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The Effect of Supplemental L-Carnitine on Growth, Proximate Composition, Survival and Cold Tolerance of Kuruma Prawn, *Marsupenaeus japonicus* (Penaeidae: Decapoda) Juveniles

Gokçen Yagcioglu and Mevlut Aktas
Faculty of Fisheries and Aquaculture, University of Mustafa Kemal, 31034, Hatay, Turkey

Abstract: Juvenile kuruma prawn, Marsupenaeus japonicus with an initial weight of 1.64±0.69 g were reared on pellets supplemented with 300, 600 and 1200 mg kg^{-1} L-carnitine for 8 weeks in 1.2 m diameter round concrete tanks. The results showed that L-carnitine supplementation did not influence weight gain and survival rate at the end of the 56 days feeding period compared with the control group ($p>0.05$). However, muscle proximate composition of the juveniles was significantly affected by L-carnitine supplementation ($p<0.05$). Lipid level decreased whilst protein content increased in those fed either 300 or 600 mg kg^{-1} L-carnitine. At the end of the feeding trial, the shrimp exposed to a cold shock to examine their tolerance. No significant effect of L-carnitine on cold tolerance M. japonicus juveniles was observed.

Key words: Marsupenaeus japonicus, L-carnitine, growth, proximate composition, cold tolerance

INTRODUCTION

L-Carnitine (γ-trimethylammonium-β-hydroxy-butyrate), an optically active, water-soluble, quaternary ammonium base, is widely distributed in the tissues of nearly all organisms. While carnitine concentrations in animal tissue are significantly higher; e.g. 640 μg g^{-1} in beef muscle, 700 μg g^{-1} in fish muscle and 2100 μg g^{-1} in sheep muscle, the carnitine concentrations in plants are usually low (1 μg g^{-1} in peanut kernels, 7-14 μg g^{-1} in wheat, 20 μg g^{-1} in alfalfa). The carnitine, which is absorbed by the intestine, is transported in the free or acetylated form in the blood. The level in tissue is 10 to 100 times higher than in the extra cellular fluid. The most significant role of carnitine in metabolism is the promotion of long-chain fatty acid transport to the site of β-oxidation. Other metabolic functions of carnitine have been reported as follows[1]. Production of metabolic energy from short and medium-chain fatty acids and branched-chain keto acids, ketone body metabolism, release of coenzyme A to facilitate pyruvate catabolism, peroxisomal β-oxidation, regulation of glycogen synthesis and ATP production, stimulation of acetoacetate oxidation and _ketogenesis and thermoregulation in brown adipose tissue.

Bilinski and Jonas[2] indicated that carnitine increased fatty acid oxidation in tissues of rainbow trout. Channel catfish (*Ictalurus punctatus*) fingerlings fed with a carnitine supplemented diet attained higher weight gains than the control[1]; Burtle and Liu[3] also reported an increase in total protein content and a decrease in lipid content of the same species. Similarly, L-carnitine increased weight gain and protein content and stimulated lipid metabolism in sea bass fry[4,5], growth rate of milkfish, *Chanos chanos* reared in semi-intensively managed ponds[6] and increased feed intake and weight gain of hybrid striped bass[7]. Another effect is a striking protection against ammonia toxicity in juvenile Chinook salmon that intraperitoneally injected with 10-16 mmol kg^{-1} L-carnitine[8]. Harpaz[9] reported that ornamental cichlid fish *Pelvicachromis pulcher* received dietary L-carnitine exhibited significantly better survival rates following exposure to a cold shock.

A limited number of studies have focused on the effects of L-carnitine in crustaceans. The effects of L-carnitine were investigated on growth, survival and body composition of juvenile *Penaeus monodon* with significant effects only on body composition[10]. Yet, Groth[11] and Groth *et al.*[12] found no statistical difference on weight gain supplemental L-carnitine for the same species. Speegle *et al.*[13] reported significant interaction of protein and L-carnitine on weight gain for *Litopenaeus vannamei*. Additionally, L-carnitine promoted the growth on *Penaeus indicus* and *Macrobrachium idella idella*[14].

Corresponding Author: Gokçen Yagcioglu, Faculty of Fisheries and Aquaculture, University of Mustafa Kemal, 31034, Hatay, Turkey

Kuruma prawn, *Marsupenaeus japonicus* is widely distributed in the eastern Mediterranean coast of Turkey and it is commercially important species for culture. It is known that increasing of cold tolerance of shrimp during the winter months could prove management and financial profit to farmers. The purpose of present study was to determine the effects of dietary L-carnitine on growth performance, survival, proximate composition as well as cold tolerance of juvenile *M. japonicus* when fed different levels L-carnitine diets.

MATERIALS AND METHODS

Experiment I. Rearing of *M. japonicus* with diets containing different levels of L-carnitine: *M. japonicus* juveniles were collected from the Yumurtalık Bight of the Northeastern Mediterranean Sea (Iskenderun Bay) in July 2004 by beach seine. Shrimps were transported in oxygenated seawater to the Yumurtalık Marine Research Station (Faculty of Fisheries, University of Cukurova) Adana, Turkey and selected for their length and healthy appearance. The animals were acclimated to the experimental conditions for two weeks before the experiments. The experiment was conducted in twelve round concrete tanks (1.2 m in diameter and 70 cm water depth, no substrate in the bottom). Inflow of fresh seawater was maintained at 0.5 L min^{-1} throughout the experiment. Ranges of physicochemical parameters such as temperature, pH, salinity (YSI Model 30 Salinometer, Yellow Springs Instrument USA) and dissolved oxygen were checked daily throughout the experiment. Thirty shrimp (average weight 1.64±0.69 g) were stocked into each tank and each L-carnitine dosage was tested in triplicate. Shrimp were taken from the acclimation tank by net, adhesive water was removed with filter paper and then weighed individually on an electronic balance to the nearest 0.01g (Scaltec, SBA 53). The dietary treatments were formulated to contain L-carnitine concentrations of either 300, 600 or 1200 mg carnitine kg^{-1} diet. Sufficient water and L-carnitine (L-carnitine, Merck-Schuchardt, Germany) were added to dry ingredients to form a soft dough. The resultant dough was passed through a 2 mm diameter die, using the mincer attachment on a commercial food mixer. The pellets were air dried at 40°C and stored at 4°C until used. Composition of the experimental diet is given in Table 1. Ingredients were obtained from a factory manufacturing fish food (Kılıc Fish Food Production Corporation, Milas, Mugla, Turkey).

Leaching rate of L-carnitine from the pellets was determined using the method described by Groth (13). Before the feeding trial, four mesh baskets containing 50 g of feed pellets were submersed into an aquarium (water level 50 cm, salinity 38 ppt and temperature 28 °C) and 15, 30, 45 and 60 minutes later the baskets were lifted and the remaining feed dried, weighed and analysed for carnitine.

Table 1: The ingredient composition of the experimental diet.

Ingredient	Incorporation (g kg^{-1})
Fish meal	310
Soy-bean meal	260
Corn bran	200
Cotton seed cake	125
Fish oil*	84
Premix**	10
DCP	10
Protein	396.7
Lipid	124

*Fish oil was obtained from a factory processing anchovy meat for fishmeal, Sinop, Turkey. **Premix: 5.000.000 IU Vitamin A, 1.250.000 Vitamin D, 12.500 mg Vitamin E, 1.250 mg Vitamin K_3, 750 mg Vitamin B_1, 2.000 mg Vitamin B_2, 15.000 mg niacin, 5.000 mg cal pan, 1.750 mg Vitamin B_6, 8 mg Vitamin B_{12}, 375 mg Folic acid, 25 mg Biotin, 50.000 mg Vitamin C, 225.000 mg Choline chloride, 12.500 mg carophyll red, 2.500 mg carophyll yellow, 50.000 mg Mn, 50.000 mg Fe, 50.000 mg Zn, 10.000 mg Cu, 150 mg Co, 800 mg I, 150 mg Se.

Dietary carnitine concentrations were analysed according to the methods of Parvin and Pande and contained 18 mg carnitine kg^{-1} in basal (control) diet. The diets were broadcasted evenly over the tank surface three times daily (10.00, 16.00 and 22.00) at 10% body weight. At bi-weekly intervals, all the shrimp were counted and weighed to determine survival and growth rate throughout the study. After the treatments, 12 shrimps of pooled samples from all the shrimp in each experimental groups were taken for proximate composition analysis at the end of the feeding experiment. Proximate analysis of shrimp was done on the basis of percentage lipid, moisture, ash and protein. Moisture content[15] and crude protein was estimated according to the Kjeldahl method Lipid extractions were carried out according to modified Bligh and Dyer method[3,16].

Experiment II: Determination of cold tolerance: The experiment was carried out in 12 plastic boxes (10-L), which were placed in thermostatically controlled water bath (±0.5°C) in 2×1×0.5 m fibreglass tank. Aeration was provided to all boxes to supply oxygen for shrimp and minimize both vertical and horizontal thermal stratification. Each plastic box was covered with a transparent lid to prevent prawns from escaping and facilitate observation. Water temperature in the bath was controlled by a 1-kW thermoregulator against cold water from a refrigerated recirculation system. At the end of experiment I.; totally 120 (30 shrimp from each treatment group) shrimp were randomly selected and 10 shrimp stocked into each plastic box in order to determine cold tolerance in tree replicates. Temperature of control and tree experimental groups simultaneously decreased by 2°C h^{-1} from 28 to 6°C and

then from 6 to 28°C again during a period of 11 has described in Harpaz *et al.*, (10). Shrimps were kept at final temperature (6°C) for five hours before the temperature was increased to 28°C again. Shrimp behaviour and mortality were closely monitored during the course and the next day.

All statistical tests were performed using SPSS version 11 statistical software a one-way Analysis of Variance (ANOVA) model was used. Any significant difference was determined at 0.05-probability level by Duncan test.

RESULTS

Experiment I: The different treatment groups did not differ in their water quality parameters which were all within the range considered as reasonable for growth of the shrimp (O_2> 5.5 mg L^{-1}; salinity 39 ppt; temperature 28°C and water exchange rate 1 L min^{-1}). While uneaten pellets stayed firmly in water for one h, L-carnitine concentration decreased from 1200 to 310 mg kg^{-1} during the first 15 min Fig. 1. This rate declined to 190 and 0 mg kg^{-1} after 30 and 60 min, respectively.

Shrimp cultured neither of the diets showed significant difference between each other at final day of the experiment Table 2 ($p>0.05$). The highest average survival 75.55% and growth rate (2.96 g day^{-1}) was found in the group fed the diet containing 600 mg kg^{-1} of L-carnitine with still non-significant difference from the others ($p>0.05$).

Dietary L-carnitine had significant effect on proximate composition of muscle of shrimp $p<0.05$. Protein content was higher in the group fed with 600 mg kg^{-1} L-carnitine (23.85 g) and this was followed by the groups fed 0, 300 and 1200 mg kg^{-1} L-carnitine treatments. Lipid content was, however, lower in those fed with 300 and 600 mg kg^{-1} L-carnitine compared to the highest carnitine level (1200 mg kg-^{1}). Total ash content was low in all the groups fed L-carnitine compared to that of the control. Water content of the muscle was highest in those fed 1200 mg kg^{-1} L-carnitine ($p<0.05$) Table 3.

Experiment II: No differences in cold tolerance and survival rate were observed between the groups ($p> 0.05$). The shrimp in all the groups displayed normal activity between 28 and 18°C. The activities became slower and swimming movements disordered when the temperature fell down to 14°C. At 12°C, the shrimp showed loss of balance and some laid down laterally on the bottom of the box. At 10°C, when touched with a glass pipette the pleopods were hardly moving. Only a few of them were able to move at 8°C and complete cessation of movement

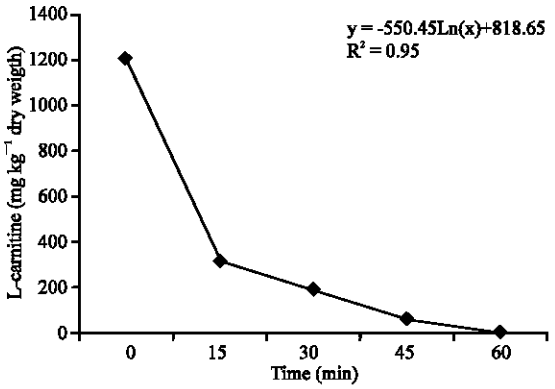

Fig. 1: L-carnitine leaching from pellets

Table 2: Growth performance and survival rate of *M. japonicus* fed different levels L-carnitine*.

	Initial weight (g)	Final weight (g)	Biomass (g)	Survival rate (%)	Weight gain(%)
Control	1.55±0.06^{a}	2.94±0.19^{a}	56.56±5.31^{ab}	64.44±7.69^{a}	89.67
300 mg kg^{-1}	1.54±0.04^{a}	2.80±0.11^{a}	54.28±9.70^{b}	64.44±10.18^{a}	81.89
600 mg kg^{-1}	1.53±0.05^{a}	2.96±0.22^{a}	66.86±2.95^{a}	75.55±6.93^{a}	93.44
1200 mg kg^{-1}	1.56±0.02^{a}	2.73±0.20^{a}	53.41±4.40^{b}	67.77±9.62^{a}	70.50

*Means of three replicates. ±Values with different superscripts in the same row differ significantly. Means±S.D.

Table 3: Proximate composition of the muscle of *M. japonicus* fed different doses of L-carnitine (%)*

	Crude protein	Crude fat	Crude ash	Moisture
Initial	19.34±5.65^{d}	1.79±0.11^{b}	1.20±0.00^{b}	77.67±0.17^{a}
Control	20.96±0.45^{c}	1.73±0.13^{b}	1.32±0.02^{a}	75.98±0.35^{b}
300 mg kg^{-1}	22.17±0.42^{b}	1.67±0.13^{b}	1.24±0.01^{b}	74.93±0.27^{c}
600 mg kg^{-1}	23.85±0.27^{a}	1.65±0.14^{b}	1.04±0.01^{c}	73.44±0.30^{d}
1200 mg kg^{-1}	18.85±0.41^{d}	2.28±0.23^{a}	1.07±0.03^{c}	77.79±0.20^{a}

*Means of three replicates. Values with different superscripts in the same row differ significantly

was observed at 6°C. One mortality occurred in the group fed 1200 mg kg^{-1} L-carnitine during the five-hour holding period. The shrimp behaviour was the same during the temperature increase back to 28°C.

DISCUSSION

Controversial results have been reported on the effects of carnitine on growth and survival of shrimps. For example, Schmekel[10] and Groth[11] found no statistical difference on the growth and survival rate of *P. monodon* fed with different levels of L-carnitine added diets, whereas Jayaprakas and Sambhu[14] reported enhanced growth and FCR in *P. indicus* fed on 500 mg kg^{-1} L-carnitine. Our results with *M. japonicus* juveniles also indicated non-significant effects of carnitine on growth and survival of this species. Similar contradictory results have also been reported for some fish species. Although supplemental L-carnitine induced growth in sea bass[4], African catfish[17], Sea bream[18,19], tilapia

Oreochromis niloticus×*O. aureus* hybrids[20], European sea bass[17], dietary L-carnitine did not effect weight gain in Atlantic salmon[21], rainbow trout[22]. Thus, it appears that the effects of dietary L-carnitine on growth performance differs among finfish species as well as crustacean species.

Whereas, the differences among experimental groups are not statistically important, the present study showed that the highest and lowest dosage of L-carnitine resulted in the growth retardation. Torreele *et al.*[23] and Focken *et al.*[24] pointed out a linear dose/response relationship, while other studies indicated non-linear or even negative responses between L-carnitine dosage and growth performance[9,18,20,17,25,26]. Even though optimum level of L-carnitine supplementation appeared to be 600 mg kg^{-1} in the present study, the effect was insignificant compare with the control ($p>0.05$). This level is similar to that found by Jayaprakas and Sambu[14] for *P. indicus* (500 mg kg^{-1}) and Groth *et al.*[12] for *P. monodon* (500 mg kg^{-1}). Reports regarding L-carnitine effects on proximate composition of shrimp or fish are also contradictory. Dietary carnitine did not alter tissue composition of rainbow trout[24], hybrid tilapia[20], hybrid striped bass[7] or European sea bass[17]. On the other hand, L-carnitine reduced tissue lipid concentrations in fingerling of Robeo rohita[27], Atlantic salmon[17], channel catfish[3], European sea bass[4] and tilapia[26]. Lipid contents increased in muscle and liver of red sea bream fed dietary L-carnitine[17]. The present results indicate that within the experimental levels adopted (300 and 600 mg kg^{-1}), dietary L-carnitine was beneficial in terms of decreasing lipid and increasing protein concentrations in the muscle of juvenile *M. japonicus*. Groth[11] reported that the proximate composition of *P. monodon* did not differ between control and L-carnitine diets, but dry matter, crude protein and gross energy content increased significantly with carnitine supplementation. Another study[12] showed that dietary L-carnitine increased protein but decreased fat content in *P. monodon*. An increase in dry matter and protein contents and a decrease in lipid content in *P. indicus*[14]. Our results support the idea that L-carnitine may favour transport of long-chain fatty acids across the inner mitochondrial membrane and increases fatty acids oxidation. Thus, L-carnitine has a decreasing effect their availability and deposition in the target lipid storage tissues. L-carnitine has known to be a beneficial agent for fish during exposure to stress inducing conditions. Harpaz *et al.*[9] found that addition of 1000 mg kg^{-1} L-carnitine to diet of cichlid fish *Pelvicachromis pulcher* promoted survival rate when exposed to a cold shock. It was shown that gills and skin of guppies (*Poecilica reticulata*) were protected under the heat stress by means of carnitine[27]. There is no information on protective effect of L-carnitine in shrimp or decapods in general. Our study showed that dietary L-carnitine does not increase tolerance of *M. japonicus* juveniles to cold at least under the experimental conditions used in the present investigation.

CONCLUSION

In conclusion, the present study shows that supplemental dietary L-carnitine does not have any expected beneficial effects on improving growth, survival and cold tolerance under these conditions. Moreover, based on the present results, consumption of a carnitine-suplemented diet has a clear effect on the proximate composition of the muscle of kuruma prawn, *Marsupenaeus japonicus*.

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