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Research Article

The effects of L-carnitine levels on population growth of rotifer *Brachionus plicatilis* fed with *Chlorella vulgaris*

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Abstract

In this study, S-type rotifer *Brachionus plicatilis* were enriched with 10 levels (0, 0.001, 0.01, 0.1, 1, 10, 100, 500, 1000 and 1500 mg L-carnitine per L) of L-carnitine supplements dissolved in *Chlorella vulgaris* culture medium in a 10 mL individual culture trial for 7 days. The initial rotifer density was 1 individual mL^{-1}. The trials were conducted to 200×0^{6} cell mL^{-1} at food density at 25±1ºC under an axenic condition in the laboratory. L-carnitine enrichment has shown considerable influence on the population growth of rotifer *Brachionus plicatilis*. As a result, the maximum number of individuals was determined as 1560 ± 10.00 rot./mL in the group enriched with 100 mg/L L-carnitine supplement and the lowest number of individuals was determined 376.67±25.17 rot./mL in the group enriched with 1500 mg/L L-carnitine addition (p<0.05). The maximum growth rate was found to be 0.63±0.02 rot/ day in the group enriched with 100 mg/L L-carnitine addition (p<0.05). The maximum doubling time was 1.31±0.02 rot./ day for the group enriched with 1000 mg/L L-carnitine (p<0.05). The results suggested that L-carnitine could be a positive factor to enhance reproduction and population growth on enriched *Brachionus plicatilis* under the optimum concentration.

Keywords: Rotifer, *Brachionus plicatilis*, L-carnitine, Population growth

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Introduction

For larval production of marine fish and crustacea, rotifers are used most widely as the early larval diet. Thus, rotifer is a key factor in the production of marine fish larvae (Bengtson, 2003). Food and feeding regime are important factors in zooplankton cultures such as rotifer and artemia. These organisms must be enriched for fish larvae through feeding on unicellular algae, baker's yeast, fish oil and commercial enrichment products (Fukusho, 1985; Hoff and Snell, 1987; Kissil and Koven, 1990; Sorgeloos *et al*., 2001; Ferreira *et al*., 2008; Estudillo-del Castillo *et al*., 2009; Mæhre *et al*., 2013). Because the ingredients that compose their body change according to the food ingested, the nutritional quality of food that they are fed is important. The manipulations of nutrition and growth enhancement for producing higher quantity and quality live food are important challenges (Harpaz, 2005). L-carnitine, as supplements of a vitamin-like nutrient, has attracted many researchers for decades because of its important function of adopting the oxidation of long chain fatty acids by the mitochondria and stimulating protein sparing action by increasing energy derived from lipids (Fathi and Farahzadi, 2014). L-carnitine is a naturally occurring substance required for energy metabolism in mammals. It is produced by the body and is available in the diet mainly in products of animal origin. L-carnitine is essential for transport of the long chain fatty acids across the mitochondrial membrane for subsequent fat degradation and energy production (Voet and Voet, 1994). Another important function of L-carnitine is the ability to shuttle short chain fatty acids from inside the mitochondria to the cytosol. Therefore, L-carnitine is responsible for maintaining energy metabolism of the whole body (Bremer, 1997). The potential positive effects of L-carnitine on fat turn over have been published under the intensive scientific investigation. Recently, L-carnitine related studies were carried out on more than 15 fish species (Harpaz, 2005; Savaş and Çiçek, 2010). However, contradicting results have been reported even with the same fish species. Hence, this study aims to investigate the effects on different L-carnitine ratios on the increase of rotifer population (*B. plicatilis*, type S).

Materials and methods

S-type (100-210 μm) marine rotifer *Brachionus plicatilis* was obtained from Mediterranean Fisheries Research, Production and Training Institute, Beymelek Center Unit, Antalya, Turkey. The trials were carried out at Isparta University of Applied Sciences, Egirdir Fisheries Faculty. Rotifers were cultured at room temperature under continuous illumination during 24 hours at laboratory. In this study, sea water was used and filtered through sand, cartridge, ultraviolet (280-315 nm) and biological filters and regulated the

salinity at 0.25‰, temperature 25±1°C, dissolved oxygen 8.3 to 14.6 mg/L and pH 7.5 ± 0.5. L-carnitine was obtained from Lonza Company (Lonza Inc., Basel, Switzerland).

At the beginning of the experiments, rotifers were enhanced with 10 levels (0.001, 0.01, 0.1, 0, 1.0, 10, 100, 500, 1000, 1500 mg L-carnitine per L) of L-carnitine supplements dissolved in *Chlorella vulgaris* culture medium. One egg-bearing female rotifer was stocked individually to sterile medium enriched with different levels of L-carnitine. The experiments were started with 10 individuals in a culture volume of 10 mL for all groups. The trials were conducted 200×10^6 cell mL^{-1} at food density. All treatments were made in triplicate. The L-carnitine was added only on the first day. The trials were carried out in the laboratory for 7 days.

Values obtained in *B. plicatilis* culture were calculated according to the following equations reported by James and Rezeq (1988).

$K= (\ln Nt– \ln No) /t$

Doubling time$=(t\times\ln 2)/ (\ln Nt–\ln No)= \ln 2 /K$

K=Growth rate (division/ day),

No=Initial rotifer density (individual/ mL),

Nt=max. rotifer density after t day (individual/ mL),

t=Maximum number of days reached to individual/ mL.

The statistical analysis of the experimental data was carried out using SPSS 23.0 software (SPSS, Inc. USA). The parameters for each feeding regime were compared using one-way analysis of variance. Differences were considered to be significant when $p<0.05$ using a Tukey test.

Results

As a result, it has been determined that the optimum concentration of L-carnitine for rotifer *Brachionus plicatilis* is 100 mg/L (Table 1). The maximum number of individuals was determined as 1560±10, 00 rot./mL in the group enriched with 100 mg/L L-carnitine and the lowest number of individuals was determined 376.67 ± 25.17 rot./mL in the group enriched with 1500 mg/L L-carnitine ($p<0.05$).

The minimum growth rate was found to be 0.45±0.06 / day in the group enriched with 1500 mg/L L-carnitine (Table 2). The maximum growth rate was found to be 0.63±0.02 / day in the group enriched with 100 mg/L L-carnitine ($p<0.05$).

The minimum doubling time was 1.10±0.01 / day for the group enriched with 100 mg / l L-carnitine (Table 3). The maximum doubling time was 1.31±0.02 / day for the group enriched with 1000 mg/L L-carnitine ($p<0.05$).

Table 1: Total number of rotifers cultured in different L-carnitine concentrations (rot./mL).

	Control	0.001 mg/L	0.01 mg/L	0.1 mg/L	1 mg/L	10 mg/L	100 mg/L	500 mg/L	1000 mg/L	1500 mg/L
Initial	10	10	10	10	10	10	10	10	10	10
Day 1	15.33± 0.58^{bc}	12.67± 0.58^{de}	13.67± 0.58^{cde}	13.33± 0.58^{cde}	16.67± 1.15^{b}	19.67± 1.53^{a}	14.67± 1.15^{bcd}	12.33± 0.58^{def}	11.67± 0.58^{ef}	10± 0.00^{f}
Day 2	40.00± 10.00^{bc}	29± 1.00^{cde}	33± 1.73^{bcde}	23.33± 1.53^{def}	56.67± 5.77^{a}	43.33± 5.77^{ab}	36.67± 5.77^{bcd}	24.33± 3.06^{def}	21± 1.00^{ef}	11.33± 0.58^{f}
Day 3	57.33± 5.77^{d}	60.67± 1.15^{cd}	65.33± 2.52^{cd}	73.33± 5.77^{bc}	93.33± 5.77^{a}	90± 10.00^{a}	86.67± 5.77^{ab}	37.67± 2.08^{e}	35± 5.00^{e}	19± 1.00^{f}
Day 4	133.33± 15.28^{d}	118.33± 2.89^{d}	126.67± 5.77^{d}	180.00± 10.00^{c}	253.33± 11.55^{a}	216.67± 15.28^{b}	266.67± 5.77^{a}	130± 5.00^{d}	113.33± 5.77^{d}	36.67± 2.31^{e}
Day 5	250.00± 0.00^{e}	223.33± 5.77^{f}	253.33± 5.77^{e}	300± 10.00^{d}	330± 10.00^{c}	380± 10.00^{b}	610± 10.00^{a}	310± 10.00^{cd}	296.67± 5.77^{d}	103.33± 5.77^{g}
Day 6	393.33± 5.77^{cd}	333.33± 5.77^{e}	370± 10.00^{d}	410± 10.00^{c}	513.33± 11.55^{b}	503.33± 5.77^{b}	1066.67± 5.77^{a}	500± 10.00^{b}	393.33± 5.77^{cd}	283.33± 11.55^{f}
Day 7	733.33± 5.77^{e}	736.67± 5.77^{f}	746.67± 5.77^{de}	763.33± 5.77^{d}	873.33± 5.77^{c}	936.67± 5.77^{b}	1560± 10.00^{a}	850± 10.00^{c}	686.67± 5.77^{f}	376.67± 25.17^{g}

*Different latter in the same column indicates significant differences (p<0.05)

Table 2: Growth rate of rotifer cultured in different L-carnitine concentrations (division/day).

	Control	0.001 mg/L	0.01 mg/L	0.1 mg/L	1 mg/L	10 mg/L	100 mg/L	500 mg/L	1000 mg/L	1500 mg/L
Day 1	0.21± 0.02^{bc}	0.12± 0.03^{cde}	0.16± 0.01^{bcd}	0.14± 0.05^{bcd}	0.26± 0.02^{ab}	0.34± 0.07^{a}	0.19± 0.01^{bcd}	0.10± 0.08^{cde}	0.08± 0.05^{de}	0.00± 0.01^{e}
Day 2	0.46± 0.01^{bc}	0.35± 0.00^{de}	0.40± 0.00^{cd}	0.28± 0.06^{f}	0.58± 0.03^{a}	0.49± 0.02^{b}	0.43± 0.01^{bc}	0.30± 0.02^{ef}	0.25± 0.00^{f}	0.04± 0.01^{g}
Day 3	0.42± 0.02^{c}	0.45± 0.03^{c}	0.47± 0.01^{bc}	0.50± 0.02^{abc}	0.56± 0.02^{a}	0.55± 0.04^{a}	0.54± 0.03^{ab}	0.33± 0.04^{d}	0.31± 0.02^{d}	0.16± 0.03^{e}
Day 4	0.52± 0.02^{c}	0.49± 0.01^{c}	0.51± 0.02^{c}	0.58± 0.00^{b}	0.65± 0.01^{a}	0.62± 0.02^{ab}	0.66± 0.00^{a}	0.51± 0.01^{c}	0.49± 0.03^{c}	0.26± 0.02^{d}
Day 5	0.54± 0.02^{bc}	0.52± 0.01^{c}	0.54± 0.02^{bc}	0.57± 0.01^{bc}	0.58± 0.05^{bc}	0.61± 0.02^{ab}	0.69± 0.02^{a}	0.57± 0.01^{bc}	0.57± 0.02^{bc}	0.39± 0.06^{d}
Day 6	0.52± 0.01^{bc}	0.50± 0.01^{c}	0.52± 0.04^{bc}	0.53± 0.02^{bc}	0.56± 0.01^{b}	0.56± 0.01^{b}	0.67± 0.02^{a}	0.56± 0.01^{b}	0.52± 0.01^{bc}	0.48± 0.01^{c}
Day 7	0.54± 0.04^{b}	0.54± 0.00^{b}	0.54± 0.03^{b}	0.54± 0.03^{b}	0.56± 0.00^{ab}	0.57± 0.01^{ab}	0.63± 0.02^{a}	0.56± 0.03^{ab}	0.53± 0.01^{bc}	0.45± 0.06^{c}

*Different latter in the same column indicates significant differences (p<0.05)

Table 3: Doubling time of rotifers cultured in different L-carnitine concentrations (day).

	Control	0.001 mg/L	0.01 mg/L	0.1 mg/L	1 mg/L	10 mg/L	100 mg/L	500 mg/L	1000 mg/L	1500 mg/L
Day 1	3.24± 0.05^{g}	5.86± 0.01^{c}	4.44± 0.01^{e}	4.82± 0.01^{d}	2.71± 0.16^{h}	2.05± 0.09^{i}	3.62± 0.02^{f}	6.61± 0.13^{b}	8.99± 0.10^{a}	0.69± 0.01^{j}
Day 2	1.50± 0.09^{e}	1.95± 0.04^{bcd}	1.74± 0.42^{cde}	2.45± 0.07^{ab}	1.20± 0.02^{e}	1.42± 0.11^{de}	1.60± 0.06^{de}	2.34± 0.06^{abc}	2.80± 0.02^{a}	1.61± 0.02^{de}
Day 3	1.66± 0.01^{cd}	1.54± 0.06^{cd}	1.48± 0.01^{cd}	1.39± 0.02^{cd}	1.24± 0.06^{d}	1.26± 0.22^{d}	1.28± 0.02^{cd}	2.09± 0.02^{bc}	2.21± 0.02^{b}	4.32± 0.15^{a}
Day 4	1.34± 0.12^{bc}	1.40± 0.01^{bc}	1.37± 0.03^{bc}	1.20± 0.02^{bc}	1.07± 0.26^{c}	1.13± 0.02^{bc}	1.06± 0.18^{c}	1.35± 0.02^{bc}	1.43± 0.08^{b}	2.67± 0.02^{a}
Day 5	1.29± 0.05^{b}	1.34± 0.21^{b}	1.29± 0.06^{b}	1.22± 0.08^{bc}	1.19± 0.03^{bc}	1.14± 0.06^{bc}	1.01± 0.01^{c}	1.21± 0.06^{bc}	1.23± 0.02^{bc}	1.78± 0.09^{a}
Day 6	1.32± 0.05^{ab}	1.38± 0.01^{a}	1.34± 0.06^{a}	1.31± 0.06^{ab}	1.23± 0.11^{ab}	1.24± 0.03^{ab}	1.04± 0.26^{b}	1.24± 0.02^{ab}	1.32± 0.02^{ab}	1.45± 0.08^{a}
Day 7	1.29± 0.06^{b}	1.29± 0.13^{b}	1.29± 0.03^{b}	1.28± 0.06^{bc}	1.24± 0.12^{bc}	1.22± 0.03^{bc}	1.10± 0.01^{c}	1.25± 0.02^{bc}	1.31± 0.02^{b}	1.53± 0.02^{a}

*Different latter in the same column indicates significant differences (p<0.05)

Discussion

Our study has demonstrated that supplement of L-carnitine in culture medium resulted in significant responses in doubling time and individual growth of enriched rotifers. Since most rotifer mechano and chemoreceptors sensitive to environmental stimulation are in direct contact with external medium (Clement *et al*., 1983). Therefore, it is possible that rotifers exposed to L-carnitine supplemented culture medium were affected in a direct way. However, L-carnitine-supplemented culture conditions might change the normal interaction between the microenvironment and rotifers, either detrimental or beneficial.

The best population density was determined in 100 mg l^{-1} L-carnitine treated rotifers. Moreover, the improved reproduction derived from increasing energy supply through enhanced lipid composition was stimulated by optimum level of supplemental L-carnitine. Higher fecundity usually resulted in shorter lifespan of these microanimals because the sources are directly used in reproduction process and caused reducing the availability for growth and maintenance (Snell and King, 1997). It is possible that over accelerated lipid catabolism, stimulated by over dose of supplemental L-carnitine, may result in an abnormal metabolic state and greatly shortened lifespan. Øie and Olsen (1997) reported that higher food ration resulted in higher growth rate, and then rotifers responded by a higher egg ratio. However, 100 mg L^{-1} treatment resulted in a considerable higher egg ratio and population growth in the present study. Enhancing reproduction may not always increase the growth rate and population density of the rotifer, but may alter the lifespan of the organism.

According to Zhang *et al*. (2005), L-carnitine supplement to algae cultures (concentration tested from 0.001 to 1000 mg L^{-1}), in individual (1 rotifer) and batch trials with *B. rotundiformis* fed *Chlorella vulgaris* (individual) or *Nannochloropsis oculata* (batch) had a positive effect on rotifer reproduction and population growth. The individual treatments with 1, 10 and 100 mg L-carnitine L^{-1} had significant increases in population densities, while treatment with 1000 mg L^{-1} showed a significant decrease in population density. These results may be dependent on the type of rotifer, culture conditions and nutrients used, but the obtained results in our study were similar to other findings. This led to suggest that the L-carnitine was vital for lipid metabolism enhancement and consequently improving reproduction and growth.

Compared to De Wilde *et al*. (2010), rotifer density increased during the trial, and the same treatments (1, 10 and 100 mg L-carnitine L^{-1}) performed better than the other treatments. These findings not only confirm the trends they found, but also suggest that the optimal dose of L-carnitine is most likely found in the range of 1–100 mg L^{-1}. However, De Wilde *et al.* (2010)

did not use *Brachionus plicatilis*, but they worked on the other species, *B. rotundiformis*.

Enriched rotifers with L-carnitine showed positive and significant responses on population growth, reproduction and individual growth under the optimum L-carnitine concentration. The results obtained in this study suggested that the optimum concentration of supplemented L-carnitine in culture medium was 100 mg L^{-1}. These enriched rotifers with L-carnitine as feed may further influence fish larval growth in some aspects.

Cost effectiveness is another important issue to be considered. According to the calculations, despite the high price of carnitine, the farmer would still benefit from supplementing the diet with L-carnitine (Harpaz, 2005).

Optimum amounts of L-carnitine addition to rotifers led increasing in population density and growth. The optimum L-carnitine concentration can provide energy to rotifers. If L-carnitine is used in practice in hatcheries in commercial scale, it is thought to contribute to economic efficiency at high rates.

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