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Role of Dietary L-carnitine on the Monosex Culture of Male Mossambique tilapia *Oreochromis mossambicus* (Peters)

S SUNIL KUMAR and V JAYAPRAKAS

Department of Aquatic Biology and Fisheries, University of Kerala, Beach P.O. Box 1603, Thiruvananthapuram 695 007, Kerala

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The influence of L-carnitine on the growth rate and body composition of male Mossambique tilapia (*Oreochromis mossambicus*) was assessed by feeding the biosynthetic growth promoter through 40% protein diet. L-carnitine at dosages, *viz.*, 150 ppm (T1), 300 ppm (T2), 500 ppm (T3), 700 ppm (T4) and 900 ppm (T5) were compared with the carnitine free control diet by culturing the fish in cement tanks. T5 (900 ppm) showed significantly ($P < 0.01$) superior growth and better food conversion efficiency and digestibility. RNA/DNA ratio of muscle and liver was high in T4 while it was high in T5 in the brain. Higher values of protein (64.8%), carbohydrate (2.74%), moisture (84.28%), ash (3.6%), fiber (4.76%) contents and lower fat content (2.13%) were recorded in T5. Carnitine incorporation showed a marked influence in lipid metabolism. The growth promoting mechanism of L-carnitine in male Mossambique tilapia is discussed in the light of its possible role for the improvement of fish production in aquaculture.

Key Words : L-carnitine, Tilapia, Conversion efficiency, RNA/DNA ratio

Introduction

Fish culture is considered today as one of the most promising source of fish for the future. Aquaculturists now exercise greater control over fish production by adopting several management measures to maximise fish yield. Recently there has been a growing interest in the use of growth promoters in aquaculture. Growth promoters and feed additives play a prominant role in intensive aquaculture practices for maximising fish production. Several substances including hormones, antibiotics and other chemical compounds are found to have anabolic effect. Very recently, carnitine a biosynthetic product has been subjected to tests for evaluating its growth promoting potential in chicken, rat, dog and pig (Feller & Rudman 1988; Gunther 1990a). Of the two forms of carnitine *viz.*, L-carnitine and D-carnitine, only L-carnitine can be used as a dietary supplement. L-carnitine is mainly engaged

in the transport of long chain fatty acids from the cytoplasm to the mitochondrial matrix where they are metabolised by beta oxidation enzymes (Fritz & Yue 1963; Bremer 1983). Considering its vital role the effect of L-carnitine on growth, food utilization, RNA-DNA content and body composition of male Mossambique tilapia, *Oreochromis mossambicus*, was evaluated.

Materials and Methods

Experimental Setup

The experiment was carried out in cement tanks each measuring $20m^2$ (5 × 4 × 1m) area for a period of 98 days. Three replications of each dosage are maintained. The tanks were cleaned, dried, and filled with fresh water to a depth of 0.75m prior to stocking fish. Level of water in the tanks was maintained at 75 ± 5cm throughout the period of the experiment.

Fry (2-2.6g) of *O. mossambicus* were manually sexed and only the males were used for the experiment. After recording the individual length and weight, the fishes were randomly transferred to the experimental tanks at a density of 25/tank. The treatments were assigned to the tanks at random. Fishes were fed twice daily at a rate of 5% of body weight. Quantity of feed was readjusted after every fortnightly sampling based on the weight of the fish. Part of the water in the tanks was changed periodically to avoid deterioration of water quality.

Diet Preparation

The fish meal based pelleted diet having 40% protein was formulated following the square method (Hardy 1980) and prepared. The proportion of the various ingredients and the proximate composition of the diet are given in table 1. L-carnitine obtained from Sigma chemical Co., St. Louis, U.S.A. was incorporated into the cooked and cooled feed dough at 150 ppm (T1), 300 ppm (T2), 500 ppm (T3), 700 ppm (T4), and 900 ppm (T5) levels. A control diet (40% protein) devoid of carnitine was also prepared.

Table 1 *Proportion of feed ingredients and the proximate composition of formulated diet*

Feed ingredients	Proportion	Protein content (%)
Rice bran	13.24	1.15
Tapioca flour	13.24	0.29
Fish meal	36.76	20.07
Groundnut oil cake	36.76	18.49
Total	100.00	40.00
Proximate composition of diet (%)		
Protein	40.00	
Fat	9.28	
Carbohydrate	12.23	
Fibre	4.21	
Ash	2.61	

Water Quality Monitoring

Water samples were collected fortnightly and analysed for temperature, *p*H, dissolved oxygen, free carbondioxide, total alkalinity, total hardness and primary productivity following APHA (1992) procedures.

Sampling

Fishes were sampled every 14th day and a minimum of 20 fishes were randomly collected and measured the individual length and weight. After the rearing period of 98 days, all the fishes were caught by draining the tanks and final length and weight were

measured. The specific growth rate (SGR) was calculated as

$$SGR\ (\%) = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

where W_1 is the weight at time T_1 and W_2 is the weight at time T_2

Tissue Body Indices

On termination of the experiment 5 fishes from each treatment were dissected out. The viscera, liver, kidney, gonad and brain of the fish were removed and weighed, the body indices such as viscero-somatic index (VSI), hepato-somatic index (HSI), reno-somatic index (RSI) and cerebro-somatic index (CSI) were calculated using the following method :

Tissue Body Index

$$= \frac{\text{Weight of Tissue (g)}}{\text{Total Weight of Fish (g)}} \times 100$$

Statistical Analysis

Analysis of variance was employed to test the statistical significance in growth performance of fish in length and weight and the means were compared by Duncan's multiple range test (Steel & Torrie 1980).

Food Consumption and Conversion Efficiency

A short term experiment was conducted in triplicate in the laboratory to study the feed intake, conversion efficiency and digestibility for one month in plastic troughs of 50 litre capacity. Five fishes were kept in each trough. Individual length and weight were recorded prior to stocking and were fed at the rate of 10% of body weight per day in the various treatments. To determine digestibility, fishes were provided with weighed amount of feed daily. The unconsumed food and faecal matter were collected, dried and weighed.

The feed intake, conversion efficiency and assimilation efficiency were calculated as given below :

Food Intake = Dry Weight of Diet Given – Dry Weight of Left over Diet

Food Conversion Efficiency (FCE)

$$= \frac{\text{Wet Weight Grain (g)}}{\text{Dry Weight of Feed (g)}} \times 100$$

Assimilation Efficiency

$$= \frac{\text{Assimilation (g)}}{\text{Food Intake (g)}} \times 100$$

Protein Efficiency Ratio (PER)

$$= \frac{\text{Body Weight Gain (g)}}{\text{Protein Intake (g)}}$$

The supplementary diet and the faecal matter collected from different treatments were analysed for protein, lipid and carbohydrate content following AOAC (1984) procedures. The apparent digestibility was calculated as follows :

Nutrient Digestibility

$$= \frac{\text{Nutrient Intake} - \text{Nutrient in Faeces}}{\text{Nutrient Intake}} \times 100$$

Estimation of Nucleic Acid and Blood Biochemistry Parameters

The DNA and RNA contents of muscle, liver and brain of the various treatments were estimated following the methods proposed by Curlewis and Stone (1987).

The blood collected from the branchial artery was analysed for total lipid, phospholipids (Corner et al. 1961), cholesterol, triglycerides (Bucolo & David 1973) and protein (Donman et al. 1971). Total lipids were extrapolated from the values of triglycerides, phospholipids and cholesterol (Corner et al. 1961).

Proximate Composition

Fish muscle was analysed for protein, fat, carbohydrate, moisture, fibre and ash contents following AOAC (1984) procedures.

Statistical Analysis

Analysis of variance was employed to test the statistical significance in length, weight, tissue indices and proximate composition of fish muscle and the means were compared by Duncan's multiple range test (Steel & Torrie 1980).

Results

Growth of Fish

Fish in all treatments showed significantly superior growth than that of control ($P < 0.01$). The growth in length and weight increased with an increase in the level of carnitine in the diet with high average percentage growth in T5 (Figures 1 and 2). The highest specific growth rate (SGR) was recorded in T5 and lowest in T0.

The water temperature ranged between 25-28°C and *p*H ranged between 7.18-8.7. Dissolved oxygen ranged between 7.8-10.4 ppm (mean 9.1 ppm) while total alkalinity ranged from 32.5-40 ppm. Other parameters like total hardness ranged between 30.3-47.8 mg $CaCO_3$ l^{-1}, free carbondioxide between 0-1.8 ppm (mean 0.9 ppm) and primary

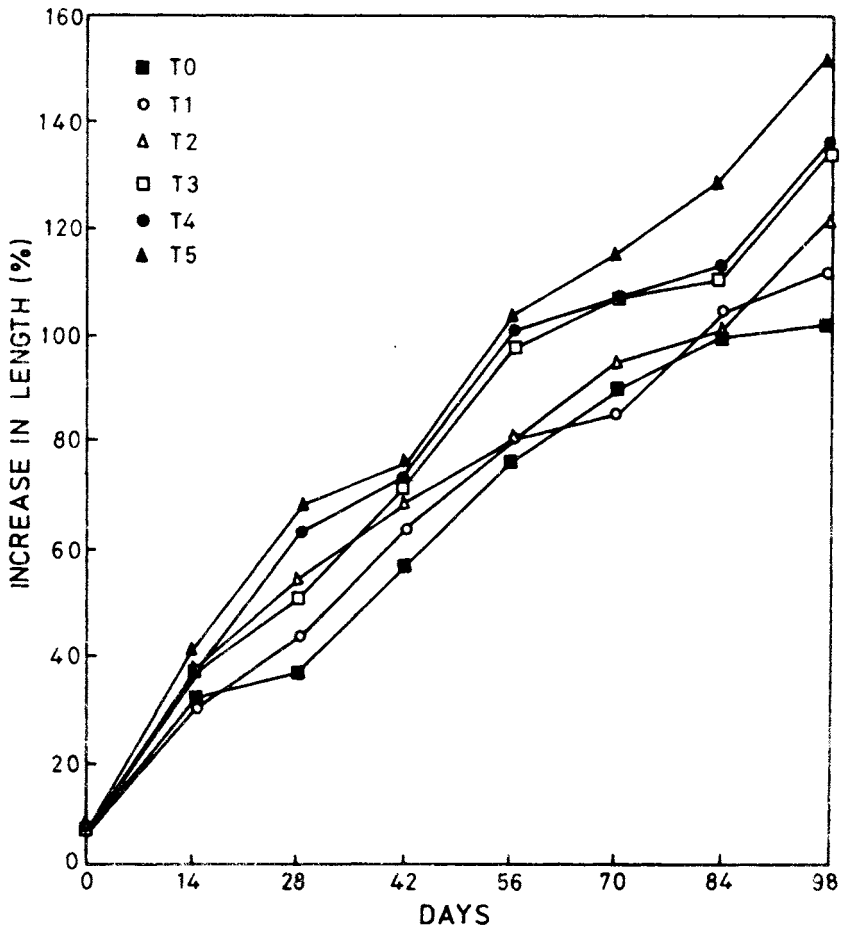

Figure 1 Average percentage increase in length of *O. mossambicus* fed different levels of carnitine

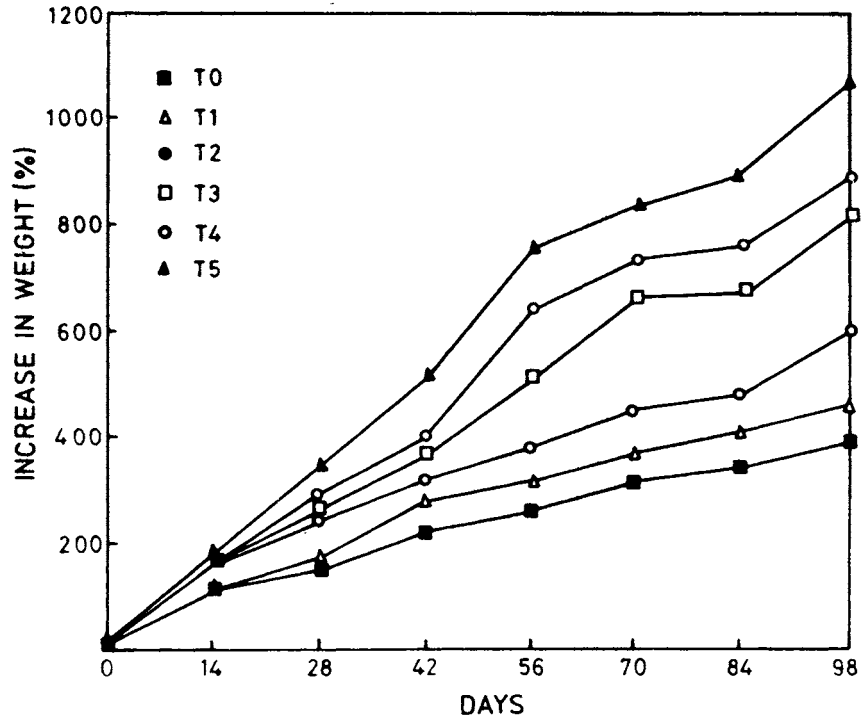

Figure 2 Average percentage increase in weight of *O. mossambicus* fed different levels of carnitine

productivity ranged between 2.1-2.8 mg C day^{-1}.

Tissue Body Indices

Viscero-somatic index (VSI) and hepato-somatic index (HSI) showed high values in control fish (T0) and lowest values in T4. In the case of reno-somatic index and gonado-somatic index highest values were found in T1 while lowest values were recorded in T0. Cerebro-somatic index was high in T5 and low in T0

Food Consumption, Conversion Efficiency and Assimilation

Maximum food intake was observed in the fishes in T5 (table 2) Highest values of assimilation efficiency, net growth efficiency and gross growth efficiency (71-75%, 74.45% and 53.42%) were observed in T5 and the lowest values (59.78%, 5.54% and 3.31%) were noticed in the control fish (T0). Protein efficiency ratio was highest in T5 (22.3%) and lowest in control (0.9125). Apparent protein, fat and carbohydrate digestibilities were highest in T5 and lowest in T0 (table 3).

Nuclic Acid Content and Blood Biochemistry Parameters

RNA content was higher than DNA content in all the three tissues (table 3). RNA/DNA ratio was high in T4 (10.63 mg g^{-1} and

Table 2 *Growth and feed utilization of O. mossambicus fed different levels of carnitine*

	Treatment					
Parameters	T0	T1	T2	T3	T4	T5
Initial length (cm)	5.29+0.12	5.29+0.12	5.29 + 0.12	5.29 + 0.12	5.29 + 0.12	5.29 + 0.12
Initial weight (g)	2.22+0.15	2.22+0.15	2.22 + 0.15	2.22 + 0.15	2.22 + 0.15	2.22 + 0.15
Final length (cm)	10.64^{d}+0.38	11.21^{cd}+0.54	11.7^{bc}+0.55	12.32^{b}+0.66	12.38^{b}+0.62	13.25^{a} + 0.65
Final weight (g)	11.90^{a}+1.03	13.42^{cd}+0.92	16.65^{c}+ 1.25	20.49^{b} + 1.65	21.87^{b} + 1.85	26.00^{a} + 2.15
Net weight gain	9.68	11.2	13.43	18.27	19.65	23.73
% weight gain	81.34	83.45	85.82	89.16	89.85	91.41
Specific growth rate (%)	1.71	1.84	1.99	2.27	2.23	2.51
Survival (%)	95	90	95	100	95	90
Feed consumed (c)	11.01 + 0.01	13.9 + 0.49	14.98 + 0.6	13.64 + 0.29	15.08 + 0.06	16.69 + 0.06
Conversion efficiency (%)	5.54 + 0.71	12.94 + 1.04	31.36 + 0.23	34.36 + 0.23	52.13 + 1.27	74.45 + 1.19
Protein efficiency ratio	0.19	2.92	7.81	7.96	13.62	22.3
Protein digestibility (%)	84.68 + 3.86	86.8 + 2.12	87.6 + 3.42	94.16 + 5.61	91.7 + 4.24	96.47 + 4.21
Fat digestibility (%)	79.37 + 2.31	85.26 + 3.12	82.11 + 4.21	89.02 + 3.42	89.12 + 5.22	92.21 + 3.82
Carbohydrate digestibility (%)	72.13 + 6.75	74.89 + 2.53	83.32 + 1.46	83.49 + 2.21	81.11 + 5.09	86.03 + 4.45

*Significant ($P < 0.01$); a, b, c, d - Means with the same superscript do not differ (Duncan's multiple range test)

Table 3 *Nucleic acid content (mg.g^{-1} tissue) of O. mossambicus fed different levels of carnitine*

Treatment	Muscle			Liver			Brain		
	RNA	DNA	RNA/DNA RATIO	RNA	DNA	RNA/DNA RATIO	RNA	DNA	RNA/DNA RATIO
T0	18.58	4.68	3.97	32.4	7.45	4.34	24.7	13.17	1.88
SD(±)	1.76	1.16		2.35	0.61		4.06	1.5	
T1	24.23	5.18	4.68	40.07	9.53	4.25	41.67	11.98	3.47
SD(±)	2.98	1.2		4.8	2.35		2.2	0.04	
T2	29.72	5.6	5.31	50.02	12.9	3.87	54.53	13.8	3.95
SD(±)	2.73	0.83		4.69	1.8		5.3	1.6	
T3	41.1	4.2	9.78	50.5	16.05	3.14	63.06	11.89	5.3
SD(±)	1.9	1.42		1.9	2.3		6.6	4.2	
T4	63.06	5.93	10.63	96.36	13.53	7.12	81.1	13.81	5.87
SD(±)	2.5	1.56		7.7	5.4		0.04	0.2	
T5	51.33	6.76	7.59	90.5	17.88	5.06	79.39	13.45	5.89
SD(±)	7.4	1.24		1.25	0.7		3.5	0.6	

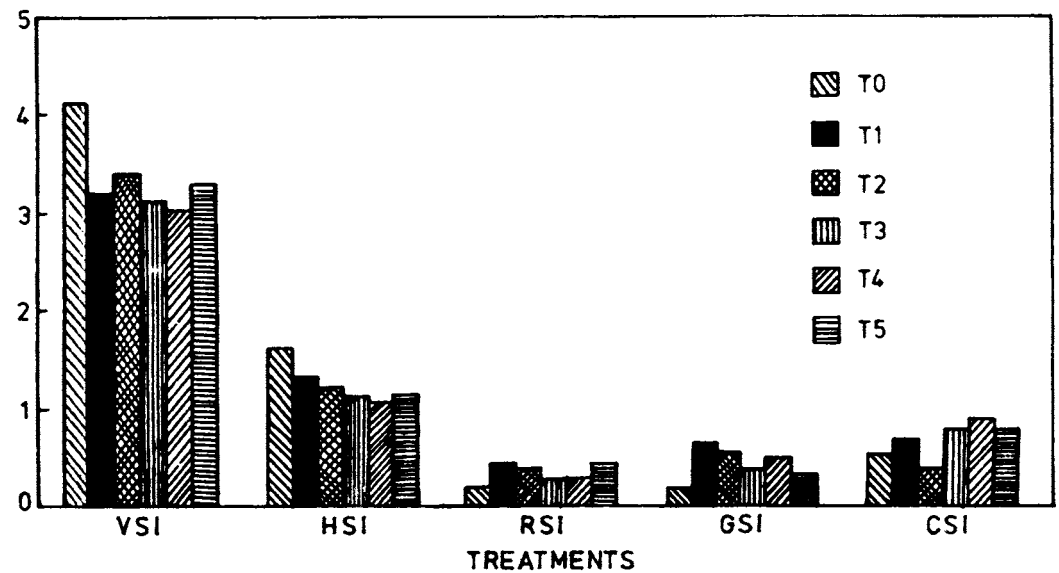

Figure 3 Tissue body indices of *O. mossambicus* fed different levels of carnitine diet

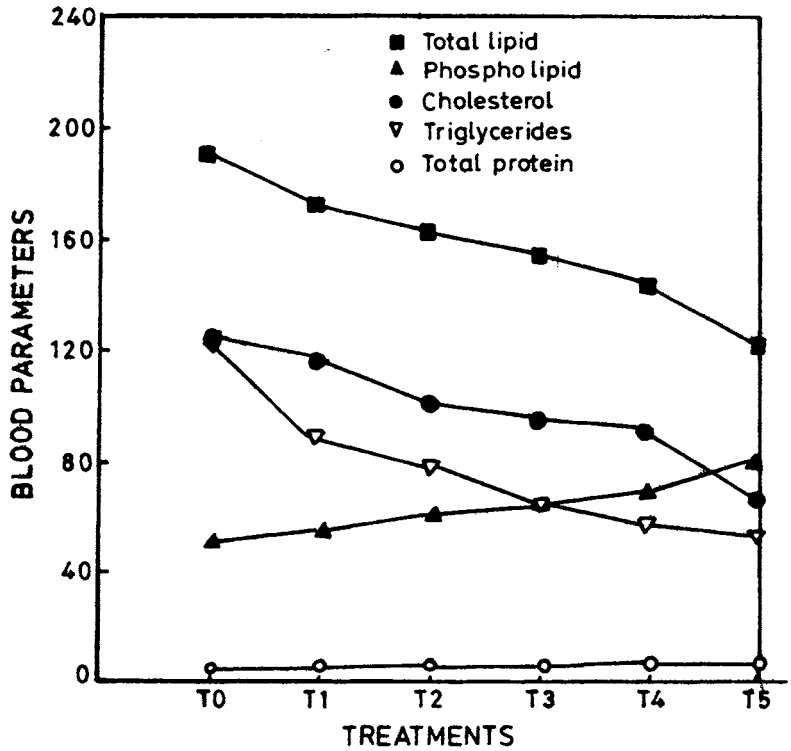

Figure 4 Blood biochemistry parameters of *O. mossambicus* in different treatments

low in T0 (3.974 $mg.g^{-1}$ tissue). In liver highest RNA/DNA ratio was recorded in T4 (7.12 $mg.g^{-1}$ tissue). In the case of brain the RNA/DNA ratio was found to be high in T5 (5.89 $mg.g^{-1}$ tissue). The highest values for total lipid, cholesterol and triglycerides were recorded in T0 and the lowest values were recorded in T5 (figure 4). Phospholipids and total protein were found to increase with increase in the levels of carnitine.

Body Composition

Highest protein content was recorded in T5 (64.8%) and lowest in T0 (44.25%) (table 4). Percentage fat content decreased with increase in the level of carnitine with highest value in T0 (3.2-05%) and lowest value in T5 (21.3%). Carbohydrate content showed the highest value in T5 (2.74%) and lowest value in T3 (0.64%). Ash and fibre contents increased with increase in the level of carnitine. The protein, carbohydrate, ash and fibre contents showed significant ($P < 0.01$) difference in all treatments than the control.

Discussion

The growth promoting effect of L-carnitine has been demonstrated in fishes such as trout (Bilinski & Jonas 1970), sea bass, *Dicentrarchus labrax* (Santulli & D'Amelio 1986a & 1986b; Santulli et al. 1988 & 1990), Nile tilapia (Gunther 1990b), African catfish (Torreele et al. 1991) and *Labeo*

Table 4 *Proximate body composition (%) of O. mossambicus in the various treatments*

Proximate composition	Treatments					
	T_0	T_1	T_2	T_3	T_4	T_5
Moisture content	76.64	78.1	81.89	82.89	81.08	84.28
SD(±)	0.12	0.23	0.42	0.6	0.32	0.42
Protein	44.25^{b}	48.06^{b}	51.9^{b}	49.09^{b}	61.8^{ab}	64.8^{a}
SD(±)	1.93	1.22	1.71	1.43	2.7	1.48
Fat	3.21^{a}	2.51^{a}	2.5^{a}	2.48^{a}	2.35^{a}	2.13^{a}
SD (±)	0.91	0.64	0.74	0.52	0.63	0.53
Carbohydrate(%)	2.47^{ab}	1.07^{b}	0.76^{b}	0.64^{b}	1.38^{b}	2.74^{a}
SD(±)	0.66	0.24	0.13	0.21	0.5	0.43
Fibre(%)	2.03^{c}	2.23^{c}	3.16^{b}	3.8^{b}	3.7^{b}	4.76^{a}
SD(±)	0.2	0.54	0.65	0.42	0.57	0.6
Ash(%)	1.73^{cd}	2.03^{bcd}	2.43^{bc}	2.66^{b}	1.56^{d}	3.6^{a}
SD(±)	0.16	0.16	0.69	0.61	0.12	0.52

Significant ($P < 0.01$); a, b, c, d - Means with the same superscript do not differ (Duncan's multiple range test)

rohita (Renuka 1993). In the present study, all levels of L-carnitine supplementation resulted in growth enhancement over the control indicating its positive effect on fish growth. In Nile tilapia, highest level of carnitine supplementation (900 ppm) did not increase weight to the extent that resulted due to 300 ppm treatment (Gunther 1990 b). This suggests that the effect of L-carnitine in promoting growth is dose dependent.

Renuka (1993) observed the lowest VSI value in rohu under the higher level of carnitine treatment (750 ppm). The lower HSI value in the present study is an indication of fat depletion from liver due to the effect of carnitine. Higher food conversion efficiency was exhibited in all the groups fed L-carnitine than that of control. Similar high FCE was observed in *Clarias gariepinus* (Torreele et al. 1991). Protein efficiency ratio was highest with diet having 900 ppm L-carnitine which induced the maximum growth indicating better utilization of protein from it. Similar high PER value with highest growth was reported in *L. rohita* (Renuka 1993). High RNA and DNA contents were noticed in the muscle, liver and brain of carnitine treated fish compared to the control. Santulli and D'Amelio (1990) had reported enhanced nucleic acid and protein synthesis in the sea bass fed carnitine incorporated diets.

Santulli and D'Amelio (1986) reported increase in the levels of phospholipids and protein content while the levels of the total lipid, free cholesterol and triglycerides decreased in the plasma of carnitine treated sea bass. The present study also showed a fall in total lipid, free cholesterol and triglycerides in the plasma of L-carnitine fed fishes from that of the control.

Increase in the muscle protein content was observed in all the carnitine treated groups than the control. Increased protein content was observed in the tissues of sea bass *D. labrax* (Santulli & D'Amelio 1986b; Santulli et al. 1990) in *O. niloticus* (Gunther 1990a) and in *L rohita* (Renuka 1993). Carnitine stimulates protein synthesis by providing carbon skeleton for the synthesis of amino acid (Emaus & Bieber 1983). This mechanism explains the increased protein content in the muscle *O. mossambicus*.

According to Santulli and D'Amelio (1986a), lipid content of L-carnitine treated sea bass fry was 27% less than that of the control fish. Similar fall in lipid content was observed in *O. niloticus* (Gunther 1990b) and in *L. rohita* (Renuka 1993) when fed with L-carnitine incorporated diets. Lipid reduction and growth improvement are the result of a more efficient use of dietary lipid. These findings indicate that diet incorporated with L-carnitine seem to reduce undesirable lipid accumulation in the tissues of cultured fishes. Consequently more energy becomes available for anabolic processes.

As L-carnitine is mainly involved in regulation of fat burning, protein can be spared for growth. It is known that lipid content of the tissue of cultured fishes is higher than that of fishes of the same species living in natural environment (Oshima et al. 1983). Thus biosynthetic growth promoter like L-carnitine, at very low levels of incorporation in the diet increases the efficiency of food utilization, enhances protein synthesis leading to high growth rate.

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