversion efficiency may be attributed to enhanced digestion and assimilation of food as a result of carnitine administration. Enhanced feed conversion efficiency and digestibility were also observed in *M. idella idella* fed carnitine-incorporated diets (Saliny 1994). L-carnitine improved digestive enzyme activities in the stomach, hepatopancreas and intestine of *P. indicus*. Increased protein and lipid digestibility observed in the carnitine-treated prawns may be attributed to enhanced protease and lipase activities in the stomach and intestine. Thus, it can be inferred that dietary carnitine has a stimulatory effect on digestive enzymes in *P. indicus* as observed in *M. idella idella* (Saliny 1994).

The increased RNA, DNA content and RNA/DNA ratio observed in the hepatopancreas and muscle of carnitine-treated prawns (Figs. 2 and 3) indicate enhanced protein synthesis in these tissues leading to better growth. The high muscle protein content found in carnitine-treated prawns is indicative of enhanced protein synthesis. Carnitine plays a major role in lipid metabolism (Borum 1987; Torreele et al. 1993, Burtle and Liu 1994; Keshavanath and Renuka 1995) by transporting fatty acids from the cytoplasm to the mitochondrial matrix where they are metabolized by beta oxidation. Santulli et al. (1990) suggested that carnitine stimulates the TCA cycle for the production of alpha ketoglutarate and acyl group flux into mitochondria, a theory that implicates

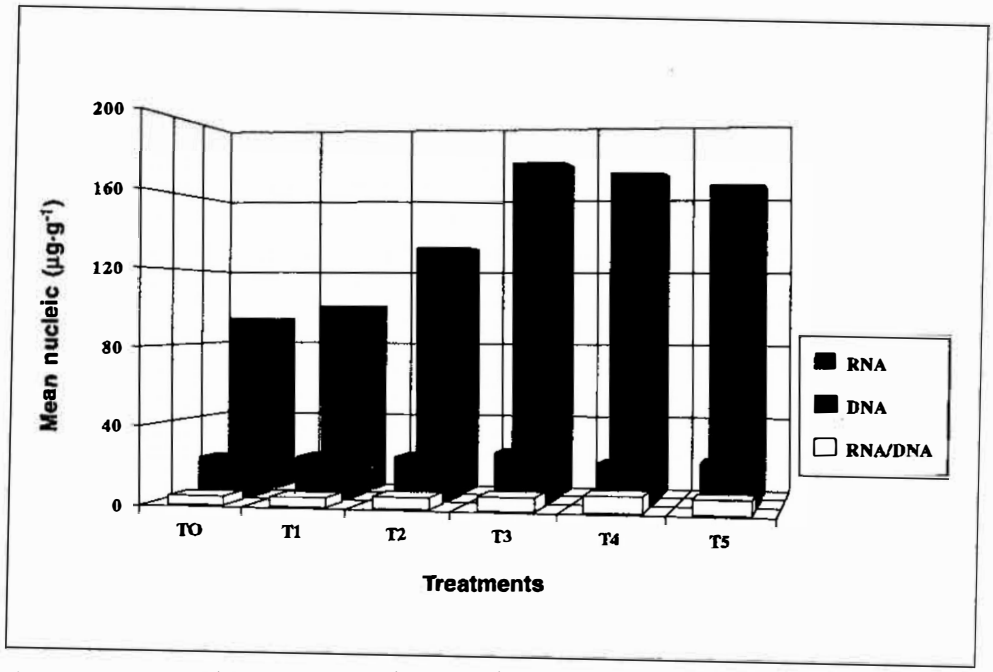

Fig. 2. Effect of L-carnitine on nucleic acid content in the muscle of *P. indicus*.

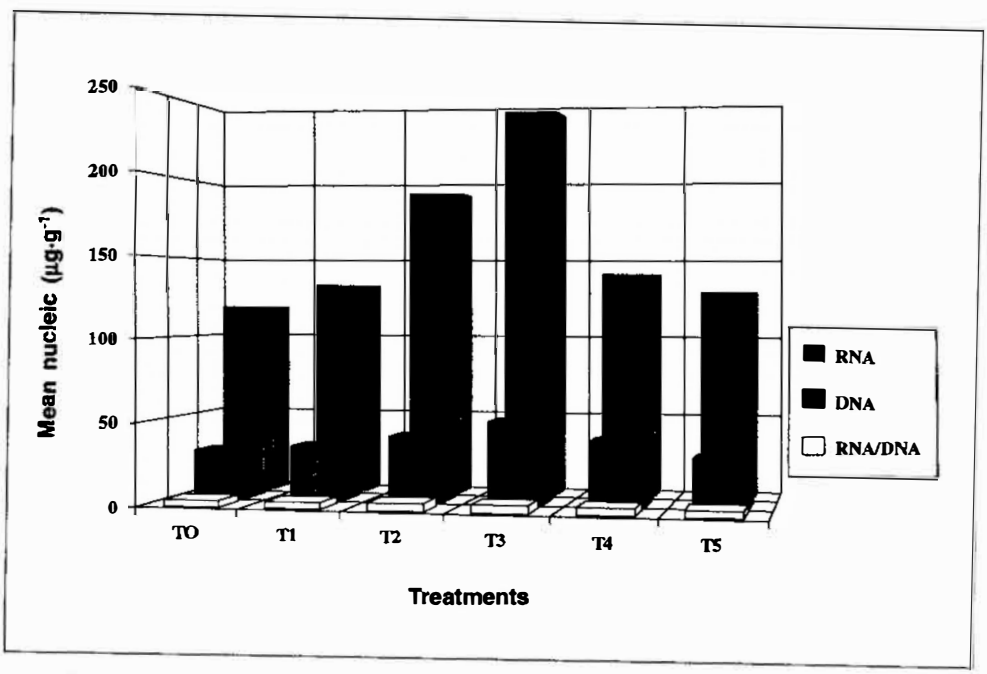

Fig. 3. Effect of L-carnitine on nucleic acid content in the hepatopancreas of *P. indicus*.

Table 2. Effect of L-carnitine on the growth and survival of *P. indicus*.

Parameter		f value	Treatment											
			T0		T1		T2		T3		T4		T5	
			M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Initial length	(cm)		2.60	0.31	2.60	0.31	2.60	0.31	2.60	0.31	2.60	0.31	2.60	0.31
Net weight gain	(g)		0.13	0.03	0.13	0.03	0.13	0.03	0.13	0.03	0.13	0.03	0.13	0.03
Final length	(cm)	2.18[1]	10.8^{a}	1.44	11.5^{ab}	1.73	12.6^{b}	2.03	13.0^{b}	2.07	12.03^{b}	2.21	11.14^{ab}	1.07
Final weight	(g)	9.71[2]	9.07^{a}	1.67	9.81^{a}	1.81	11.61^{b}	1.84	14.82^{c}	2.86	12.81^{b}	1.87	10.8^{a}	1.79
Net weight gain	(g)		8.94	2.13	9.68	2.10	11.48	3.19	14.69	2.86	12.68	3.01	10.67	2.11
SGR	(%)		3.53	0.83	3.60	1.11	3.74	0.91	3.94	0.96	3.82	0.83	3.68	0.81
Survival	(%)		96.00	4.00	96.00	3.08	88.00	4.50	96.00	6.00	92.00	4.50	88.00	6.00

[1] $P<0.05$; [2] $P<0.01$; SGR (%) = $\log_e W2 - \log_e W1$/time 100

Means with the same superscript do not differ from each other (Duncan's multiple range test).

Table 3. Effect of L-carnitine on feed utilization in *P. indicus*.

Parameter			Treatment											
			T0		T1		T2		T3		T4		T5	
		f value	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Feed consumption	(g)	8.91[2]	10.82[a]	1.89	11.78[ab]	1.44	12.58[b]	1.86	14.03[cd]	1.95	13.13[c]	1.44	11.5[ab]	1.38
Assimilation	(g)		7.94	1.32	8.85	1.22	9.37	1.02	10.62	1.43	10.14	1.57	8.64	1.28
Production	(g)		4.42	0.93	4.59	1.07	4.73	0.88	5.81	0.99	5.49	0.73	4.82	0.89
Conversion efficiency	(%)	6.14[2]	37.85[a]	3.11	38.96[a]	2.89	40.59[b]	4.36	42.41[bc]	2.58	41.81[b]	4.61	41.91[b]	4.86
Assimilation efficiency	(%)		73.38	3.87	75.12	4.51	74.48	2.73	75.69	3.01	77.22	3.71	75.13	3.86
PER	(%)	4.86[2]	11.05	1.91	11.47	2.38	11.82	1.74	14.52	0.86	13.72	1.39	12.05	2.11
APD	(%)	3.70[2]	68.71[a]	4.81	71.34[b]	3.81	71.86[b]	3.99	74.81[c]	3.17	72.86[b]	3.61	71.11[b]	1.89
AID	(%)		81.81[a]	4.83	82.43[a]	1.86	83.18[a]	4.11	85.36[b]	3.82	84.19[b]	2.71	80.13[a]	3.88

PER = Protein efficiency ratio; APD = apparent protein digestibility; AID = apparent lipid digestibility

[2] $P<0.01$

[a,b,c] Means with the same superscript do not differ from each other (Duncan's multiple range test).

Table 4. Effect of L-carnitine on digestive enzymes activity in the stomach + hepatopancreas and intestine of *P. indicus*.

Tissue	Enzyme activity	f value	Treatment T0 M	T0 SD	T1 M	T1 SD	T2 M	T2 SD	T3 M	T3 SD	T4 M	T4 SD	T5 M	T5 SD
							Amylase							
Stomach +	TA	31.37^{2}	413.12	5.07	448.19	6.03	473.16	5.08	503.17	5.93	500.28	6.07	453.33	5.03
hepato-	Sp.A	0.81^{NS}	5.71	1.62	5.76	1.14	5.21	1.28	5.82	1.33	5.36	1.42	5.15	1.21
pancreas	TA	37.17^{2}	439.36	6.73	463.17	6.71	498.33	7.12	583.17	4.37	573.31	3.86	496.39	4.17
Intestine	Sp.A.	0.096^{NS}	10.15	1.29	10.45	2.81	10.00	3.07	11.40	2.83	11.48	3.01	10.08	1.86
							Protease							
Stomach +	TA	27.17^{2}	231.87	3.48	244.21	4.17	288.17	3.93	381.12	4.07	323.01	3.86	281.14	4.98
hepato-	Sp.A	0.07^{NS}	3.20	0.17	3.13	0.21	3.54	0.36	3.95	0.73	3.44	0.47	3.83	0.52
	TA	17.11^{2}	330.17	4.43	341.52	4.56	368.17	4.38	404.11	4.21	363.13	4.37	341.21	4.16
Intestine	Sp.A.	0.09^{NS}	7.63	1.11	7.70	1.22	7.22	1.27	7.90	0.93	7.21	0.78	6.93	0.73
							Lipase							
Stomach +	TA	2.93^{1}	5.38	0.07	6.39	0.07	7.41	0.07	7.52	0.06	0.39	0.06	9.40	0.07
hepato-	Sp.A	0.11^{NS}	0.08	0.03	1.20	0.04	1.01	0.09	1.04	0.31	1.80	0.58	1.90	0.76
	TA	3.11^{1}	2.48	0.03	4.59	0.02	5.60	0.02	5.61	0.01	6.41	0.07	7.39	8.01
Intestine	Sp.A.	0.71^{NS}	0.80	0.07	0.91	0.06	0.88	0.40	1.00	0.99	0.04	0.08	1.10	0.07

TA = Total activity; Sp.A = Specific activity
NS = Non significant; $^{1}P<0.15$; $^{2}P<0.01$

Table 5. Effect of L-carnitine on body composition of *P. indicus*.

Parameter		f value	Treatment											
			T0		T1		T2		T3		T4		T5	
			M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Moisture	(%)	12.31^{2}	74.33	3.31	76.39	2.86	73.28	3.44	71.31	3.87	73.92	3.53	74.11	3.41
Dry matter	(%)	12.76^{2}	25.67^{ab}	2.73	23.61^{a}	1.86	26.72^{b}	2.03	28.69c	1.74	26.08^{b}	2.07	25.89^{ab}	2.33
Protein	(%)	12.31^{2}	66.31^{a}	1.86	69.21^{b}	2.04	70.77^{b}	2.81	73.33^{c}	2.03	72.19^{bc}	1.86	69.34^{b}	1.44
Lipid	(%)	2.17^{1}	2.69^{c}	0.81	2.43^{bc}	0.48	2.00^{b}	0.73	1.04^{b}	0.67	1.02^{b}	0.88	0.93^{ba}	0.17
Glycogen	(%)	1.71^{NS}	0.93	0.11	0.98	0.33	1.07	0.46	1.33	0.51	0.92	0.55	0.91	0.03
Fiber	(%)	1.8^{NS}	2.73	0.51	2.81	0.12	3.41	0.93	3.63	0.82	3.04	0.14	3.01	0.15
Ash	(%)	0.97^{NS}	3.02	0.19	4.87	0.28	3.93	0.67	3.88	0.71	4.01	0.33	3.73	0.44

Expressed on dry weight basis.
NS = Non significant; [1] $P<0.05$; [2] $P<0.01$
[a,b,c] Means with the same superscript do not differ from each other (Duncan's multiple range test).

the production of non-essential amino acids by carnitine. It also helps in the transportation of acetylic groups from the mitochondria to the cytoplasm where they are utilized by the fatty acid synthetase complex. Thus, carnitine supplementation in the diet enhances the rate of fatty acid oxidation and increases the metabolic flux in the Krebs cycle, releasing additional energy for metabolic processes, sparing protein for growth. This is evident from the lipid depletion observed in the muscle of carnitine-treated *P. indicus*. Similar decrease in the muscle lipid content has been reported in *M. idella idella* (Saliny 1994).

It is known that the lipid content of tissues of cultured fishes is higher than those living in their natural environment (Oshima et al. 1983). Dietary supplementation of L-carnitine may reduce unnecessary lipid accumulation in the tissues of pond-raised shrimps, an essential attribute of the cultured species. The results of the present study reveal that L-carnitine could help shrimp farming by increasing yield through higher growth and improving organoleptic characteristics.

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