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Effect of L-Carnitine on Colour, Digestive Enzymes and Growth of the Electric Yellow Cichlid, *Labidochromis caeruleus*

Müge Aliye Hekimoğlu and Sırma Sönmez
Aquaculture Deptartment, Faculty of Fisheries, Ege University, Izmir, Turkiye.

ABSTRACT

This 40-day feeding trial was aimed to determine the effective rate of L-carnitine on the growth of electric yellow cichlid (*Labidochromis caeruleus*) fry (n: 395, initial weight:0,46 g, initial length: 2.5 cm). They were randomly placed in 50 l aquariums. Based on the basal diet (55% crude protein+14% oil), the four experimental diets were designed with supplementation of L-carnitine (0, 500, 1000, 2000 mg/kg). They were fed 10% of their weight every day. Sampling was done at the beginning and the final day of the experiment for digestive enzymes (protease, lipase, amylase), weight and length, body colour and survival rate. The image analysis method was used to determine skin colour. The chemical analysis method was used to determine the activity of enzymes. The results showed that supplementation of 2000 mg/kg of dietary significantly increased weight, length and specific growth rate ($P < 0.05$). While no change in colour was observed in the control group at the end of the experiment, it was determined that carnitine could have a 27% effect on the colour change of fish in the other groups. Better results were obtained between the L-carnitine groups compared to the control group for in terms of digestive enzymes, while and the difference between the groups was found to be statistically significant ($p \leq 0.05$). The results show that L-carnitine did not play an important role in the survival of fish. As a result, the addition of L-carnitine to fish feed positively affects the growth of fish and as expected, the growth of fish increases as the amount of L-carnitine in the feed increases.

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Authors' Contribution
MAH planned, funded and wrote this study. She analyzed the colour of fish and did statistics. SS planned and prepared feed supplemented with L-carnitine and took care of the fish.

Key words
Feed supplement, L-Carnitine, Digestive enzymes, Fish skin colour, Cichlid, *Labidochromis caeruleus*

INTRODUCTION

The electric yellow cichlid (*Labidochromis caeruleus*), a species that can be kept in aquariums, belongs to the Cichlidae family, which is the most diverse (Kocher, 2004; Kuraku and Meyer, 2008). The protective behaviour of the young of this fish species with bright yellow colour has a special place in the aquarium sector (Bangerder, 2007; Oliver, 1975; Wand, 2005). In terms of aquaculture, the colour of aquarium fish is one of the most important features that increase the sale of that fish. For this reason, from the breeder's perspective, encouraging the distinctive colours of the fish to appear most brightly and attractively during the growth period is the most natural way to increase sales of the fish from a commercial point of view. For this purpose, natural or unnatural sources of carotene are added to the feed of aquarium fish as an additive. To balance the feed cost, the breeders want the fish to benefit from the carotene added feed at the maximum level and the fish to reach the sales qualities as soon as possible. This situation also depends on the healthy digestion of the given feed by the fish at the maximum level. Therefore, it is vital to determine the activity of digestive enzymes from living cells, which have a catalysis role in biochemical reactions during the growth period of fry. It is essential to monitor the changes due to digestive enzymes activities in defining the growth performance, survival rate and nutritional conditions of fish in developing new feeding regimes and providing optimal breeding conditions (Yungul and Ozdemir, 2017). Studies conducted for this purpose have focused on fish in the larval stage, which has economic importance: *Sparus aurata* (Suzer *et al*., 2007), *Dicentrarchus labrax* (Aksu, 2008; Suzer *et al*., 2011), *Pagellus erythrinus* (Suzer, 2003) *Diplodus puntazzo* (Aktulun, 2007), *Oncorhynchus mykiss* (Akpınar *et al*., 2009), *Dentex dentex* (Suzer *et al*., 2014), *Sardinus pilcardus* (Noda and Murakami, 1981), *Solea solea* (Clark *et al*., 1985), *Anguilla japonica* (Chiu and Pan, 2002), *Merluccius productus* (An *et al*., 1994), *Gadus morhua* (Amiza *et al*., 1997; Gjellesvik *et al*., 1992), *Chelon labrosus* (Pujante *et al*., 2017), *Labeo rohita, Sardinella longiceps, Liza subviridis, Rastrelliger kanagurta* (Nayak *et al*., 2003)

L-carnitine, chosen as an additive in the study, is a bioactive substance. Its main task is to convert fatty acids

* Corresponding author: muge.aliye.hekimoglu@ege.edu.tr
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into energy (Taşbozan and Gökçe, 2007). L-carnitine increases the reproductive rate of fish, encourages spermatogenesis, increases the viability, and the benefit of protein in the feed, provides effective use of the oil in the feed, increases the growth rate, strengthens the immune system, improves the tolerance against toxic ammonia and xenobiotics, as well as the physical and chemical properties of water. It has a positive effect on issues such as increasing the adaptation to chemical changes (Harpaz, 2005; Dikel, 2007; Taşbozan and Gökçe, 2007). This substance has been involved in nearly 60 studies in aquaculture in many countries to date. Bilinsky and Jonas were the first to use L-carnitine in aquaculture (Dikel, 2007). Other studies are concerned with the effect of L-carnitine on fish such as the one on the growth of the fish (on *Sparus aurata* Tasbozan, 2005; on *Oncorhynchus mykiss* Dikel *et al*., 2010; *Rachycentron canadum* Marques *et al*., 2021), its action against toxic levels of ammonia and some other elements (on catfish Burtle and Liu, 1994), its effect on water temperature changes (on *Pelvicachromis pulcher* Harpaz *et al*., 1999), its effect on reproduction (on *Poecilia reticulata* Schreiber *et al*., 1997; Dzikowski *et al*., 2001), its use for manipulating metabolic stress (*Pimephales promelas* Chen *et al*., 2021), and finally its effect on biochemical and antioxidant response (*Larimichthys crocea* Li *et al*., 2021). In the literature search, no previous study has been found on the effect of L-carnitine on electric yellow cichlid fish. The uniqueness of this study comes from determining the effects on the young individuals by feeding them with L-carnitine feed in the breeding of electric yellow cichlid, which has commercial value and is widely sold.

MATERIALS AND METHODS

Experimental design

Four experimental groups and three replicates of each group were created. Experimental groups were designed according to the amount of L-Carnitine added to the diet.

Trial feed preparation

Fish diets for the experiment were prepared using commercial juvenile trout chow with a grain size of 1 mm (55% crude protein, 14% fat, 1.5% crude fibre, and 11% ash). L-carnitine (98% crystallised: Roche, Co., Mannheim, Germany) was added to the trout feed: 500 mg/kg for Group A, 1000 mg/kg for Group B and 2000 mg/kg for Group C studies of Burtle and Liu (1994) and Harpaz *et al*. (1999). L-carnitine was not added to the control group (Group D). For this, the aquarium solution of L-carnitine was mixed, and powdered feed, and left to dry for 12 h at room temperature in a dark environment with suitable ventilation. Enzymatic UV Test Kit (Roche, catalogue number 11-242-008-001) was used according to Wieland *et al*. (1985) and Tasbozan (2005) to measure the actual amount of L-carnitine mixed into the feeds after drying. The prepared foods were kept in a refrigerator at +4 °C in plastic containers until the day of the experiment.

Fish maintenance

The healthy electric yellow cichlid juveniles (n: 395, initial body mean weight and length: 0,46±0,09 g and 2.5±0.02 cm) were put randomly into 12 glass aquaria (32 fish for each 50 L aquarium). Aquaria were kept for 50 days under a photoperiod regime (12 h light:12 h darkness) and high quality clean and well-aerated water. Water temperature and pH were kept at 25±1°C and 7.01 ± 0.32, respectively.

Feeding protocol

The fish were fed with up to 10% of their weight every day. Uneaten foods were siphoned 1 h later from aquaria.

Sampling schedule and analytical procedures

At the end of the experiment, fish were anaesthetised using 2 ml/L pure clove oil (Hekimoglu *et al*., 2012) and weighed and their length measured. Pictures were taken for colour analysis. Then, three fish were randomly taken from each aquarium for enzyme analysis. Growth, feed utilisation, and biometric indices were computed according to the following equations:

Weight gain (%) = $[(W_T-W_0) /W_0] \times 100$

Specific growth rate (%/day)= $[(LnW_T- LnW_0)/_T]\times100$

Feed conversion ratio (FCR)= Feed given (dry weight) / body weight gain (wet weight)

Survival rate (%) = $(N_T/N_0) \times 100$

where, W_T = body weight at a specific time (day); W_0 = initial body weight; T = the trial period in days (d); N_0 = initial number of fish; N_T = final number of fish; W = fish weight (g); L = fish length (cm), N_T=Number fish at a specific time; N_0=Initial number fish.

Enzymatic analysis

Groups of *L. caeruleus* were sampled from each experimental tank, and the gastrointestinal system was dissected on a glass maintained at 0 ºC. Homogenates were used, and samples were taken at the same hour before food distribution. Samples were collected and homogenised in 5 volumes v/w of ice-cold distilled water. Extracts utilised for enzyme assays were obtained after homogenisation of fish (35 mg ml-1) in cold 50 mM Tris-HCl buffer, pH 8.0, followed by centrifugation (13.500xg; 30 min 4°C). Total protease activity was assayed using casein as the substrate (Walter, 1984). Amylase activity was measured using starch as the substrate (Métais *et al*., 1968). Lipase activity

was analysed using Mckellar *et al*. (1986) as modified by Versaw *et al*. (1989), using β-naphthyl caprylate as substrate. One unit of lipase activity was defined as 1 mg of β-naphthol released per minute. Enzyme activities were expressed as specific activities, i.e. $U.mg^{-1}$ soluble protein. Protein was determined by the Bradford procedure (Bradford, 1976).

Color analysis

The fish was photographed at the beginning and end of the trial. In order not be affected by the ambient light, a vertical picture was taken of the fish, which was placed on a white background in a dark environment. Codak brand camera was used for picture shooting. Then, each picture was transferred to the computer and the dorsal region, and the tail stem region was marked with Microsoft 7 Professional Paint (version 6.1) program. These sections were analysed with the Color Analysis Professional program (Version 6, Roy Leizer), a program designed to analyse colour groups according to the visible spectrum. The resulting colours were grouped and ranked from the more dominant colour to less significant (Table II). Among the results obtained, the most dominant colour groups were evaluated.

Statistical analysis

Before statistical analysis, all data were tested for the normality of distribution and the homogeneity of variances. The data were statistically analysed using ANOVA and the treatment means were compared by Duncan's multiple range test. For enzyme and colour results, differences between groups were tested with Kruskal Wallis analysis of variance, one of the nonparametric tests. The significance level was taken as 0.05 in the analysis. All statistical analyses in the research were made using the IBM SPSS Version 25 program. Data were presented as mean ± SD values, and differences were considered statistically significant at $P < 0.05$.

RESULTS

Growth and survival

Growth parameters of electric yellow cichlid fed with different level of L-carnitine supplement are shown in Figure 1. Compared to the control group, all the levels of L-carnitine supplementation significantly increased the final body weight and final standard length, average live weight gain and specific growth rate. In terms of these measured values, while the groups were homogeneous at the beginning of the experiment, there was a difference between the groups at the end of the experiment ($p<0.05$). It was found that the values of the proportional body weight gain in the A, B, C, and D groups were 0.31 ± 0.05^{bd}, 0.34 ± 0.04^{bd}, 0.46 ± 0.06^{acd}, 0.27 ± 0.04^{abc}, respectively. When the specific growth at the end of the experiment was taken into consideration, group C was higher than the others. (A, B, C, D respectively 0.86 ± 0.06^{bd}, 0.92 ± 0.05^{bcd}, 1.16 ± 0.05^{cd}, 0.76 ± 0.03^{abc}).

The FCR values found at the end of the experiment were found to be 1.42 ± 0.04, 1.42 ± 0.03, 1.39 ± 0.05 and 1.27 ± 0.05, in groups A, B, C, D, respectively.

Table I. Actual amount determined in feed after addition of L-Carnitine.

Group	L-Carnitine (%)
A	1.66
B	3.52
C	2.34
D	1.45‡

‡ Although L-carnitine was not added, it was found to be naturally present in the control feed.

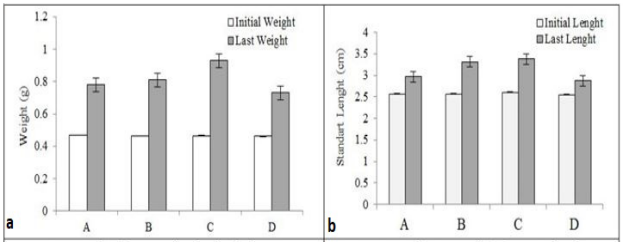

Fig. 1. Effect of L-carnitine on the body weight (a) and length (b) of *Labidochromis caeruleus*.

Digestive enzyme activities

The activities of the protease, amylase, lipase are presented Figure 2.

On the final day for total protease the results were as follows. The protease activities increased 36% in Group A, 79% in Group B, 109% in Group C and 26% in Group D. Group C reached the highest value compared to the other groups and was significantly different from the other groups ($p\leq0.05$). For lipase Group A increased by 60%, group A by 75%, Group C by 80%, and Group D by 13%. Group D reached a lower mean value than the other groups ($p\leq0.05$). For amylase, Group A increased by 65%, Group B increased by 71%, Group C increased by 76%, Group C increased by 49%. Group D was significantly different from the other three groups, with the lowest mean ($p\leq0.05$) when the means were compared. Group C and Group B gave higher mean values than the other groups ($p\leq0.05$).

Color

In the determinations made according to the Color

Analysis Professional program, 4 colour groups and the sub-colours under these colour groups were detected. Their RGB (red, green, blue) and HSL (hue, saturation, lightness) values are given in the table below. At the beginning of the experiment, the fish had the brass subgroup colour of the yellow colour group with a density of over 50% as the dominant colour. At the end of the experiment, the colours found for each group are given in Table II. A small amount of green group colour, which was not seen in group D, was detected.

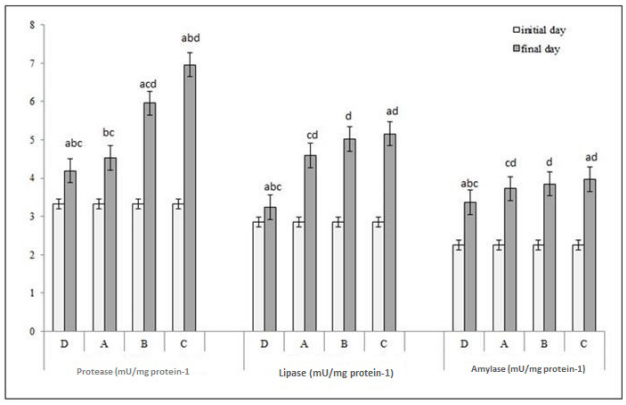

Fig. 2. Effect of L-Carnitine on specific activities of total protease, lipase and amylase in *Labidochromis caeruleus*. Results are expressed as means±SD (n=9) (P< 0.05). (lower letters indicate between group differences).

Table II. Determineded colour groups and RGB-HSL values of the trial fish.

	Red	Green	Blue	Hue	Saturation	Lightness
Yellow	188	202	138	73	38	67
Green	139	151	117	76	48	30
Orange yellow	152	156	69	63	39	44
Orange brown	128	114	67	62	32	38

While yellow (brass) was the dominant colour in all groups initially, it was determined that it decreased considerably in each group at the end of the experiment. Although it decreased compared to baseline in group D, it was found to be more than 50% concentrated in the dorsal and ventral regions (Fig. 3).

Orange yellow (dark tan) was found in all regions of group C fish at a meagre rate (20%) at the beginning of the experiment. At the end of the experiment, it was determined that the same rates were maintained in the same regions. This colour, which was not found in the other groups initially, showed itself at the end of the experiment. This colour appeared over 50% in the caudal region of group A and the ventral region of group B.

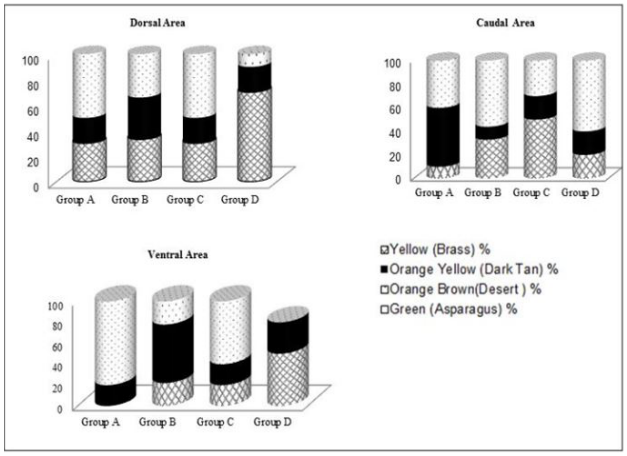

Fig. 3. Effect of L-carnitine on colour pattern distribution in fish at the end of the trial (%).

Orange brown (desert) was initially found in the caudal region at a meagre rate (20%) only in group D. At the end of the experiment, this colour appeared. It was detected as 50% or more in the dorsal and ventral regions in groups A and C, and 50% or more in the caudal region in groups B and D. In general, it was seen that this colour was more dominant in group A than in other groups. It was observed that this colour was the dominant colour in group C following group A. In groups B and D, it was detected intensely only in the caudal region.

According to the analysis results, a correlation test was performed to measure the effect of L-carnitine supplemented feeds on the colour of the fish. Accordingly, the correlation between L-carnitine and the colours on the upper part of the dorsal fin, the dorsal region, the ventral region, and the orange brown (desert) colour dominant at the base of the tail were found 0.6, 0.7, 0.2, respectively. There was no significant difference between the groups regarding all these characteristics ($p>0.05$).

Survival

When counting the survivors at the end of the experiment, groups A, B, C and D are found to be 90%, 89%, 87% and 83%, respectively. Although carnitine had an effect on survival ($R2=0.74$), the results were not significant in terms of difference between groups ($p\geq0.05$).

DISCUSSION

In the present study, electric yellow cichlid displayed noticeably increased growth performance (WG, SLG, SGR) than the control group. All levels of L-carnitine

administration resulted in an appreciable effect on the fish. Considering these results on growth performance and survival rate; *Labeo rohita* (Keshavanath and Renuka, 1998), *Oreochromis niloticus* (Becker *et al*., 1999; Dikel *et al*., 2003), *Sparus aurata* (Taşbozan, 2005), *Cyprinius carpio* (Asgharimoghadam *et al*., 2014) and *Ctenopharyngodon idella* (Aksoy, 2006), *Moina micrura* (Savas *et al*., 2001) and *Sander lucioperca* (Akbari *et al*., 2014) is similar to previous studies. However, studies with *Oncorhynchus mykiss* (Schuhmacher and Gropp, 1998; Ozoria *et al*., 2012), *Clarias gariepinus* (Ozoria *et al*., 2012), *Oreochromis mossambicus* (Tekle, 2004; Yang *et al*., 2009), *Marsupenaeus japonicus* (Yağcıoğlu and Aktaş, 2006), *Carassius auratus* (Arslan, 2012) that concluded L-carnitine did not affect growth. So some studies have reported contrasting results regarding our findings related to increased growth rate.

It has been obtained from studies in species such as *Scophthalmus maximus* (Fernandez *et al*., 1992), *Clarias gariepinus* (Torreele *et al*., 1993), *Oreochromis niloticus* x *Oreochromis aureus* hybrids (Becker *et al*., 1999), *Morone chrysops* x *Morone saxatilis* hybrids (Twibell and Brown, 2000), *Sparus macrocephalus* (Ma *et al*., 2008), *Cirrhinus mrigala* (Singh *et al*., 2008), *Oncorhynchus mykiss* (Haji-abadi *et al*., 2010) and *Bidyanus bidyanus* (Yang *et al*., 2012) that L-carnitine increases body weight gain. Our study, which was similar to these studies in terms of results, determined that all groups had more weight gain than the control group. In addition, there was more growth in length than in the control group. Contrary to these studies, the negative effects of L-carnitine on body weight gain were observed in hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) (Schlechtriem *et al*., 2004), *Clarias gariepinus* (Yilmaz *et al*., 2004), Caspian white fish (*Rutilus frisii kutum*) (Nekoubin *et al*., 2012) and *Oreochromis niloticus* (Erdogan *et al*., 2015).

This result in the experiment, shows similarities to the study of *Oreochromis niloticus* (El-Sayed *et al*., 2010) and *Oreochromis mossambicus* juveniles (Jayaprakas *et al*., 1996) were fed L-carnitine supplemented feed, bodyweight increased as L-carnitine amount. Nevertheless, contrary to our findings, study of Zang *et al*. (2005) (*Brachionus rotundiformis*) and Aksoy (2006) (*Ctenopharyngodon idella*) reported that L-carnitine had no effect on fish weight.

Gaylord and Gatlin (2000a,b) reported that the amount of fat in the feed they gave hybrid striped bass (*Morone chrysops* x *Morone saxatilis*) affected growth while adding l-carnitine was not influential. However, We think that the growth performance of our fish is also related to the good use of the amount of oil that we added to their feed in the growing period. Harpaz (2005) and Chen *et al*. (2020a,b) showed carnitine supplementation affect the growth of fish because it increased the utilization of energy as a result of the increased fatty acid oxidation in the cell. Our study in this situation parallels with some studies; for example, Torreele *et al*. (1993) stated that the benefit to be gained by adding L-carnitine to African catfish (*Clarias gariepinus*) feeds on the level of fat in the feed. In their study, it was determined that the growth was increased when the addition of L-carnitine at 500 mg/kg or more and the 9.6% fat ratio was combined, or the combination of 684 mg/kg L-carnitine and above with 15.5% oil level was effective on the growth of fish. In a similar study, the body composition was improved by adding 10% fat and 1000 mg of l-carnitine kg^{-1} to the feeds of juvenile *Cyprinus carpio* (Sabzi *et al*., 2017). Our study found that the feed's growth values were increased compared to the control group when the fat ratio in the feed was kept at 14%, and L-carnitine was added to the feed at different rates.

Another value in growth performance is SGR, and in this study as well, it was determined that L-carnitine had a positive effect on SGR. The SGR finding of this study; *Huso huso* (Mohseni *et al*., 2008), *Sander lucioperca* (Akbari *et al*., 2014), *Sparus aurata* (Taşbozan, 2005), *Ctenopharyngodon idella* (Aksoy, 2006), *Cyprinus carpio* (Asgharimoghadam *et al*., 2014), *Oncorhynchus mykiss* (Haji-Abadi *et al*., 2010) and *Oreochromis niloticus* (El-Sayed *et al*., 2010) support the previous studies. Group A, B and C in our experiment showed that they utilize the feed better with the FCR value higher than the control group.

In our study, we reached a similar conclusion with Bilguven (2002) finding that feeding with L-carnitine feed increases the amylolytic activity of rainbow trout fry, and we found that our experimental groups had more enzyme activities than the control group. When the amount of L-carnitine in the feed of *Oreochromis mossambicus* fry is increased, digestive enzymes also increase (Jayaprakas *et al*., 1996). This information explains that the enzymes in our study increased more than the control group depending on the amount of L-carnitine. Renuka (1992) proved the growth parameters of *Labeo rohita* increases parallel to the enzyme activity increases. Our trial results support a similar situation. This can be explained as follows: L-carnitine increases the growth rate by stimulating the energy metabolism. Our finding that the amount of L-carnitine added to the diet increases the enzyme activities in fish is similar to the findings of the study of Sanches *et al*. (2021) with nil tilapia juveniles. In adult *Dicentrarchus labrax*, the addition of L-carnitine increases the activity of the enzyme responsible for the digestion of fats 3 times, but there is no change in the fat levels in the tissues (Dias *et al*., 2001). In our study, the lipase enzyme increased between 1.5 and 2 times in groups with L-carnitine addition. Addition of

0.02% L-carnitine to feed containing 14% fat to be used in the feeding of juvenile largemouth bass (*Micropterus salmoides*) increases oil utilization and growth (Chen *et al*., 2020a,b). In our experiment, group C fed with 0.02% L-Carnitine added feed showed higher growth performance than the other groups. It has been reported that the amylase enzyme activity in *Oncorhynchus mykiss* offspring is quite high, which increases as the fish grows and reaches a maximum when it reaches 100 g (Yungul *et al*., 2017). In this study, a positive correlation was found between the amount of amylase enzyme and live weight in all groups. According to these results, we can conclude that L-carnitine added feeds are generally better digested.

L-carnitine, which plays an important role in fat metabolism, also increases the effect of fat-soluble carotenoids on the color of fish (Sigugisladottir *et al*., 1997; Shadidi *et al*., 1998). At the end of our study, it was determined that L-carnitine may have a 27% effect on the color of fish ($p>0.05$).

CONCLUSION

It was concluded that adding L-carnitine to feed had many positive effects such as on the increase in growth and more intense colour of electric yellow cichlid fry in the developmental period used in this study. As a result we can suggest that 2000 mg/kg L-carnitine has a positive effect on growth for electric yellow cichlid juveniles´ growth.

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Statement of conflict interest

The authors have declared no conflict of interest.

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