

Effect of Dietary L-carnitine on Growth and Reproductive Performance of Male *Oreochromis mossambicus* (Peters)

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Cultured male tilapia was fed diets supplemented with various levels of L-carnitine for 252 days and its growth and reproductive performance were evaluated. Carnitine administration produced growth enhancement, increasing with dosage. Carnitine stimulated feed conversion efficiency, better digestibility and high enzyme activity. The RNA-DNA and protein contents in the muscle and liver also increased concomitant with high growth rate. Carnitine induced lipid catabolism leading to reduction in lipid content of cultured fish, using lipid as energy source while sparing protein for anabolic processes. Significantly high GSI, sperm cell concentration, motility and percentage viability of the spermatozoa in carnitine treated tilapia were observed.

Key words : L-carinitine, Growth, reproduction, *Oreochromis mossambicus*.

L-carnitine is a product derived from the anabolic activity of tissue and is mainly engaged in the transport of the long chain fatty acids from the cytoplasm to the mitochondrial matrix where they are metabolised by beta-oxidation enzymes (Bremer, 1983). It also transports the acetylic groups from the mitochondrion to the cytoplasm where they are utilised by the fatty acid synthetase complex (Santulli & D'Amelio, 1986 a&b). L-carnitine plays a fundamental role in energy supply in fishes. L-carnitine has been shown to increase oxidation of free fatty acids in trout muscle mitochondria (Bilinski & Jones, 1970), stimulate lipid metabolism and increase growth of sea bass fry (Santulli & D'Amelio, 1986 a&b). It also enhances protein synthesis by providing carbon skeletons for biosynthesis of certain amino acids (Emaus & Bieber, 1983). L-carnitine improves growth, increases protein and nucleic acid contents in the muscle, liver and brain of Indian major carp fry (Renuka, 1993). Although,

carnitine is biosynthesised, cultured fishes seem to have lower L-carnitine content (Santulli & D'Amelio, 1986 a&b) and therefore, may need dietary supplementation. Considering its vital role, we have undertaken research to study its effect on growth and reproduction of male tilapia and its possible utilization in aquaculture.

Materials and Methods

The study was conducted in the Department of Aquatic Biology and Fisheries, Trivandrum. Fry of *Oreochromis mossambicus* were procured from a fish pond and cultured in cement tanks (20 m² area) having a water level of 75±5 cm for a period of 252 days. Uniform sized fry (5.3±0.12 cm; 2.22±0.15 g) were randomly stocked (25 nos) in each tank. Three replicates of each treatment were maintained. L-carnitine was purchased from Sigma Chemicals Co., St. Louis, U.S.A. Fish meal based supplementary diet having 40% protein was prepared and used for carnitine incorporation. The

supplementary diet was prepared by mixing with 36.76% fish meal, 36.76% groundnut oil cake, 13.24% rice bran and 13.24% tapioca flour along with 0.03% vitamin mineral mix. Before pelletizing, the required quantity of the carnitine was sprayed on to the cooked and cooled dough. The dough was then pelletized and the pellets were oven-dried and stored in airtight containers. The dosages of carnitine were selected based on preliminary screening experiments. Five dosages of carnitine viz., 150 (T₁), 300 (T₂), 500 (T₃), 700 (T₄) and 900 (T₅) ppm with a control (T₀) were fed at 5% of the body weight once daily. The fishes were sampled every fortnight and the ration was re-adjusted based on the growth rate. On termination of the experiment, all fishes were collected, the total lengths and weights measured.

The activity of digestive enzymes, viz: amylase, protease and lipase in the anterior, middle and posterior regions of the intestine were assayed. Protein content of the crude enzyme extract was estimated by Lowry *et al.* (1951). Strumbouer & Hofer's (1986) method was used for amylase assay and the unit amylase activity was calculated as mg maltose liberated in 10 min at 30°C. The casein digestion method (Kunitz, 1974) was used for protease assay. Amount of tyrosine liberated in 15 min was estimated to calculate unit protease activity. Bier's (1962) titrimetric method was employed for lipase assay. Lipase activity was calculated as the ml of 0.025 N NaOH required to neutralise the fatty acids liberated during 18 h of incubation at 30°C. The enzyme activity was calculated as enzyme unit g⁻¹ tissue.

DNA and RNA contents of the dorsal body muscle and liver were estimated following the methods of Carlewis & Stone (1987). The total protein, lipid, carbohydrate, fibre and ash contents of muscle and

liver were analysed following AOAC (1985) procedures. A short-term experiment was conducted in fibre glass tanks (100 litres) using 10 fish each in triplicate for 30 days to assess the food intake, conversion efficiency, protein efficiency ratio, apparent protein and lipid digestibility (De Silva, 1989) of the carnitine fed and control fishes. In this experiment, the fishes were fed with the diet at 10% of the body weight daily for 30 days. The unconsumed feed and faecal matter were removed separately every day and the feed consumption was estimated after termination of the experiment. Feed conversion efficiency (FCE) and protein efficiency ratio (PER) were calculated as follows:

$$\text{FCE (\%)} = \frac{\text{Wet weight gain (g)}}{\text{Feed consumed (g)}} \times 100$$

$$\text{PER (\%)} = \frac{\text{Increase in body weight (g)}}{\text{Protein consumed (g)}} \times 100$$

Apparent protein and lipid digestibility were estimated by using the formula;

$$\text{Apparent nutrient digestibility (\%)} = \frac{\text{Nutrient in feed} - \text{Nutrient in excreta}}{\text{Nutrient in feed}} \times 100$$

The nutrient contents (protein and lipid) of the feed and excreta were analysed following the AOAC procedures (AOAC, 1985).

The male reproductive performance of the cultured fishes were evaluated using the testes of 30 fishes from each treatment. The colour and stage of maturity of testes were recorded and gonado-somatic index (GSI) was worked out. Milt was collected into 1.5 ml Eppendorf tubes and kept in the refrigerator (4°C). The spermatozoa concentration was determined using improved Neubauer counting chamber (Buyukhatipoglu & Holtz, 1978). Eosin nigrosin dye exclusion method (Turner, 1986) was used for estimating the viability of spermatozoa.

The duration of motility in fertilizing solution (3 g Urea and 4 g NaCl per litre distilled water) was determined using the standard method (Erdahl, 1986). All these observations were made within two hours of the collection of milt. The data were statistically analysed using ANOVA (Snedecor & Cochran, 1968) and the treatment means were compared by Duncan's multiple range test (Steel & Torrie, 1980).

Results and Discussion

The data summarised in Table 1 indicate that all levels of L-carnitine administration resulted in an appreciable effect on tilapia growth. The observed differences were statistically significant ($P < 0.05$). The fish receiving 900 ppm carnitine (T_5) exhibited significantly superior growth throughout (Fig. 1) showing 26.78% increase in the mean weight over the untreated fish. The mean specific growth rates were higher than that observed in the control fish with maximum in T_5 (1.47%) and

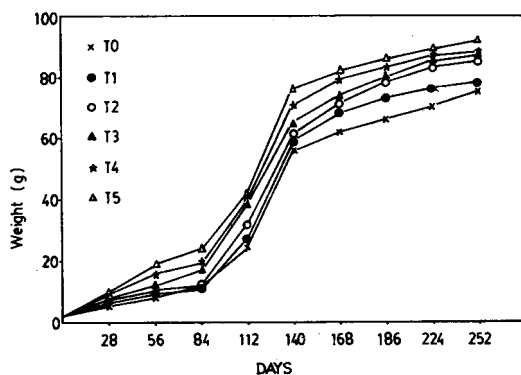


Fig. 1. Growth of *Oreochromis mossambicus* fed L-carnitine.

minimum in control (1.39%) showing that carnitine promoted the growth of the tilapia. Growth promoting effect of L-carnitine has been previously demonstrated in trout (Bilinski & Jonas, 1970), sea bass (Santulli & D'Amello, 1986 a, b). Nile tilapia (Gunther, 1990 b), African cat fish (Torrel *et al.*, 1991) and rohu (Renuka, 1993). In the present investigation, the

Table 1. Effect of L-carnitine on growth and survival of *Oreochromis mossambicus*

Parameters	Treatments											
	T ₀		T ₁		T ₂		T ₃		T ₄		T ₅	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Initial length, cm	5.30	±0.12	5.30	±0.12	5.30	±0.12	5.30	±0.12	5.30	±0.12	5.30	±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	19.74	±1.98	20.02	±2.01	20.60	±1.18	22.20	±1.17	22.64	±2.00	23.60	±1.78
Final weight, g	75.05	±2.31	78.65	±3.40	85.85	±2.43	86.81	±2.11	86.48	±3.48	91.83	±3.91
Net weight gain, g	72.83		76.43		83.63		84.59		84.26		89.61	
Percentage weight gain, %	3280.63		3542.79		3867.11		3910.36		3795.49		4036.48	
S.G.R., %	1.71		1.83		1.90		2.26		2.33		2.51	
Survival, %	95.00		90.00		95.00		90.00		95.00		100.00	

Table 2. Effect of L-carnitine on feed utilization of *Oreochromis mossambicus* over 30 days

Parameters	Treatments											
	T ₀		T ₁		T ₂		T ₃		T ₄		T ₅	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Food consumption, g	11.01	±0.01	13.90	±0.49	14.98	±0.60	13.64	±0.29	15.08	±0.6	16.69	±0.06
Assimilation, g	6.66	±0.33	9.03	±0.18	9.96	±0.08	9.27	±0.02	10.45	±0.25	11.98	±0.30
Feed conversion efficiency, %	20.17	±0.71	20.98	±1.04	23.74	±0.23	24.11	±0.23	25.07	±1.27	25.67	±1.19
Assimilation efficiency, %	59.78	±3.12	64.58	±0.93	66.45	±3.63	67.93	±1.32	69.33	±1.96	71.75	±1.52
Protein efficiency ratio, %	10.11		10.73		10.81		10.92		11.11		11.76	
Protein digestibility, %	84.68	±3.86	86.80	±2.12	87.60	±3.42	94.16	±5.61	91.70	±4.24	96.47	±4.21
Lipid digestibility, %	79.37	±2.31	85.26	±3.12	82.11	±4.21	89.02	±3.42	89.12	±5.21	92.21	±3.82
Carbohydrate digestibility, %	72.13	±6.75	74.89	±2.53	82.32	±1.46	83.49	±2.21	81.11	±5.09	86.03	±4.45

highest level of carnitine supplementation (900 ppm) brought the maximum growth indicating that the increment in growth observed is concomitant with the dosage of L-carnitine used. Hence it may be sug-

gested that the highest dosage of carnitine tested produced the maximum growth and further studies are required to optimise the dosage of carnitine required to maximise growth.

Table 3. Effect of L-carnitine on proximate composition of *Oreochromis mossambicus*

Treatments	Muscle						Liver					
	Moisture %	Protein %	Lipid %	Glycogen %	Fibre %	Ash %	Moisture %	Protein %	Lipid %	Glycogen %	Fibre %	Ash %
T ₀	74.61 ±2.17	65.13 ±3.41	5.22 ±0.51	3.43 ±0.11	5.89 ±0.89	10.17 ±1.18	59.13 ±1.18	73.31 ±2.17	3.81 ±0.73	3.74 ±0.07	-	1.27 ±0.07
T ₁	75.01 ±3.12	68.90 ±2.12	4.82 ±0.78	3.81 ±0.33	6.11 ±0.81	13.13 ±2.29	58.10 ±1.23	74.11 ±3.18	3.21 ±0.55	3.94 ±0.83	0.07 ±0.01	1.11 ±0.00
T ₂	73.41 ±3.14	69.71 ±2.71	4.31 ±0.71	5.21 ±0.13	6.11 ±0.87	14.16 ±2.31	50.70 ±0.73	79.13 ±1.17	3.10 ±0.67	4.12 ±0.18	0.08 ±0.03	1.21 ±0.01
T ₃	78.00 ±2.78	70.11 ±2.86	4.21 ±1.10	3.27 ±0.71	6.30 ±0.93	12.73 ±1.13	58.13 ±1.21	78.18 ±3.13	2.81 ±0.48	4.20 ±0.48	0.10 ±0.01	1.02 ±0.03
T ₄	76.11 ±5.11	70.50 ±1.74	4.01 ±1.12	2.81 ±0.83	6.81 ±0.67	13.30 ±0.81	60.17 ±0.71	76.11 ±1.16	2.66 ±0.71	4.71 ±0.55	0.07 ±0.00	1.01 ±0.00
T ₅	78.11 ±4.93	72.00 ±3.18	3.61 ±0.93	3.11 ±0.17	7.10 ±0.71	9.90 ±0.73	57.11 ±0.93	81.11 ±4.18	2.41 ±0.05	4.80 ±0.91	0.02 ±0.00	0.87 ±0.02

Expressed on dry weight basis

Table 4. Effect of L-carnitine on reproductive performance of male *Oreochromis mossambicus*

Treatment	Weight of testis, g	Colour of testis	Stage of maturity	GSI (%)	Sperm cell concentration sperms ml ⁻¹ **	Motility in fertilising solution* sec	Viability (%)
Control (T ₀)	0.10±0.00 c	Pale yellow	Ripe	0.35±0.003	24.4×10 ⁹ cd ±0.1414×10 ⁹	226.0±3.807 b	82.94±0.336 b
150 ppm (T ₁)	0.10±0.11 c	"	"	0.38±0.005	24.46×10 ⁹ cd ±0.207×10 ⁹	23.00±3.804 a	82.94±0.554 b
300 ppm (T ₂)	0.10±0.00 c	"	"	0.44±0.00	26.68×10 ⁹ cd ±0.130×10 ⁹	238.4±4.219 a	83.46±0.926 b
500 ppm (T ₃)	0.165±0.00 c	"	"	0.43±0.012	27.5×10 ⁹ c ± 0.158×10 ⁹	238.4±4.219 a	83.46±0.926 b
700 ppm (T ₄)	0.33±0.003 b	"	"	0.94±0.025	29.52×10 ⁹ b ±0.192×10 ⁹	236.8±4.436 a	84.90±0.894 cd
900 ppm (T ₅)	0.45±0.00 a	"	"	1.048±0.049	39.66×10 ⁹ a ±0.230×10 ⁹	238.8±2.167 a	91.46±1.725 a

* P < 0.05; ** p < 0.01; a, b, c - Means with the same suffix do not differ (Duncan's multiple range test), n = 30

L-carnitine also significantly influenced the food consumption and

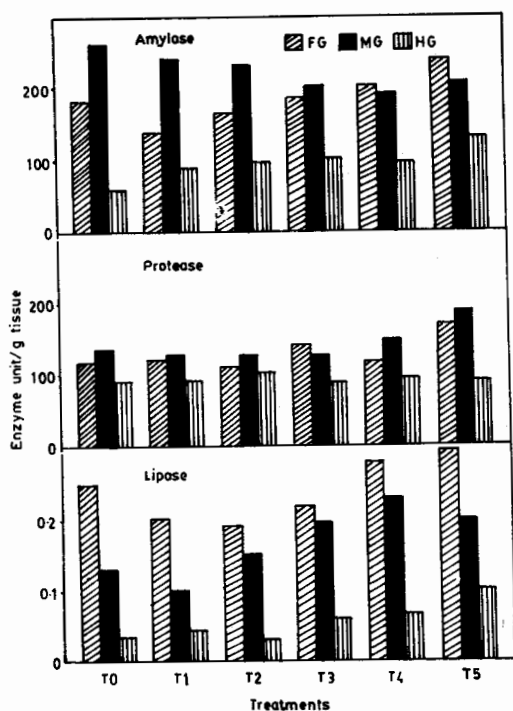


Fig. 2. Activity of digestive enzymes in *Oreochromis mossambicus* fed L-carnitine.

utilization of *O. mossambicus*. The fish fed 900 ppm (T₅) carnitine showed higher feed conversion efficiency (25.67%), protein efficiency ratio 11.76, apparent protein (96.47%) and lipid (92.21%) digestibility than control (Table 2). The digestive enzyme activity in the three intestinal regions (Fig. 2) shows that fish treated with 900 ppm carnitine exhibited higher protease and lipase activity in the foregut than control. In the *O. niloticus*, Gunther (1990 b) observed an increase in the activity of the digestive enzymes of the L-carnitine treated fish which led to enhanced growth. Renuka (1993) suggested that carnitine promoted growth in *Labeo rohita* through improved protease activity in the alimentary canal. In the present study also such an increase in the rate of enzyme activity was noticed which could stimulate enhanced food utilization and growth.

The RNA-DNA contents in both the muscle and liver were found to be high in the carnitine fed fish with highest values in

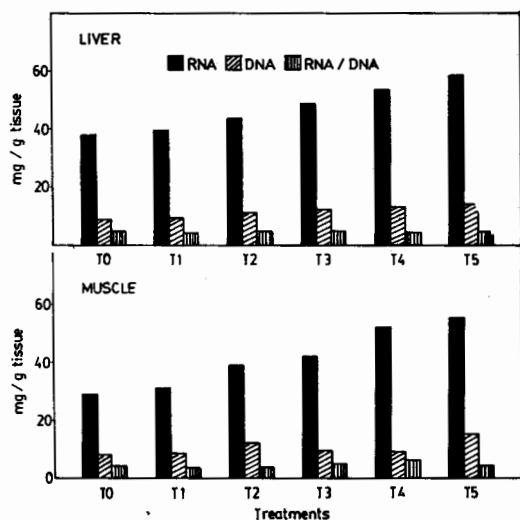


Fig. 3. Nucleic acid contents of *Oreochromis mossambicus* fed L-carnitine.

the 900 ppm level (Fig. 3). L-carnitine may stimulate growth by enhancing RNA-DNA synthesis in the growing tissues. Santulli & D'Amelio (1986 a&b) and Renuka (1993) reported high RNA-DNA ratio in carnitine treated sea bass and *L. rohita* respectively. The protein contents in the muscle and liver increased with increase in the level of L-carnitine indicating that carnitine may have promoted increased protein synthesis (Table 3). Similar results have been recorded in *L. rohita* (Renuka, 1993). Lipid contents decreased in the treated fish over control in both muscle and liver tissues. The decrease in lipid content may be due to an enhancement of beta oxidation in those tissues. Santulli & D'Amelio (1986 a&b) also observed lipid depletion in growing seabass with L-carnitine supplemented diets and suggested that L-carnitine promoted lipid metabolism. That it can be suggested that diets supplemented with L-carnitine may reduce lipid accumulation in tissues and promote growth.

The reproductive potential of L-carnitine treated tilapia is presented in Table 4.

Although gonad was at the ripe stage in all treatments, significantly higher GSI and spermatozoa concentration ($p < 0.01$) were observed in the fish treated with 900 ppm L-carnitine. The duration of motility and percentage viability of spermatozoa were also high in the 900 ppm carnitine fed fishes. The difference in the motility and viability of spermatozoa were found to be statistically significant ($p < 0.01$). No previous literature is available on the influence of L-carnitine on the reproductive performance of fishes. However, Chao *et al.* (1987) suggested a positive relationship between quality of feed and sperm viability in tilapia. The increased spermatozoa concentration, enhanced sperm motility and high percentage viability of spermatozoa observed in the present study could be attributed to the improved growth rate consequent on administration of L-carnitine.

L-carnitine was thus found effective to limit undesirable lipid accumulation in the tissues of farmed fish. It increases growth rate by stimulating energy metabolism. It also enhances the sperm cell concentration, motility and viability of spermatozoa of male fish. Thus L-carnitine may help fish rearing by increasing growth rate of fish.

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