

DETERMINATION OF GROWTH PERFORMANCE AND FEED UTILIZATION OF FRY OF GOLDFISH, *CARASSIUS AURATUS* (ACTINOPTERYGII: CYPRINIFORMES: CYPRINIDAE) FED L-CARNITINE-SUPPLEMENTED DIETS

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Background. A quest continues for alternative feed additives to the content of feeds used in global ornamental fish farming. This study was initiated to see the effect L-carnitine on the growth, feed utilization, and survival rates of goldfish, *Carassius auratus* (Linnaeus, 1758), one of the most cultivated aquarium fish species in the world. This experiment was carried out since there was no previous study on the effect of the L-carnitine on goldfish.

Materials and methods. In this study, five isonitrogenous, isolipidic, and isoenergetic diets (40% protein, 6% lipid, and 14.82 MJ · kg⁻¹ digestible energy, respectively) were formulated. The diets were supplemented with L-carnitine at the dose of 250, 500, 750, and 1000 mg · kg⁻¹ with a non-supplemented diet as control. Goldfish fry was reared in a 65 L glass tank containing aged water. Each dietary treatment was tested in triplicate groups of 20 fish per glass tank. Experimental fish (0.311 ± 0.01 g initial weight) were fed the diets twice daily to apparent satiation for 84 days. In the experiment, diets were evaluated based on growth performance and diets utilization.

Results. Supplemental dietary L-carnitine has beneficial effects on improving growth performance, feed conversion ratio, specific growth rate, and protein efficiency ratio in goldfish fry.

Conclusion. This study provides first data on the effect of L-carnitine on growth, feed utilization, and survival rates of goldfish. Future research should focus on the growth performance, and feed utilization parameters of other ornamental fish of different conditions of L-carnitine supplemented diets.

Keywords: *Carassius auratus*, feed utilization, goldfish, growth, L-carnitine

INTRODUCTION

In recent years, the importance of global ornamental fish farming and trade is increasing day by day. All species of goldfish in trade in the world has reached a volume of more than 1 billion dollars annually in more than 125 countries. (Anonymous 2016). These fish are one of the most preferred fish species by people, because of their varieties and attractive colors, and high tolerance to environmental effects. For this reason, it is very important for the ornamental fish farming that goldfish can reach the market size in a short time by feeding with low-cost feed. (Gümüş et al. 2016).

If L-carnitine is defined simply, it can be explained as amino acid and vitamin-like nutrient elements associated with B-group vitamins. It is an essential element mainly involved in the conversion of fatty acids into energy. In another definition, L-carnitine is not exactly an amino acid, because it does not work in protein synthesis. However, it is said to be grouped under this title because of

its similarity with amino acids (Bremer 1983, Shigenaga et al. 1994).

L-carnitine is synthesized from lysine and methionine in a way dependent on iron and ascorbate. L-carnitine provides the passage of fatty acids through the mitochondria membrane. It is necessary for the transfer of long-chain fatty acids through the membrane (He and Dahl 2000, Harpaz 2005).

The addition of L-carnitine in fish feeds plays an important role in promoting growth, the protein-protective effect of fat, and ultimately reducing fat accumulation in the body (Harpaz 2005, Yang et al. 2009).

Many studies are aiming to use L-carnitine in fish nutrition, involving the following fish species, silver perch, *Bidyanus bidyanus* (Mitchell, 1838) (see Yang et al. 2012); beluga sturgeon, *Huso huso* (Linnaeus, 1758) (see Mohseni and Ozório 2014); juvenile common carp, *Cyprinus carpio* Linnaeus, 1758 (see Sabzi et al. 2017); juvenile yellow princess, *Labidochromis caeruleus* Fryer, 1956 (see Sönmez unpublished*); juvenile

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** Sönmez S. 2018. L-Karnitin ilaveli yemlerin sarı prenses (*Labidochromis caeruleus* Fryer, 1956) yavrularının büyümesi ile artırılmış su sıcaklığının yaşama oranına etkisi. [Effects of dietary L-carnitine supplementation on growth performance and increased water temperature on survival rate of juvenile yellow princess (*Labidochromis caeruleus* Fryer, 1956)]. MSc thesis, Ege University, İzmir, Turkey. [In Turkish.]

black seabream, *Acanthopagrus schlegelii* (Bleeker, 1854) (see Jin et al. 2019); and brook trout, *Salvelinus fontinalis* (Mitchill, 1814) (see Paşlı unpublished*). However, no study with *Carassius auratus* juveniles has been carried out.

This study was intended to investigate the effects of L-carnitine on feed consumption, live weight increase, survival, and feed evaluation of goldfish, *Carassius auratus*, which is one of the most cultivated goldfish species in the world. We assumed that our study would be useful since no research publication related to feeding to L-carnitine supplemented feeds for goldfish fry, has been available. The fry is one of the most important stages of goldfish cultivation.

MATERIAL AND METHODS

Preparation of feeds. The diets to be used in the experiment were prepared based on fish meal and soybean meal. They contained 40% crude protein and represented 14.65 MJ · kg⁻¹ of digestible energy, as defined by Yanar et al. (2008) and Silva et al. (2010) as the nutritional requirement of goldfish. For the experiment, feeds with the mean digestible energy content of 40% crude protein, 6% crude lipid, and 14.65 MJ · kg⁻¹ were formulated (Table 1). The feed of the experimental groups was prepared by formulating in five different ways, including the ratios of 0, 250, 500, 750, and 1000 mg · kg⁻¹ of L-carnitine. The nutritional content has been verified and the results are shown in Table 2.

Experimental conditions. This study was carried out in a laboratory of the Faculty of Fisheries Research, Akdeniz University. Experimental fish were obtained from the Kepez Unit of the Ministry of Agriculture and Forestry Mediterranean Fisheries Research Production and Training Institute. The fry had fed and adapted to new conditions in 250 L fiber-glass for two weeks. At the end of the two weeks, goldfish fry with the mean live weight of 0.311 ± 0.01 g and a total length of 2.68 ± 0.04 cm were placed randomly in 15 aquaria, with 20 fry per tank. In the study, 15 glass aquaria (65 L) (70 × 30 × 40 cm) were used. The experiment was carried out with 3 replications. During the experiment, the lighting was set with fluorescent light for 10 h at day (0800–1800 h) and 14 h at night (1800–0800 h). Aquarium water was heated with thermostat heater at 24°C and aerated with stone aquarium diffuser. All groups were fed by hand, two times a day (0800 h and 1700 h) for 84 days until they were satiated. The unused feed and the fish feces accumulated on the bottom of the tank were removed by siphoning one hour after the last feeding of the day. The amount of water decreased by siphoning in the experiment tanks was completed with aged water (approximately 1/3). Water temperature, pH, and dissolved oxygen were measured daily using the WTW multi-oxygen meter (WTW Wissenschaftlich-

Table 1
Composition of diets used in the L-carnitine feeding experiment on *Carassius auratus* L-carnitine contents

Component	Composition of diets [%]				
	Diet 0 (Control)	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal	46.0	46.00	46.0	46.00	46.0
L-carnitine	0.0	0.25	0.5	0.75	1.0
Soybean meal	26.5	26.00	25.5	25.00	24.5
Corn meal	9.5	8.75	8.5	8.25	9.0
Corn starch	2.0	3.00	3.5	4.00	3.5
Fish oil	3.0	3.00	3.0	3.00	3.0
Vitamin premix ¹	2.0	2.00	2.0	2.00	2.0
Mineral premix ²	3.0	3.00	3.0	3.00	3.0
Methionine	0.5	0.50	0.5	0.50	0.5
Lysine	0.5	0.50	0.5	0.50	0.5
Sodium chloride (NaCl)	1.0	1.00	1.0	1.00	1.0
CaHPO ₄ ·2H ₂ O ³	4.0	4.00	4.0	4.00	4.0
Carboxymethyl cellulose	1.0	1.00	1.0	1.00	1.0
Cellulose	1.0	1.00	1.0	1.00	1.0
Total	100.0	100.00	100.0	100.00	100.0

L-carnitine contents in diets [mg · kg⁻¹]: Diet 0 = 0, Diet 1 = 250, Diet 2 = 500, Diet 3 = 750, and Diet 4 = 1000;

¹ per kg mix: 4 000 000 IU vitamin A, 600 000 IU vitamin D₃, 40 000 mg vitamin E, 2400 mg vitamin K₃, 5000 mg vitamin B₁, 8000 mg vitamin B₂, 4000 mg vitamin B₆, 12 mg vitamin B₁₂, 40 000 mg vitamin C, 50 000 mg niacin, 1400 mg folic acid, 8000 mg calcium D-pantothenate, 50 mg D-biotin, 40 000 mg inositol.

² per kg mix: 60 000 mg manganese, 10 000 mg iron, 75 000 mg zinc, 5000 mg copper, 1000 mg cobalt, 2500 mg iodine, 100 mg selenium, 65 000 mg magnesium.

³ Calcium hydrogen phosphate.

* Paşlı E. 2019. L-Karnitin ve koenzim Q10 ilaveli yemlerle beslenen kaynak alabalık (*Salvelinus fontinalis* Mitchill, 1814)'larında büyüme performansının belirlenmesi üzerine bir araştırma. [An investigation on determination of using diets with L-carnitine and coenzyme Q10 on growth performance of brook trout (*Salvelinus fontinalis* Mitchill, 1814)]. MSc thesis. Recep Tayyip Erdoğan University, Rize, Turkey. [In Turkish.]

Table 2
Proximate composition of diets used in the L-carnitine feeding experiment on *Carassius auratus* (% wet weight)

Component	Composition of diets [%]				
	Diet 0 (Control)	Diet 1	Diet 2	Diet 3	Diet 4
Crude protein	40.74 ± 0.77	40.16 ± 0.04	40.54 ± 0.57	40.53 ± 0.79	40.89 ± 0.34
Crude lipid	6.4 ± 0.19	5.94 ± 0.21	5.96 ± 0.37	5.93 ± 0.11	5.96 ± 0.07
Crude ash	15.88 ± 0.31	16.01 ± 0.26	16.10 ± 0.42	16.38 ± 0.60	16.31 ± 0.21
Moisture	9.34 ± 0.16	9.53 ± 0.20	9.62 ± 0.26	9.64 ± 0.16	9.68 ± 0.24
Dietary energy [MJ·kg ⁻¹]	14.90	14.85	14.81	14.76	14.75

Values represent the mean value ± standard deviation of three replicate analyses; L-carnitine contents in diets [mg·kg⁻¹]: Diet 0 = 0, Diet 1 = 250, Diet 2 = 500, Diet 3 = 750, and Diet 4 = 1000;

Technische Werkstätten GmbH, Germany). During the experiment, the amount of dissolved oxygen was 5.17 ± 0.058 mg·L⁻¹, and the pH value was measured as 7.50 ± 0.017. Fish were measured in 21 day periods throughout the experiment. Before weighing, they were anesthetized using clove oil (v/v, 1/20) to reduce the stress of the fish (Gümüş et al. 2016).

Fish growth parameters such as specific growth rate (SGR) [%day⁻¹] (Brown 1957), condition factor (CF) (Le Cren 1951), and survival rate (SR) [%] (Çelikkale 1994) were calculated according to the following equations:

$$\text{SGR} = 100(W_F - W_I) \times T^{-1}$$

$$\text{CF} = 100W_F \times L_T^{-3}$$

$$\text{SR} = 100 N_F \times N_I^{-1}$$

where W_F is the final weight, W_I is the initial weight, T is time [days], L_T is the total body length, N_F is the final fish number, N_I is the initial fish number. The weight and length values are expressed in grams [g] and centimeters [cm].

The feed utilization values, such as feed conversion ratio (FCR) (Halver and Hardy 1982) and protein efficiency ratio (PER) (de Silva and Anderson 1994) were calculated according to the following equations:

$$\text{FCR} = I_F \times (W_F - W_I)^{-1}$$

$$\text{PER} = (W_F - W_I) \times I_p^{-1}$$

where I_F is the feed intake [g], and I_p is the protein intake [g].

Chemical analysis. Chemical analysis of experiment feeds was done according to the rules of the AOAC (Association of Official Analytical Chemists) (Anonymous 1995). For the determination of a matter, the samples representing each group were weighed and taken into moisture containers. Then they were kept in the oven for about 12 h until they reached a constant weight in the oven set at 105 ± 2°C and then they were left in the desiccator to cool down to room temperature. The crude protein analysis was carried

out using the Kjeldahl method. Crude lipid analysis was determined by ether extraction with the help of the Soxhlet extraction system. The ash analysis was made by burning the samples in the ash oven at 550°C (Anonymous 1995).

Statistical analyses. The statistical evaluation of the data obtained from the experiment was made using the SPSS 15.0 (SPSS INC. Chicago, IL, USA) package program. After applying variance homogeneity tests to all data, variance analysis (ANOVA) was performed to determine the effect of different L-carnitine ratios on weight and length.

Duncan multiple comparison tests was applied to see differences in weight, height, specific growth rate (SGR), condition factor (CF), feed conversion ratio (FCR), and protein efficiency ratio (PER) between the groups.

RESULTS

Growth values of the experimental fish fed different feeds are given in Fig. 1. There were statistically significant differences between the weight increase values of the fish in the experiment groups ($P < 0.05$). As the L-carnitine level increased, an increase in weight values was observed. It was determined that the group fed feed containing 1000 mg·kg⁻¹ L-carnitine was the best in terms of an increase in weight.

Growth values of experimental fish fed different feeds are given in Fig. 2. Statistically significant differences were found between the growth values of fish in the experimental groups ($P < 0.05$). As the L-carnitine level increased, an increase in growth values was observed. It was determined that the group fed feed containing 1000 mg·kg⁻¹ L-carnitine was the best in terms of an increase in length.

Condition factor values of the experimental fish fed different feeds are given in Fig. 3. There were no statistically significant differences between the condition factor values of the fish in the experimental groups ($P > 0.05$). It was determined that the group fed feed containing 750 mg·kg⁻¹ L-carnitine had the best condition factor.

The specific growth rate values of the experimental fish fed different feeds are given in Fig. 4. Statistically significant differences were found between the specific growth rate values of fish in the experimental groups ($P < 0.05$). It was

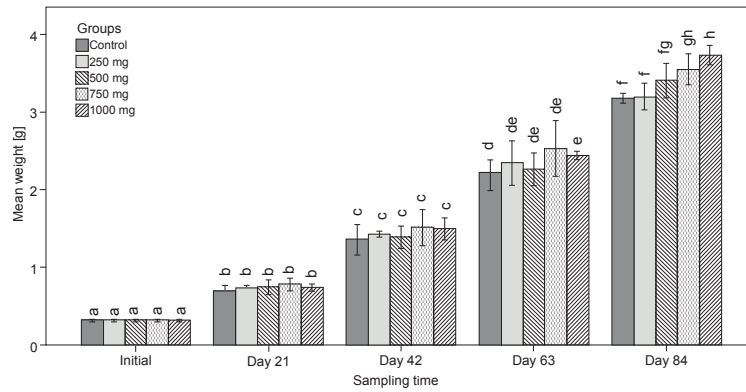


Fig. 1. Growth values of by weight of *Carassius auratus* used in the L-carnitine feeding experiment (Different letters indicate statistical significance at 95% confidence interval)

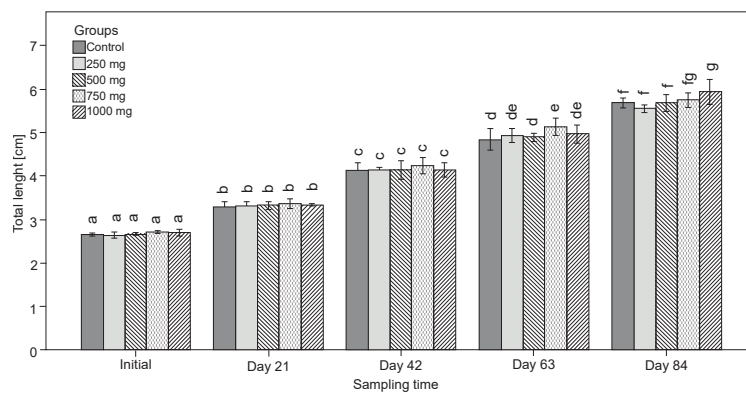


Fig. 2. Growth values of by length of *Carassius auratus* used in the L-carnitine feeding experiment (Different letters indicate statistical significance at 95% confidence interval)

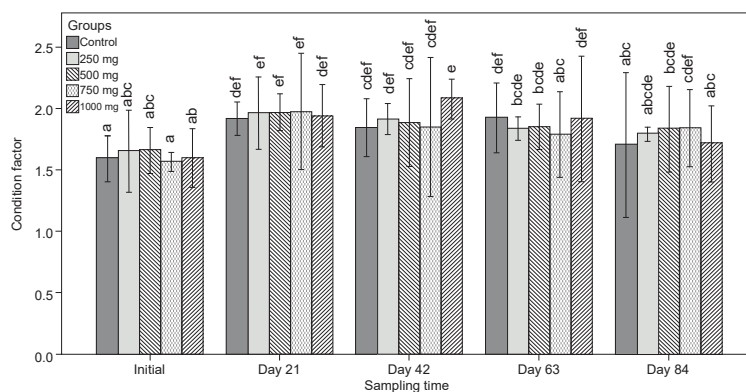


Fig. 3. Condition factor values of *Carassius auratus* used in the L-carnitine feeding experiment (Different letters indicate statistical significance at 95% confidence interval)

determined that the group fed feed containing 1000 mg · kg⁻¹ L-carnitine had the best specific growth rate.

Feed conversion ratio values of the experimental fish fed different feeds are given in Fig. 5. Statistically significant differences were found between the feed conversion ratio of fish in the experiment groups ($P < 0.05$). It was determined that the group fed feed containing mg · kg⁻¹ L-carnitine had the best feed rate.

The protein efficiency ratio values of the experimental fish fed different feeds are given in Fig. 6. Statistically significant differences were found between the protein efficiency ratio values of the fish in the experiment groups ($P < 0.05$). It was determined that the group fed feed containing 1000 mg · kg⁻¹ L-carnitine had the best feed rate.

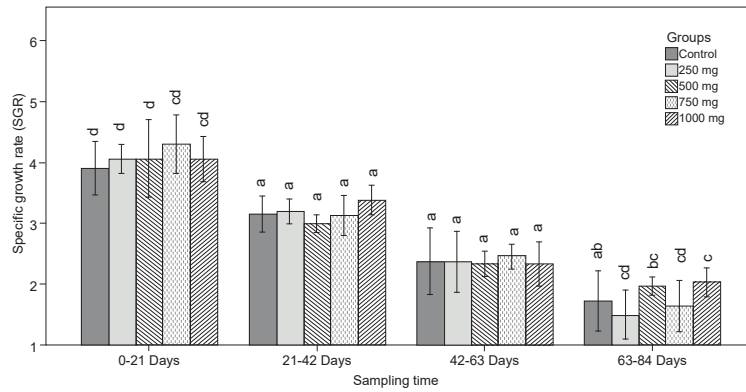


Fig. 4. Specific growth rate values of *Carassius auratus* used in the L-carnitine feeding experiment (Different letters indicate statistical significance at 95% confidence interval)

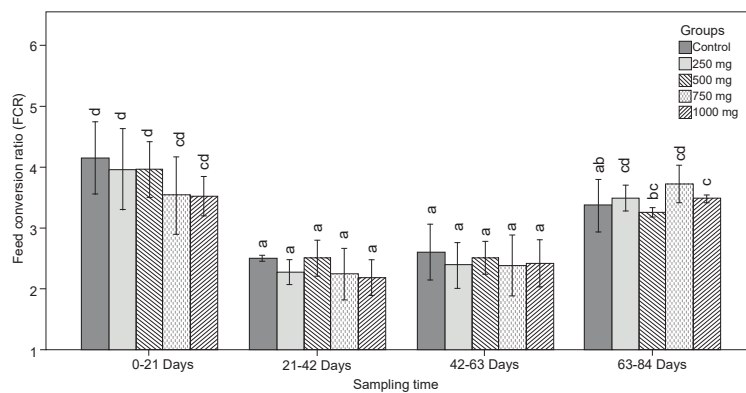


Fig. 5. Feed conversion ratio values of *Carassius auratus* used in the L-carnitine feeding experiment (Different letters indicate statistical significance at 95% confidence interval)

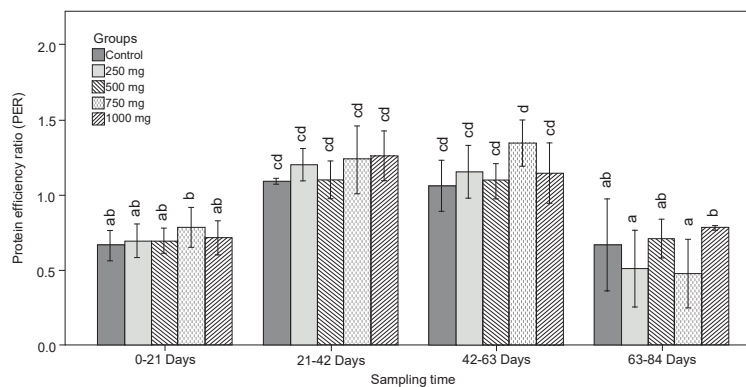


Fig. 6. Protein efficiency ratio values of *Carassius auratus* used in the L-carnitine feeding experiment (Different letters indicate statistical significance at 95% confidence interval)

DISCUSSION

As determined in the presently reported study, L-carnitine had a positive effect on fish growth. Similar results have also been reported in rohu, *Labeo rohita* (Hamilton, 1822) (see Keshavanath and Renuka 1998); red sea bream, *Pagrus major* (Temminck et Schlegel, 1843) (see Chatzifotis et al. 1995); Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852) (see Jayaprakas

et al. 1996); sea bass, *Dicentrarchus labrax* (Linnaeus, 1758) (see Santulli and D'Amelio 1986); African catfish, *Clarias gariepinus* (Burchell, 1822) (see Torreele et al. 1993); hybrid striped bass, *Morone chrysops* (Rafinesque, 1820) × *Morone saxatilis* (Walbaum, 1792) (see Twibell and Brown 2000); Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (see Dikel et al. 2003); seabream, *Sparus aurata* Linnaeus, 1758 (see

Taşbozan unpublished*); grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) (see Aksoy unpublished**); black seabream, *Acanthopagrus schlegelii* (see Ma et al. 2008); rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (see Dikel et al. 2010, Haji-abadi et al. 2010); and yellow princess, *Labidochromis caeruleus* (see Sönmez unpublished***). On the other hand, the results of the presently reported study are not consistent with the results of studies conducted on rainbow trout, *Oncorhynchus mykiss* (see Rodehutschord 1995); Atlantic salmon, *Salmo salar* Linnaeus, 1758 (see Ji et al. 1996); hybrid striped bass (*Morone chrysops* ♀ × *Morone saxatilis* ♂) (see Gaylord and Gatlin 2000); sea bass, *Dicentrarchus labrax* (see Dias et al. 2001); hybrid tilapia (see *Oreochromis niloticus* × *Oreochromis aureus*) (see Schlechtriem et al. 2004); mrigal carp, *Cirrhinus cirrhosus* (Bloch, 1795) (see Singh et al. 2008); beluga sturgeon, *Huso huso* (see Mohseni et al. 2008b); hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus* (see Yang et al. 2009); and red porgy, *Pagrus pagrus* (Linnaeus, 1758) (see Nogueira et al. 2010).

When looking at the specific growth rates in the presently reported study, statistical differences were found between groups. These results are similar results have also been reported in red sea bream (Chatzifotis et al. 1995), Mozambique tilapia (see Jayaprakas et al. 1996), rainbow trout (Chatzifotis et al. 1997), common carp (Becker and Focken 1995), beluga sturgeon (Mohseni et al. 2008b), and yellow princess (Sönmez unpublished***). They reported that the specific growth rate was better in groups where L-carnitine was added. In addition, our results differ from the results reported on beluga sturgeon (Mohseni et al. 2008a) and brook trout, *Salvelinus fontinalis* (Mitchill, 1814) (see Paslı unpublished****).

When the feed evaluation rate and protein efficiency rate in this study were examined, statistical differences were observed between groups. Our findings were similar to studies on African catfish (Torreale et al. 1993), red sea bream (Chatzifotis et al. 1995; Chatzifotis and Takeuchi 1997), rainbow trout (Chatzifotis et al. 1997), hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus* (see Becker et al. 1999), rohu (Keshavanath and Renuka 1998), sea bass (Dias et al. 2001), grass carp (Aksoy unpublished**), beluga sturgeon (Mohseni et al. 2008a, 2008b), mrigal carp (Singh et al. 2008), brook trout (Paslı unpublished****). In addition, our results differ from those conducted on carp (Becker and Focken 1995) and hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus* (Yang et al. 2009).

In our study, the survival rate of fish was 100%. We believe that the reason for such a high survival rate might be the following factors a short experimental period, low stocking density, and adherence to hygienic requirements.

CONCLUSION

Supplemental dietary L-carnitine has beneficial effects on improving growth performance and feed conversion ratio in goldfish fry. When the level of L-carnitine supplement dietary increased, there was an increase in weight and length growth values, condition factor, and specific growth rate. Future research should focus on the growth performance and feed utilization parameters of other ornamental fish of different conditions of L-carnitine supplemented diets.

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*** See footnote on page 349.

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