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Research Article

Effects of L-Carnitine and L-Arginine enriched *Nannochloropsis oculata* on fatty acid profiles and growth of *Artemia franciscana*

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Abstract

Nutritional value and some special properties of Artemia have led to this creature being considered as a living food and biological model in aquaculture industry and research. L-carnitine and L-arginine as nutritional supplements are conditionally essential nutrients that play vital role in energy production and fatty acid metabolism. In this study, effects of different concentrations (0, 1, 10, 100 and 1000 mg/L) of L-carnitine and L-arginine on growth process and profile of fatty acids of *A. franciscana* were investigated separately. Artemia larvae were fed using L-carnitine and L-arginine enriched with *N. Oculata* microalgae separately for 30 days. Results of this study showed that total share of SAF saturated fatty acid of *A. franciscana* fed with *N. oculata* rich in L-carnitine was 157.99 mg/g and rich in L-arginine 148.64 mg/g and total share of MUFA rich in L-Carnitine was 74.89 mg/g and rich in L-arginine 78.87mg/g (p<0.05). Also in biometric results, double mutation of the 1000 mg/L-carnitine concentration treatment on the sixth day compared to the third confirmed the high effect of this supplement in instar 6 stage (p<0.05). Results of this study showed that the use of appropriate and complete diets as well as growth stimulants can be effective in increasing the qualitative and quantitative volume of production in aquaculture farms, including zooplankton such as *A. franciscana*, which have many applications as live food.

Keywords: *A. franciscana*, *N. oculata*, L-carnitine, L-arginine, Fatty acid profile, Growth

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Introduction

The brine shrimp Artemia is a key feed for larval stages of various commercially farmed shrimp and fish species all over the world. However, the increasing global demand for brine shrimp cysts could be a bottleneck for the continued growth of aquaculture industry, because its supply comes from harvesting wild populations, which can sometimes be unpredictable and vary considerably depending on environmental conditions (Sorgeloos *et al.*, 2001). In current aquafeed industry, protein is the most expensive macronutrient in feeds formulation. The increasing cost and limited supplies of traditional protein sources, such as fishmeal, have increased the trend of using high carbohydrate or fat diets in aquafeeds production to maximize a "protein sparing effect" (Francis and Turchini, 2017). However, the use of high-fat diets (HFD) has caused some metabolic diseases, including excess lipid accumulation in the liver and abdominal adipose tissues (Cao *et al.*, 2019), which impair fish immune functions and resistance to stress (Dai *et al.*, 2018). Therefore, improved lipid catabolism has been an important research topic in order to ensure efficient energy supply so as to promote protein deposition and reduce fat accumulation. L-carnitine (L-β-hydroxy-γ-N, N, N-trimethylaminobutyric acid) is a functional additive, which plays an essential role in translocation of long-chain fatty acids (LCFAs) from cytosol into mitochondrial matrix for subsequent β-oxidation, thus allowing cells to break down fat and obtain energy (Porter *et al.*, 2017). In all animals, including fish, l-carnitine can be synthesized naturally from lysine and methionine (Li *et al.*, 2019). During past decades, the effects of dietary L-carnitine on nutrient metabolism have been studied extensively in different farmed fish species. L-carnitine is reported to reduce body lipid content (Yang *et al.*, 2012; Sharifzadeh *et al.*, 2017), and to increase body and muscle protein content, showing a significant protein sparing effect (Li *et al.*, 2019). Moreover, L-carnitine also affect glucose metabolism by influencing blood glucose level and tissues glycogen content (Sharifzadeh *et al.*, 2017). In a study by Lee *et al.* (2014), effects of 1, 10, 100 and 1000 mg/L doses of L-carnitine directly and with intracellular enrichment on microalgae *Dunaliella tertiolecta* on growth, survival and precocious maturity of artemia were investigated. 1000 doses of this substance had the best effect on puberty and 100 doses had the best effect on the overall growth of Artemia. Also Adloo *et al.* (2017) showed that enriching Artemia with HUFA, PUFA and MUFA fatty acids increased its nutritional quality for use as a live food in the diet of western white shrimp *Penaeus vannamei*. In view of the above, the aim of the present study was to evaluate improvement of growth indices, reproductive performance and homogeneity, as well as improvement of performance of fatty acid profiles in *A. franciscana* fed by the microalgae *N.*

oculata enriched with L-arginine and L-carnitine supplements in cells microalgae and obtaining functional correct dose.

Materials and methods

Experimental design

This study was conducted in two periods of 86 days in winter 2016 and winter 2017 in Aquatic Live Fish Laboratory and Phycolab of Islamic Azad University, Bandar Abbas Branch and Pollution Assessment Laboratory of Bandar Abbas Environmental Organization and Quality Control Laboratory of Tehran University in a completely randomized block design with 9 treatments. Treatments include, treatment 1 (NCH.LC1) Artemia fed with L-carnitine enriched with *N. oculata* 1, treatment 2 (NCH.LC10) L-carnitine-enriched *N. oculata* 10, treatment 3 (NCH.LC100) L-carnitine-enriched *N. oculata* 100 and treatment 4 (NCH.LC1000) Artemia fed with L-carnitine *N. oculata* 1000, and also treatment 5 (NCH.LA1) of Artemia fed with L-arginine enriched with *N. oculata* 1, treatment 6 (NCH.LA10) Artemia fed with *N. oculata* enriched with L-arginine 10, treatment 7 (NCH.LA100) Artemia fed with L-arginine enriched with L-arginine 100, treatment 8 (NCH.LA1000) Artemia fed with L-arginine enriched with L-arginine 1000 with treatment 9 as control. Each treatment had three replications with a total of 27 replications.

Hatching operation, release and breeding of Artemia

To perform this part of laboratory operation, *A. franciscana* cyst was prepared from Jask Port, a subset of Dr. Gorgij's workshop. The amount of nauplius determined was 500 units per liter of water (Lavens and Sorgeloos, 1996) and as a result, in the present study, 20.000 units were estimated for each zogg, which according to the number of zoggs, the total number was 1080.000 nauplia, which was equivalent to 12 grams of high quality cyst with 90% hatch percentage. After hatching, exposure to phototropism phenomenon was done to accumulate the nauplius and create a homogeneous mixture of nauplius-water, then this mixture was transferred to each zogg, and the correct weighting of nauplius was obtained in replicated treatment zoggs.

Preparation and cell count of stock of microalgae

In the present study after preparing the stock, *N. oculata* microalgae was cultured. Stocks were obtained from Phycolab Center for Reproduction and Reconstruction of Persian Gulf Aquatic Reserves, Shahid Kolahy Port, Minab County. The amount of stock was 1 liter separately. After preparation and sealing away from direct light, stocks were immediately transferred to Phycolab of Live Food Laboratory of Islamic Azad University of Bandar Abbas and aerated at medium light intensity of 2000 lux with fluorescent lamps measured by a lux meter. The

formula used for calculating the number of algal cells on a neobar slide (hemocytometer) with 25 squares was: Total number of cells in 25 squares=number of cells in 5 squares randomly×5 Number of algal cells per 1 mL of sample (culture medium or zoggs)=10^4×Total number of cells per 25 squares (Haran *et al.*, 1992).

Cultivation of microalgae

For algae culture Guillard's culture medium (Guillard's f/2) containing 3 ml of vitamin-nutrients (per liter of water and algae) and 1 ml of silicate (per liter of water and algae) was used. The water used for the culture environment of Gillard Sea was purified, which was prepared from Persian Gulf Ecological Research Institute, Bandar Abbas, and was sterilized by autoclave at 121.5°C and pressure of 2 atmospheres for 20 minutes. Ambient temperature after the end of this part of the operation was 25°C. Light intensity was 3500±350 lux. Light period was 12 hours of darkness and 12 hours of light (7 pm to 7 am dark and 7 am to 7 pm light). It was considered for each algal group to reach the static phase, which was achieved by daily counting of algal cells.

Enrichment of microalgae with supplements to feed Artemia

Enrichment of *N. oculata* microalgae with dietary supplement of L-carnitine and L-arginine with four different doses of 1, 10, 100, 1000 mg/L was done separately for each microalga. For this purpose, serial dilutions were prepared at the beginning of the work. We did it for supplements. Different doses were prepared by first diluting 10000 mg of pure L-carnitine in an Erlenmeyer flask containing 1000 ml of distilled water until we reached a dose of 10000 mg per liter (ppm). Then 100 ml of this solution was prepared in Erlenmeyer containing 900 ml. Enter a liter of distilled water to obtain a dose of 1000 mg/L. These steps were continued consecutively until a dose of 1 mg/L and a total of 4 one-liter Erlenmeyers were obtained from dilutions of 100, 10, 1 and 1000 L-carnitine. Exactly the same operation was performed to prepare serial dilutions of L-arginine. For daily algae culture, we prepared a sample and cell count from Erlenmeyers in which stock was cultured and mass-produced. When the number of *N. oculata* cells reached 6×9 (phase static), we enriched them with different concentrations of the specified supplements.

Initiation of Artemia active feeding with L-Carnitine and L-Arginine enriched microalgae treatments

After releasing newly hatched nauplii into the zoggs marking the zoggs as treatments and replications was done. After 8 hours when the yolk sac was absorbed and active swimming started (Lavens and Sorgeloos 1996), the microalgae *N. oculata* enriched with L-carnitine and L-arginine with different concentrations of 1, 10, 100, 1000 mg/L

was added to the treated zoggs with three replications for each treatment.

Measurement of growth factors, fecundity, survival and resistance to environmental stress

-Calculation of survival percentage (Mazurkiewicz *et al*., 2017), SVR =(S-D)/S ×100
-Calculation of absolute fecundity Equivalence (Biswas 1993), F = N/G
-Calculating relative fecundity (Biswas 1993), R = F/ TW
-Ammonia stress (Mugnier and Justou 2004), T =S/D×100
-Oxygen stress (hypoxia, Mugnier *et al*., 2008), T =S/D×100

Determination of fatty acid composition of experimental samples

To extract fat, one gram of the sample was transferred to the decanter. Then 7 ml of methanol was added to the sample and the decanter was shaken vortex vigorously for 1 minute. Then 14 ml of chloroform solution was added and shaken vigorously again for 1 minute. Brühl (1996) method was used to esterify fat. To check and identify the fatty acids in the samples a Philips gas chromatograph (GC) equipped with BPX70 SGE capillary column (60m×0.32mm ID×0.25.m-film thickness) and FID flame ionization detector (flame ionization detector) 0.2 µl of the ester sample was injected using a microliter syringe. In this method, helium gas with a purity of 99.999 % as a carrier gas, hydrogen gas as a fuel, nitrogen with a purity of 99.9 % as an auxiliary gas and dry air were used. Fatty acids in Artemia muscle were identified and the results were reported as percentage.

Statistical method and data analysis

Statistical analysis including calculation of mean and standard deviation was performed using SPSS software. Analysis of data related to changes in growth factors, fatty acid profiles, survival, and fecundity was done through one-way analysis of variance (ANOVA) and comparison of means between treatments was performed based on Duncan's multiple range test. Existence or absence of significant difference at 5% level was done using SPSS16 and Excel software in Windows environment.

Results

Fatty acid analyzes

Nutritional effect of *N. oculata* microalgae enriched with different concentrations of supplements, L-carnitine, L-arginine on profile, *A. franciscana* percentage shows the end results of the trial period (Table 1). In this study, the mean±standard deviation of SAF was 21.361±0.454 mg/g, MUFA was 12.043±0.998 mg/g and PUFA was 7.941±0.412 mg/g in *Nannochloropsis* feeders. Total share of SAF saturated fatty acid in Artemia fed with *N. oculata* rich in L-carnitine was 157.99 mg/g and rich in L-arginine was 148.64 mg/g. Total share of MUFA

fatty acid in Artemia fed with *N. oculata* rich in L-carnitine was 89.74 mg/g and rich in L-arginine was 78.87 mg/g. The highest amount of MUFA fatty acid was observed in the diet treated with microalgae enriched with *N. oculata* at a concentration of 1000 mg/L arginine.

Table 1: Comparison of some factors of fatty acid in *Artemia franciscana* nutrient feeding algae *Nannochloropsis oculata* rich with L-Carnitine and L-Arginine supplements in different doses (mg/L).

Treatment /Index	C10 (mg/g)	C12 (mg/g)	C14 (mg/g)	C14:1 (mg/g)	C15 (mg/g)
Control	1.275±0.001^{a}	4.657±0.569^{e}	1.030±0.003^{e}	0.392±0.002^{a}	1.203±0.001^{d}
NCh.LA1	025. ±0.001^{g}	5.559±0.005^{d}	0.885±0.002^{g}	0.837±0.002^{g}	0.654±0.001^{f}
NCh.LA10	0.123±0.001^{b}	7.135±0.002^{ab}	1.222±0.003^{d}	0.282±0.002^{b}	1.855±0.001^{a}
NCh.LA100	0.022±0.001^{h}	7.326±0.025^{a}	1.772±0.002^{b}	0.053±0.002^{h}	0.455±0.002^{h}
NCh.LA1000	0.066±0.002^{f}	6.117±0.003^{c}	0.801±0.002^{h}	0.147±0.002^{c}	1.853±0.001^{a}
NCh.LC1	0.083±0.001^{e}	5.444±0.002^{b}	0.801±0.002^{h}	0.091±0.002^{f}	1.829±0.002^{b}
NCh.LC10	0.096±0.001^{d}	7.233±0.001^{a}	1.403±0.002^{c}	0.053±0.002^{h}	0.827±0.025^{e}
NCh.LC100	0.109±0.002^{c}	6.853±0.001^{b}	2.546±0.002^{a}	0.134±0.002^{d}	1.222±0.002^{c}
NCh.LC1000	0.005±0.001^{i}	7.126±0.001^{ab}	0.945±0.002^{f}	0.097±0.002^{e}	0.566±0.001^{g}

Treatment /Index	C16 (mg/g)	C16:1 (mg/g)	C17 (mg/g)	C17:1 (mg/g)	C18 (mg/g)	C18:1(n-9) (mg/g)
Control	1.820±0.002^{a}	1.500±0.001^{h}	2.314±0.001^{b}	1.126±0.002^{c}	6.864±0.055^{d}	1.942±0.001^{g}
NCh.LA1	1.800±0.001^{e}	1.685±0.002^{g}	1.587±0.001^{e}	0.887±0.002^{d}	8.942±0.002^{b}	2.285±0.005^{b}
NCh.LA10	1.721±0.001^{f}	1.832±0.002^{e}	1.553±0.001^{e}	0.694±0.004^{e}	8.950±0.002^{b}	1.965±0.003^{e}
NCh.LA100	1.839±0.579^{d}	2.002±0.002^{a}	0.681±0.001^{f}	0.364±0.003^{i}	9.285±0.344^{b}	2.231±0.001^{a}
NCh.LA1000	1.902±0.002^{c}	1.856±0.002^{d}	1.989±0.005^{c}	1.992±0.002^{a}	7.912±0.002^{c}	2.285±0.002^{c}
NCh.LC1	1.808±0.002^{e}	1.456±0.001^{i}	2.036±0.001^{c}	0.597±0.002^{g}	8.141±0.009^{c}	2.162±0.001^{d}
NCh.LC10	2.121±0.001^{a}	1.802±0.002^{f}	2.852±0.011^{a}	0.478±0.001^{h}	9.660±0.002^{a}	1.821±0.001^{h}
NCh.LC100	1.966±0.002^{b}	1.901±0.002^{b}	1.887±0.001^{d}	0.666±0.002^{f}	9.166±0.763^{b}	1.622±0.001^{i}
NCh.LC1000	1.800±0.002^{e}	1.900±0.001^{c}	0.598±0.001^{g}	1.555±0.001^{b}	9.057±0.002^{b}	1.944±0.002^{f}
Total	1.149±1.864	1.795±1.770	0.698±1.722	0.517±0.928	0.865±8.665	2.039±2.308

* Numbers (mean±SD) with dissimilar letters in each row have a significant difference (p<0.05).

Biometric results in Artemia franciscana

Nutritional effect of *N. oculata* microalgae enriched with different concentrations of L-carnitine and L-arginine on the biometry of *A. franciscana* in different periods of 30 days (Table 2).

Calculation of survival in Artemia franciscana

Nutritional effect of *Chlorella vulgaris* and *N. oculata* microalgae enriched with different concentrations of L-carnitine and L-arginine on the survival of *A. franciscana* in 30-day trial is included in the three-day time process (Table 3).

Results of comparison of the mean of some stressors, final excretory ammonia, final fresh and dry weight, and quality index in Artemia franciscana

Table 4 lists the effects of L-carnitine and L-arginine supplements on algae-fortified algae on stress factors, ammonia excretion, final weight, level of smoothness and quality index in *A. franciscana* over a 30-day trial period.

Table 2: Biometric comparison of *Artemia franciscana* fed with *Nannochloropsis oculata* rich in L-carnitine and L-arginine supplements in different doses (mg/L).

Treatment /Index	2018-02-13 (mL m)	2018-02-16 (mL m)	2018-02-19 (mL m)	2018-02-22 (mL m)	2018-02-22 (mL m)
Control	0.00±0.50^{a}	0.00±0.94^{c}	0.01±1.03^{c}	0.01±1.18^{h}	0.00±1.44^{e}
NCh.LA1	0.00±0.49^{a}	0.00±0.84^{g}	0.50±0.68^{d}	0.01±1.44^{g}	0.01±1.91^{d}
NCh.LA10	0.00±0.50^{a}	0.00±1.00^{a}	0.00±1.34^{bc}	0.00±2.35^{a}	0.01±3.34^{a}
NCh.LA100	0.00±0.50^{a}	0.00±0.90^{e}	0.00±1.43^{b}	0.01±2.19^{c}	0.02±2.92^{b}
NCh.LA1000	0.00±0.50^{a}	0.10±0.94^{c}	0.01±1.40^{b}	0.00±1.55^{f}	0.33±1.55^{e}
NCh.LC1	0.00±0.49^{a}	0.00±0.97^{b}	0.01±1.31^{bc}	0.00±1.64^{e}	0.01±1.95^{d}
NCh.LC10	0.00±0.50^{a}	0.01±0.87^{f}	0.01±1.11^{bc}	0.00±1.45^{g}	0.01±1.81^{d}
NCh.LC100	0.00±0.50^{a}	0.01±0.82^{h}	0.01±1.23^{bc}	0.01±2.11^{d}	0.01±2.91^{b}
NCh.LC1000	0.00±0.50^{a}	0.00±0.92^{d}	0.00±1.85^{a}	0.01±2.21^{b}	0.01±2.53^{c}
Total	0.006±0.502	0.056±0.915	0.331±1.266	0.409±1.791	0.661±2.265

Treatment /Index	2018-02-28 (mL L)	2018-03-03 (mL L)	2018-03-06 (mL L)	2018-03-09 (mL L)	2018-03-12 (mL L)	2018-03-15 (mL L)
Control	0.01±1.92^{e}	0.01±2.21^{f}	0.01±3.24^{e}	0.02±4.03^{g}	0.04±5.24^{h}	0.02±6.03^{g}
NCh.LA1	0.03±2.14^{d}	0.02±2.47^{e}	0.01±3.55^{c}	0.02±4.48^{d}	0.02±5.44^{g}	0.02±6.02^{g}
NCh.LA10	0.02±2.24^{c}	0.01±2.59^{d}	0.02±3.12^{g}	0.01±4.31^{e}	0.01±6.86^{d}	0.01±7.03^{d}
NCh.LA100	0.01±2.56^{a}	0.02±3.17^{b}	0.02±3.73^{a}	0.01±5.13^{c}	0.02±7.03^{b}	0.02±7.32^{b}
NCh.LA1000	0.01±1.84^{f}	0.04±2.23^{f}	0.02±2.88^{i}	0.01±4.21^{f}	0.05±6.50^{e}	0.02±6.93^{e}
NCh.LC1	0.01±2.11^{d}	0.02±3.12^{b}	0.02±3.42^{d}	0.02±5.22^{b}	0.02±6.92^{c}	0.02±7.22^{c}
NCh.LC10	0.01±1.91^{e}	0.03±2.86^{c}	0.01±3.16^{f}	0.01±5.13^{c}	0.03±6.81^{d}	0.03±6.99^{d}
NCh.LC100	0.02±2.46^{b}	0.03±3.33^{a}	0.01±3.65^{b}	0.06±6.15^{a}	0.02±7.13^{a}	0.05±7.40^{a}
NCh.LC1000	0.02±2.13^{d}	0.03±2.19^{f}	0.02±3.08^{h}	0.02±4.47^{d}	0.03±6.14^{f}	0.05±6.50^{f}
Total	0.237±2.145	0.430±2.687	0.277±3.314	0.647±4.794	0.670±6.453	0.505±6.827

* Numbers (mean±SD) with dissimilar letters in each row have a significant difference (p<0.05).

Table 3: Comparison of survival in *Artemia franciscana* fed with *Nannochloropsis oculata* rich in L-carnitine and L-arginine supplements with different doses (mg/L).

Treatment /Index	2018-02-13	2018-02-16	2018-02-19	2018-02-22	2018-02-25	2018-02-28
Control	1.00±480.00^{a}	2.08±192.33^{b}	1.00±74.00^{e}	1.52±47.33^{e}	2.08±35.33^{f}	2.08±28.33^{bc}
NCh.LA1	2.00±480.00^{a}	1.52±186.33^{c}	1.00±71.00^{f}	1.52±46.67^{e}	1.00±32.00^{f}	3.60±24.00^{cd}
NCh.LA10	3.00±480.00^{a}	2.08±181.67^{d}	1.52±1116.67^{a}	1.52±72.67^{c}	1.52±40.33^{e}	1.52±31.33^{b}
NCh.LA100	2.00±480.00^{a}	2.08±154.33^{f}	2.08±1110.33^{b}	1.52±78.67^{a}	3.00±35.00^{d}	1.00±28.00^{bc}
NCh.LA1000	3.00±480.00^{a}	2.08±197.33^{a}	2.08±96.67 ^{c}	2.08±62.67^{d}	2.51±32.33^{a}	2.51±22.33^{d}
NCh.LC1	1.00±480.00^{a}	2.08±192.00^{b}	2.08±76.33^{e}	2.08±47.33^{e}	1.00±32.00^{b}	1.00±27.00b^{cd}
NCh.LC10	2.00±480.00^{a}	2.51±182.67^{d}	1.00±97.00^{c}	1.52±75.67^{b}	1.00±69.00^{b}	6.24±57.00^{a}
NCh.LC100	1.00±480.00^{a}	1.52±159.33^{e}	1.52±192.67^{d}	1.52±48.67^{e}	1.52±35.33^{e}	2.51±23.33^{cd}
NCh.LC1000	2.00±480.00^{a}	1.52±132.33^{g}	1.52±32.33^{g}	2.00±21.00^{f}	2.08±17.33^{g}	1.52±14.67^{e}
Total	1.687±480.00	21.035±175.37	24.410±85.22	17.831±55.63	13.253±36.52	11.517±28.44

Treatment /Index	2018-03-03	2018-03-06	2018-03-09	2018-03-12	2018-03-15
Control	1.00±23.00^{cd}	1.52±19.33^{b}	1.52±16.33^{b}	1.52±14.33^{bc}	1.52±13.33^{bc}
NCh.LA1	2.08±20.33^{de}	1.15±18.33b	1.15±15.67^{b}	1.00±13.00^{c}	1.00±12.00^{c}
NCh.LA10	1.00±26.00^{b}	1.52±21.33^{b}	2.51±18.33^{b}	2.08±16.33^{b}	1.52±15.33^{b}
NCh.LA100	1.00±24.00^{bc}	1.00±19.00^{b}	1.52±16.67^{bc}	1.15±14.33^{bc}	1.00±13.00^{bc}
NCh.LA1000	1.52±17.67^{e}	2.64±12.00^{c}	1.73±9.00^{c}	1.00±7.00^{e}	1.15±5.67^{e}
NCh.LC1	1.52±23.67^{bc}	1.52±18.67^{b}	1.52±15.67^{b}	1.52±14.00^{bc}	1.52±12.33^{c}
NCh.LC10	1.00±46.00^{a}	2.00±31.00^{a}	2.51±27.67^{a}	2.64±25.00^{a}	2.08±23.33^{a}
NCh.LC100	3.00±20.00^{e}	2.08±14.33^{c}	1.52±11.67^{c}	1.00±10.00^{d}	1.15±8.33^{d}
NCh.LC1000	1.00±9.00 ^{f}	1.52±4.33^{d}	1.00±3.00^{d}	1.00±2.00^{f}	0.57±1.67^{f}
Total	9.575±23.30	7.110±17.59	6.676±14.89	6.272±12.89	6.00±11.67

* Numbers (mean±SD) with dissimilar letters in each row have a significant difference (p<0.05).

Table 4: Evaluation of some survival indices, ammonia content, final weight, total uniformity and quality index in *Artemia franciscana* fed with *Nannochloropsis oculata* rich in supplements.

Treatment /Index	Ammonia shock losses (%)	Hypoxia shock losses (%)	Ammonia content (mg/l l)	Individual weight (µg)	Individual dry weight (µg)	Absolute Fecundity (%)	Qualitative index (g/cm)
NCh.B	18.33±2.88 ^{c}	66.16±2.88 ^{ab}	13.30±0.10 ^{g}	109.22±10.00 ^{f}	2.53±3.05 ^{cd}	2.51±2.66^{e}	3.49±0.03 ^{ab}
NCh.LA1000	21.66±2.88^{bc}	16.66±2.88 ^{ab}	85.20±0.81 ^{a}	112.14±5.13 ^{d}	2.66±1.52 ^{b}	2.51±21.66^{a}	3.69 ±0.02 ^{a}
NCh.LA100	25.00±0.00^{b}	18.33±2.88 ^{a}	43.46±0.15 ^{b}	113.35±13.22 ^{d}	2.68±3.21 ^{b}	1.00±10.00^{bc}	3.52 ±0.03 ^{ab}
NCh.LA10	23.33±2.88^{bc}	16.66±2.88^{ab}	38.53±0.10 ^{c}	111.12±2.51 ^{e}	2.66±4.16 ^{b}	2.08±7.66^{bc}	3.77 ±0.62 ^{a}
NCh.LA1	18.33±2.88^{c}	20.00±0.00 ^{a}	15.81±0.20 ^{f}	106.20 ±81.85 ^{g}	2.56 ±3.21 ^{cd}	1.52±6.33^{d}	3.80 ±0.03 ^{a}
NCh.LC1000	21.66±2.88^{bc}	16.66±2.88 ^{ab}	84.30±0.20 ^{a}	105.91±7.09 ^{g}	2.58 ±3.00 ^{a}	1.15±9.66^{bc}	3.74 ±0.05 ^{a}
NCh.LC100	31.66±2,88 ^{a}	16.66±2.88 ^{ab}	34.65±0.35 ^{d}	119.03±15.27 ^{a}	2.84 ±4.50 ^{c}	2.08±11.66^{b}	3.04±0.05 ^{c}
NCh.LC10	23.33±2.88^{bc}	11.66±2.88 ^{b}	39.45±1.42 ^{c}	109.53±35.11 ^{f}	2.51 ±3.51 ^{d}	1.52±10.33^{bc}	2.9 9 ±0.04 ^{c}
NCh.LC1	31.66±2.88 ^{a}	21.66±2.88 ^{a}	23.23±0.37 ^{e}	115.48 ±10.40^{b}	2.70±4.50 ^{b}	0.57±4.66^{de}	3.21 ±0.04 ^{c}

* Numbers (mean ± SD) with dissimilar letters in each row have a significant difference (p<0.05).

Discussion

The effects of L-carnitine and L-arginine enriched *N. oculate* on growth, reproductive activity and performance improvement of fatty acid profiles of *A. franciscana* were investigated in this study. During recent years, much research has been done in determining diets suitable food for better growth and premature maturity with reduced production costs. In this regard and in order to increase production efficiency for years, L-carnitine and L-arginine have attracted the attention of researchers as a dietary supplement, such as vitamins. In research, it was found that the microalgae *N. oculata* contains about 33% of total fatty acids, which is very similar to the results obtained in the present study. On the other hand, the total values of L-carnitine enrichment were higher than that of L-arginine enriched which according to scientific reports; indicate that L-carnitine is used only for the entry of long-chain unsaturated fatty acids into the mitochondria (Dikel *et al.*, 2010). Therefore it plays a stronger role in the elimination of fatty acids in competition with L-arginine supplementation, which this analysis fully confirms, the result of this part of the study. Also, the total fatty acid in Artemia fed with L-arginine-rich *Nannochloropsis* also showed a significant increase (p<0.05) compared to the diet with L-carnitine-rich *Nannochloropsis*. L-carnitine accompanied by activation of fatty acids to transfer the matrix into the mitochondria, for the entry of long-chain fatty acids in the form of acetyl l-carnitine is essential for mitochondria and plays an important role in the breakdown of fat into energy. Findings of Lankford and Weber (2006) showed a direct relationship between increased fatty acids and amino acids with L-arginine supplements. In a similar study, the L-carnitine containing diet in the African catfish (*Clarias gariepinus*) reduced eicosapentaenoic acid and decosahexaenoic acid, suggesting that these two unsaturated fatty acids may be used as part of the transfer of acid-converting enzyme to carboxylate (Ozrorio *et al.*, 2005), that did not match the results of this study. Also, Khamechin *et al.* (2014) studied the effect of dietary L-carnitine

supplementation on growth performance, survival rate, early maturation and fatty acids profile of parthenogenetic Artemia. Results revealed that this supplement can stimulate early sexual maturation and aggravates masculinization in all of the experimental groups in comparison with the control group. The best result, in this regard was recorded for 1000 mg concentration of L-carnitine in which at 13th day the best percentage of sexual maturation, 5.04%, was seen. These results showed that L-carnitine has significant effect on growth performance when it is exposed to 1000 mg concentration of L-carnitine. Also the fatty acids profile showed that DHA is not present in any of these fatty acids that did not correspond to the results of the present study.

Double mutation of the 1000 mg/L L-carnitine treatment on the sixth day compared to the third confirms the high effect of this supplement in Instar 6 stage. The difference of more than doubling the maximum length of Artemia fed with supplement 10 mg/L L-arginine with the control sample showed the extraordinary effect of this growth supplement on the longitudinal increase of instar 12. Also, the maximum amount of nutritional biometrics with the microalgae *N. oculata* was obtained in a diet rich in L-arginine with 100 doses which can be realized with high nutritional-absorption power of *N. oculata*. It can be concluded that dose of 1000 supplements, which is considered a high concentration, is regarded as a growth-limiting dose compared to the control and other treatments in 27-day-old adult Artemia. Also, lowest values showed a positive effect of supplements in combination with *N. oculata* compared to the control sample and the sample rich in L-arginine 1 mg/ (p<0.05). Khamechin *et al.* (2011) in a similar study measured different levels of L-carnitine, yeast and supplement-rich microalgae on Artemia biometrics and found a similar result that a concentration of 100 mg/L L-carnitine had the best effect on overall Artemia growth. In a study by Gaylord and Gatlin (2000), effects of different levels of L-carnitine (0, 500 and 1000 mg per kg) of diet containing 5 and 10% fat in hybrid striped bass (*Morone chrysops*×*M. saxatilis*) with an average initial weight of 3.3g were examined for 9 weeks and the results showed that L-carnitine supplementation at 1000 mg per kg level increased the growth rate. In another study by Dong *et al.* (2011), different concentrations of this substance were used for rotifer cultivation and it was found that L-carnitine can be considered as a growth and survival stimulant for rotifers. Also, Chen *et al.* (2019) investigated the effects of methionine enriched Artemia nauplii on growth, amino acid profiles, absorption enzyme activity and antioxidant capability of common carp (*Cyprinus carpio var. Jian*) larvae. The results demonstrated that the level of methionine in nauplii enriched with methionine increased significantly as

the level of supplemental methionine rose (p<0.05). The specific growth rate (SGR) and body length growth rate (BLGR) values in all experimental groups in the trial period were significantly higher than those in the control group (p<0.05). With supplement of methionine, the amino acid profiles vary depending on the type of amino acid and/or the level of methionine applied. Further examination revealed that activity of absorption enzymes in 800 and 1,600 mg/L groups were all significantly higher compared to other two groups (p<0.05). Similar results on antioxidant capability of fish larvae were observed among the treatments. The changes in these physiological factors allowed the control of content of supplemental methionine in Artemia nauplii for larvae and potentially the ability to improve the growth performance of common carp larvae that was similar to the results of the present study.

Among nutritional treatments of the algal group in instar 6, the lowest survival rate was related to feeding with *N. oculata* with a feeding dose of L-carnitine 1000. Also, in the instar stage 9, the lowest survival rate was related to nutrition obtained from *N. oculata* microalgae enriched with L-carnitine at a concentration of 1000 mg/L. In the instar stage 12, the highest survival rate was observed in nutritional treatments with L-arginine at a dose of 1000 and the lowest was related to the nutritional treatment at L-carnitine at a dose of 1000 (p<0.05). The dose is in fact designed to improve survival process in instar 12. A 2011 study by Dong *et al.* (2011) used different concentrations of L-carnitine in rotifer breeding and found that L-carnitine can be effective as a growth stimulant in rotifer survival. Given that L-carnitine plays an important role in acid metabolism, it has a long-chain fat and prevents the storage of fats in the form of triglycerides in the body, as a result, it protects the organism against diseases and liver damage caused by an increase in body fat, which in turn increases the body's immunity to stressful conditions and thus increases survival (Chen *et al.*, 2015). Another study by Tremblay and Bradley (1992) showed increasing survival effect of adding L-carnitine to chinook salmon diet.

There was no significant difference between doses of 10 and 100 mg/L L-arginine and 10 and 1000 mg/L L-carnitine in 30-day-old Artemia breeders fed with *N. oculata*-rich supplements, respectively (p<0.05) compared with the control sample. The highest level of fecundity was related to the sample of L-arginine-rich nutritional treatment and then L-carnitine supplementation with a concentration of 100 in the *Nannochloropsis* nutritional treatments that represented the role of L-arginine and L-carnitine supplements in improving fecundity. L-arginine and L-carnitine improved the homogeneity in a similar report (Jayaprakas and Sambhu, 1996), the effective role of L-carnitine supplementation in 4 different

concentrations of 300, 500, 700 and 900 mg/kg diet on growth and reproductive mechanism of male tilapia weighing 2.2g for 252 days and to the amount of 5% fish biomass was examined and it showed that the best amount of L-carnitine in the diet to increase reproduction and growth was 900 mg/kg of the diet so that the weight of the group fed with this concentration was 20g more than the group fed without L-Carnitine which is completely consistent with the results of the present study in terms of the type of supplement and the amount of consumed concentrations.

There was no significant difference in nutritional treatments with *N. oculata* rich in supplementation of L-arginine 1, 10, 1000 and L-carnitine 1000 mg/L nutritional treatments, while there was a significant difference with control (p<0.05). The highest quality index of productive Artemia was also seen in the same category. This category of Artemia also showed the lowest quality index, which can easily be pointed to the weak role of L-carnitine with doses below 1000 mg/L in increasing the quality of *A. franciscana*, which is produced in 30 days. The results of Becker *et al.* (1999) showed that the use of 500 mg of L-carnitine per kg of diet due to lipid oxidation, improved growth performance and protein access. The results of this study showed that dietary supplements, L-carnitine and L-arginine, were added directly in culture medium and indirectly added by enrichment, the algae were fed by the specimens. Growth stimulants can also increase qualitative and quantitative volume of production in various farms. Aquatic animals, such as zooplankton and Artemia, which have many uses as a living food, are effective in increasing aquaculture production.

References

Adloo, M.N., Salarzadeh, A.R., Aqh, N. and Bahri, A.H., 2017. Effect of Artemia biomass enriched by HUFA, PUFA and MUFA fatty acids on fatty acid composition, growth and qualitative reproduction indices of white shrimp (*Litopenaeus vannamei*). *Journal of Aquaculture Development*, 11(**2**), 79-91, in Persian.

Becker, K., Schreiber, S., Angoni, C. and Blum, R., 1999. Growth performance and feed utilization response of *Oreochromis niloticus* × *Oreochromis aureus* hybrids to L-carnitine measured over a full fattening cycle under commercial conditions. *Aquaculture*, 174(**3-4**), 313-322. DOI: 10.1016/S004-8486(99)00019-8.

Biswas, S.P., 1993. *Manual of methods in fish biology.* South Asian Publishers Pvt. Ltd., New Delhi, India, 157 P.

Brühl, L., 1996. *Official methods and recommended practices of the American Oil Chemist's Society, physical and chemical characteristics of oils, fats and waxes*, section 1. The AOCS Methods Editor and the AOCS

Technical Department, 54 P. AOCS Press, Champaign, Illinois, USA.

Cao, X.F., Dai, Y.J., Liu, M.Y., Yuan, X.Y., Wang, C.C., Huang, Y.Y., Liu, M.Y. and Jiang, G.Z., 2019. High-fat diet induces aberrant hepatic lipid secretion in blunt snout bream by activating endoplasmic reticulum stress-associated IRE1/XBP1 pathway. *Biochemistry et Biophysica Acta-Molecular and Cell Biology of Lipids*, 1864(**3**), 213-223. DOI: 10.1016/ j.bbalip.2018.12.005.

Chen, G.F., Liu, Y., Jiang, J., Jiang, W.D., Kuang, S.Y., Tang, L., Tang, W.N., Zhang, Y.A., Zhou, X.Q. and Feng, L., 2015. Effect of dietary arginine on the immune response and gene expression in head kidney and spleen following infection of Jian carp with *Aeromonas hydrophila*. *Fish and Shellfish Immunology*, 44(**1**), 195-202. DOI: 10.1016/j.fsi.2015.027.

Chen, Y., Yang, Y., Wang, Q., Lin, L., Liu, H., Guo, Z. and Zhang, D., 2019. The effects of methionine-enriched *Artemia* nauplii on growth, amino acid profiles, absorption enzyme activity and antioxidant capability of common carp (*Cyprinus* carpio var. Jian) larvae. *Aquaculture Research*, 50(**1**), 1-8. DOI: 10.1111/are.13863.

Dai, Y.J., Jiang, G.Z., Yuan, X.Y. and Liu, W.B., 2018. High-fat-diet-induced inflammation depresses the appetite of blunt snout bream (*Megalobrama amblycephala*) through the transcriptional regulation of leptin/mammalian target of rapamycin. *British Journal of Nutrition*, 120(**12**), 1422-1431. DOI: 10.1017/S000711451800288X.

Dikel, S., Ünalan, B., Eroldoğan, O.T. and Özlüer Hunt, A., 2010. Effects of dietary L-carnitine supplementation on growth, muscle fatty acid composition and economic profit of rainbow trout (*Oncorhynchus mykiss*). *Turkish Journal of Fisheries and Aquatic Sciences*, 10, 173-180. DOI: 10.4194/trifas.2010.0203.

Dong, J.Y., Qin, L.Q., Zhang, Z., Zhao, Y., Wang, J., Arigoni, F. and Zhang, W., 2011. Effect of oral L-arginine supplementation on blood pressure: a meta-analysis of randomized, double-blind, placebo-controlled trials. *American Heart Journal*, 162(**6**), 959-965. DOI: 10.1016/ j.ahj.2011.09.012.

Francis, D.S. and Turchini, G.M., 2017. Retro-engineering the protein sparing effect to preserve n-3 LC-PUFA from catabolism and optimise fish oil utilisation: A preliminary case study on juvenile Atlantic salmon. *Aquaculture*, 468(**1**),184-192. DOI: 10.1016/j.aquaculture.2016. 10.013.

Gaylord, T.G. and Gatlin, M.D., 2000. Effects of dietary carnitine and lipid on growth and body composition of hybrid striped bass (*Morone chrysops* female x *M. saxatilis* male). *Fish Physiology and Biochemistry*, 2, 297-302.

Haran, M.P., Surendran, V., Reddy, K.M. and Rao, V.S., 1992. TASPARC shrimp farm - a success story on scientific shrimp farming. *Fishing Chimes*, 12, 34-45.

Jayaprakas, V. and Sambhu, C., 1996. Growth response of white prawn, *Penaeus indicus*, to dietary L-carnitine. *Asian Fisheries Science*, 9(**3**), 209-219.

Khamehchin, Y., Manaffar, R., Tokmehchi, A., Hojati, V. and Maleki Balajoo, O., 2011. The effect f dietary L-Carnitine supplementation on the growth performance, survival rate and early maturation of *Artemia franciscana*. *Journal of Animal Biology*, 3(**4**), 29-26, in Persian.

Khamechin, Y., Manaffar, R., Tokmechi, A., Hojati, V. and Maleki Balajoo, O., 2014. The effect of dietary L-carnitine supplementation on the growth performance, survival rate, early maturation and fatty acids profile of parthenogenetic Artemia. *Journal of Marine Science and Technology*, 12(**2**), 47-56, in Persian.

Lankford, S.E. and Weber, G.M., 2006. Associations between plasma growth hormone, insulin-like growth factor-I, and cortisol with stress responsiveness and growth performance in a selective breeding program for rainbow trout. *North American Journal of Aquaculture*; 68(**2**), 151-159. DOI: 10.1577/A05-014.1.

Lavens, P. and Sorgeloos, P., 1996. *Manual on the production and use of live food for aquaculture.* FAO Fisheries Technical Paper 361, 295p. Food and Agriculture Organization of the United Nations, Rome, Italy.

Lee, B.J., Lin, J.S., Lin, Y.C. and Lin, P.T., 2014. Effects of L-carnitine supplementation on oxidative stress and antioxidant enzymes activities in patients with coronary artery disease: a randomized, placebo-controlled trial. *Nutrition Journal*, 13(**1**), 79. Published online, DOI: 10.1186/1475-2891-13-79.

Li, L.Y., Limbu, S.M., Ma, Q., Chen, L.Q., Zhang, M.L. and Du, Z.Y., 2019. The metabolic regulation of dietary L-carnitine in aquaculture nutrition: present status and future research strategies. *Reviews in Aquaculture*, 11(**4**), 1228-1257. DOI: 10.1111/raq.12289.

Mazurkiewicz, J., Hoffmann, L., Czyżak-Runowska, G., Pietrzak, M., Sierpowska, K., Andrzejewski, W. and Golski, J., 2017. The effects of diet and feeding techniques on growth factors and meat quality of common carp (*Cyprinus carpio*). *Iranian Journal of Fisheries Sciences*, 16(**2**), 639-653.

Mugnier, C. and Justou, C., 2004. Combined effect of external ammonia and molt stage on the blue shrimp *Litopenaeus stylirostris* physiological response. *Journal of Experimental Marine Biology and Ecology*, 309(**1**), 35-46. DOI: 10.1016/j.jembe.2005.03.008.

Mugnier, C., Zipper, E., Goarant, C. and Lemonnier, H., 2008. Combined effect of exposure to ammonia and hypoxia on the blue shrimp *Litopenaeus stylirostris* survival and physiological response in relation to molt stage. *Aquaculture*, 274(**2-4**), 398-407. DOI: 10.1016/j.aquaculture.2007.11.033.

Ozrorio, R.O.A., Ginneken, V.V., den Thillart, G.V., den Eertwegh, G.V., Verstegen, M.W.A. and Verreth, J.A.J., 2005. Dietary carnitine maintains energy reserves of exercised African catfish (*Clarias gariepinus*) fed high fat diets. *Scientia Agricola*, 62(**3**), 208-213. DOI: 10.1590/ S0103-90162005000300002.

Porter, C., Constantin-Teodosiu, D., Constantin, D., Leighton, B., Poucher, S.M. and Greenhaf, P.L., 2017. Muscle carnitine availability plays a central role in regulating fuel metabolism in the rodent. *Journal of Physiology*, 595(**17**), 5765-5780. DOI: 10.1113/jp274415.

Sharifzadeh, E., Yeganeh, S., Firouzbakhsh, F., Oraji, H., 2017. Effects of L-carnitine and vitamin C on the growth indices, body composition and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) juveniles. *Journal of Veterinary Research*, 72(**2**), 173-182, in Persian. DOI: 10.22059/jvr.2017.62530.

Sorgeloos, P., Dhert, P. and Candreva, P., 2001. Use of the brine shrimp, *Artemia* spp., on marine fish larviculture. *Aquaculture*, 200(**1-2**), 147-159. DOI: 10.1016/S0044-8486(01)00698-6.

Tremblay, G.C. and Bradley, T.M., 1992. L-carnitine protects fish against acute ammonia toxicity. *Comparative Biochemistry and Physiology - Part C. Toxicology and Pharmacology*, 101(2), 349-351. DOI: 10.1016/0742-8413(92)90286-g.

Yang, S.D., Liu, F.G. and Liou, C.H., 2012. Effects of dietary L-carnitine, plant proteins and lipid levels on growth performance, body composition, blood traits and muscular carnitine status in juvenile silver perch (*Bidyanus bidyanus*). *Aquaculture*, 342-343, 48-55. DOI: 10.1016/ j.aquaculture.2012.02.002.