



## Effects of Dietary L-carnitine Supplementation on Growth, Muscle Fatty acid Composition and Economic Profit of Rainbow Trout (*Oncorhynchus mykiss*)

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### Abstract

In this study, the effect of dietary L-carnitine on growth, proximate and muscle fatty acid compositions of rainbow trout (*Oncorhynchus mykiss*) were investigated. The fish were fed with diets containing 300 mg kg<sup>-1</sup> L-carnitine (LC300), other group was fed with diets containing 600 mg kg<sup>-1</sup> L-carnitine (LC600) and control group was not supplementary L-carnitine for 63 days. The weight gain of LC600 fed with L-carnitine supplemented was found to be 7.73% higher than in control group. Feed conversion ratio in LC600 (1.66) and LC300 (1.60) is better than in control group (2.00). L-carnitine supplemented groups would be lower cost of production than control. Economic conversion ratio (ECR) of LC300 (2.71 US.\$ kg<sup>-1</sup>) would represent a saving of 0.44 US.\$ kg<sup>-1</sup> compared to the control. L-carnitine administration did not affect proximate composition of muscle except for protein percent. Protein content of L-carnitine supplemented groups was higher than control group. Total saturated monounsaturated and polyunsaturated muscle fatty acid content increased by L-carnitine supplementation in rainbow trout. The results indicate that supplementation of 600 mg L-carnitine/kg diet is more effective on growth than the other L-carnitine supplementation level 300 mg kg<sup>-1</sup> and control diet in commercial cage conditions.

**Keywords:** L-carnitine, rainbow trout, growth, fatty acid, economic profit index, economic conversion ratio.

### L-carnitine'nin Gökkuşığı Alabalığının (*Oncorhynchus mykiss*) Büyümesine ve Kas Yağ Asidi Kompozisyonuna Etkileri ve Ekonomik Yararlılığı

#### Özet

Bu çalışmada, L-carnitine'nin gökkuşığı alabalığının (*Oncorhynchus mykiss*) büyümesine ve kas yağ asidi kompozisyonuna etkisi incelenmiştir. Balıklar; hiç L-carnitine eklenmeyen kontrol grubu yemleri ve 300 mg kg<sup>-1</sup> L-carnitine (LC300) ve 600 mg kg<sup>-1</sup> L-carnitine (LC600) içeren yemlerle 63 gün boyunca beslenmişlerdir. LC600 grubu kontrol grubundan %7,73 daha fazla canlı ağırlık kazanmıştır. Yem değerlendirme oranı açısından LC600 (1,66) ve LC300 (1,60) gruplarının kontrol grubundan (2,00) daha iyi olduğu bulunmuştur. L-carnitine eklenen grupların kontrol grubuna oranla daha düşük üretim maliyeti sağladığı bulunmuştur. LC300 grubunun (2,71 US.\$ kg<sup>-1</sup>) ekonomik dönüşüm oranı (EDO) kontrol grubuna göre 0,44 US.\$ kg<sup>-1</sup>lık tasarruf sağlamıştır. L-carnitine eklemenin protein oranı dışında, kaslardaki genel besin içeriğinde bir değişiklik yapmadığı saptanmıştır. L-carnitine eklenen grupların protein içerikleri kontrol grubundan daha yüksek çıkmıştır. Alabalıklarda L-carnitine eklenmesi kaslardaki total doymuş, tekli doymamış ve çoklu doymamış yağ asidi içeriklerini yükseltmiştir. Elde edilen sonuçlar, ticari kafes şartlarında, diyet 600 mg L-carnitine/kg eklenmesinin, 300 mg L-carnitine/kg eklenmesinden ve hiç eklenmemesinden daha etkili büyüme sağladığını göstermektedir.

**Anahtar Kelimeler:** L-carnitine, gökkuşığı alabalığı, yağ asidi, Ekonomik Yarar Endeksi, Ekonomik Dönüşüm Oranı.

#### Introduction

L-carnitine (L-β-hydroxy γ -trimethyl aminobutyrate) is a water-soluble quaternary amine that occurs naturally in microorganisms, plants and animals (Bremer, 1983) and synthesized from the essential amino acids lysine and methionine with the assistance of vitamin C and other compounds

produced in the body (Rebouche, 1991). It functions as a cofactor for the transport of fatty acids into the mitochondrial matrix. Increased import of fatty acids into the mitochondria for oxidation has the potential to spare the catabolism of proteins for energy. Thus, animals fed diets with elevated L-carnitine contents may have more protein energy available for growth. Several studies on pigs, foals and broiler chickens

have shown that growth performance was significantly improved by feeding dietary L-carnitine (Weeden *et al.*, 1991; Hausenblasz *et al.*, 1996; Rabie *et al.*, 1997). Studies employing carnitine in fish have indicated growth improvement in sea bass (Santulli and D'Amelio, 1986), African catfish (Torreele *et al.*, 1993), carp (Becker and Focken, 1995) red sea bream (Chatzifotis *et al.*, 1995), white prawn (Jayaprakas and Sambhu, 1996) and hybrid sea bass (Twibell and Brown, 2000). On the other hand, inclusion of different levels of L-carnitine in diet has negligible or even negative effects on growth in warm water (Ozorio, 2001; Burtle and Liu 1994; Harpaz *et al.*, 1999; Dzikowski *et al.*, 2001; Schlechtriem *et al.*, 2004) and cold water fish species (Rodehutsord, 1995; Ji *et al.*, 1996; Chatzifotis *et al.*, 1997; Gaylord and Gatlin, 2000). These differences of results between species suggest that the effects of dietary carnitine supplements are associated with different factors such as age, fish size, experiment period, feed composition and levels of supplement.

Many investigations showed that different results obtained from different levels of supplementation. Typically linear dose/response relations have been observed in a few studies when growth parameters are taken into account (Torreele *et al.*, 1993; Jayaparakas *et al.*, 1996; Focken *et al.*, 1997). A non linear dose/response curve is more typical for L-carnitine effects usually with one very efficient dose combined with neutral or even negative reactions as the concentration of additive increases (Chatzifotis *et al.*, 1995; Schreiber *et al.*, 1997; Keskevanath and Renuka, 1998; Becker *et al.*, 1999)

The researchers have been studying to replace animal protein sources with proteins derived from plant material or some feed additives for stimulate to the growth. One of these additives is L-carnitine which can increase lipid catabolism and might also lead a protein sparing effect (Harpaz, 2005). Nakagawa *et al.* (2000), pointed out that the improvement of vitamin C metabolism by *Spirulina* as feed supplement eventually activated lipid metabolism through carnitine metabolism. Several enzymes are involved in the lipid and carnitine metabolism process. For instance, carnitine palmitoyltransferase, as lipolysis enzyme, performs a function to exchange of coenzyme A for carnitine to facilitate the transfer of acyl groups into mitochondria for  $\beta$ -oxidation (Ji *et al.*, 2009; Ozório, 2009). So far, a relatively small amount of work has been done on the effects of L-carnitine on muscle fatty acid composition of fish.

The objectives of the present study were to evaluate effects on growth, economic profit analysis and proximate and muscle fatty acid composition of rainbow trout.

## Materials and Methods

The growth trial was conducted in our

experimental fresh water cage-culture facilities (Seyhan Dam Lake, Adana, Turkey). Rainbow trout were obtained from a commercial fish farm and were acclimated to experimental conditions for 2 weeks prior to the onset of the experiment. During this period, fish were kept in two cages (5x2x2 m, 20 m<sup>3</sup>) with a stocking density 1000 fish. The initial fish body weight (mean  $\pm$ SD) across all treatment was 70.3 $\pm$ 1.3 g. Triplicate groups of 50 rainbow trout were allotted to each of nine floating cages of 1 m<sup>3</sup> (1x1x1 m). Temperature and dissolved oxygen were daily measured.

Different levels of Carniking<sup>®</sup> (a commercial product used in animal feeds and containing 50% L-carnitine, 35% silica and 15% water, manufactured by Lonza Benelux) were used as a dietary L-carnitine supplement for the elevated levels of L-carnitine. Feed was in the form of sinking extruded pellets, 3 mm in diameter, manufactured for rainbow trout (Produced by Abalioglu in Turkey). Pertinent characteristics of this feed were: 44% crude protein and 20% crude fat. Experimental diets were containing either 0 (control diet with no L-carnitine supplemented), 300 mg L-carnitine kg<sup>-1</sup> (LC300) or 600 mg L-carnitine kg<sup>-1</sup> (LC600) were prepared by spraying L-carnitine onto the commercial pellets. The pellets were dried under normal air circumstances and stored at 4°C in plastic bags during the experiment. Fish were fed with experimental diets by hand two times a day at 900 and 1700 h. Each experimental diet was randomly assigned to triplicate groups. Feeding rate was 3% of body weight per day during the 63 days and the amounts of feed were adjusted weekly based on weight and mortality of fish. At the end of the growth experiment, fillets of ten fish per cage were collected, pooled and stored at -20°C, sampled for fatty acid analysis.

## Economic Profit, Proximate and Fatty Acid Analysis

Taking into account the feed price, we calculated the cost of feed required to produce 1 kg of biomass. The economic conversion ratio (ECR) was calculated with the following equation:  $ECR (US.\$ kg^{-1}) = (\text{feed cost } (US.\$ kg^{-1}) + \text{L-carnitine cost } (US.\$ kg^{-1})) \times \text{feed conversion ratio } (kg \text{ diet } kg^{-1} \text{ fish})$ , the economic profit index [EPI (US.\$ fish<sup>-1</sup>) = final weight (kg<sup>-1</sup> fish) x fish sale price (US.\$ kg<sup>-1</sup>) - ECR (US.\$ kg<sup>-1</sup>) x weight increase (kg)] developed by Martinez-Llorens *et al.*, (2007). The entire constant cost such as taxes, infrastructure, and labor costs were not included in the calculation. Rainbow trout sale price calculated at 3.5 US.\$ kg<sup>-1</sup>.

Ash and moisture contents were determined as described by AOAC (1984) and crude protein content was calculated by converting the nitrogen content determined by Kjeldhal's method (6.25 x N) (AOAC, 1984).

Muscle lipids were extracted by the method of

Bligh and Dyer (1959) and were stored under nitrogen at  $-20^{\circ}\text{C}$  for further analysis. The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14%  $\text{BF}_3$  (w/v) in methanol (IUPAC, 1979). Esterified sample was analyzed using a Thermoquest Trace gas chromatograph equipped with a Supelco-SP-2330 fused-silica capillary column (30m x 0.25 mm i.d., 0.20  $\mu\text{m}$  film thickness of polyethylene glycol) (Supelco, Inc., Bellefonte, PA, USA) and a flame-ionization detector (FID). Helium (30 ml  $\text{min}^{-1}$ ) was used as the carrier gas. The samples were injected at  $120^{\circ}\text{C}$ . After 2 min, the temperature was raised at  $5^{\circ}\text{C min}^{-1}$  to  $220^{\circ}\text{C}$  where it was kept for 8 extra min. The temperatures of the injector and detector were set at  $240^{\circ}\text{C}$  and  $250^{\circ}\text{C}$ , respectively. The identification of the methyl esters of the fatty acids was performed by external standards (Sigma Co.) and reported as percentage of total fatty acids.

### Data Sampling and Statistical Analysis

Average weights of fish were weekly determined by bulk weighing 30% of the groups. Performances of the fish were evaluated by calculating the following parameters from the data collected:

Specific growth rate (SGR body weight  $\text{day}^{-1}$ );  $[(\ln w_1 \pm \ln w_0)(t_1 - t_0) \pm 1] 100$ , where  $w_1$  and  $w_0$  are wet weight at times  $t_1$  and  $t_0$ .

Feed conversion ratio (FCR);  $(W_{\text{final}} - W_{\text{initial}})/\text{consumed feed}$ , where  $W_{\text{final}}$  and  $W_{\text{initial}}$  are live weights (g) of the fish at day initial (t) and final (T), respectively.

The mean and standard deviation ( $\pm$  SD) was calculated for all parameters in each group and the differences on growth performance were examined by comparison of mean weights of fish.

All the data were subjected to one-way ANOVA using statistical software Statistical Package for the Social Sciences (SPSS) version 11.0. Duncan's multiple range test was used to determine the differences among treatment means at 5% level of significance (Duncan, 1955).

### Results

Throughout the experiment, survival ranged from 96.00% to 100% with no statistically different among the experimental groups (Table 1) ( $P > 0.05$ ). Throughout the experiment, water temperature ranged from  $10.4$  to  $20.9^{\circ}\text{C}$  (average  $15.1 \pm 2.5^{\circ}\text{C}$ ). By the end of the feeding trial, average weight gain of the LC600 was higher than LC300 and control. The greatest weight gain  $186.6 \pm 8.9$  g was obtained in LC600 mg L-carnitine  $\text{kg}^{-1}$  diet. Total yield of LC600 ( $12.86 \pm 0.46$   $\text{kg m}^{-3}$ ) was significantly ( $P < 0.05$ ) higher than control group ( $10.85 \pm 0.49$   $\text{kg m}^{-3}$ ). Similarly total net weight gain and average individual net weight gain of LC600 were significantly higher than control group ( $P < 0.05$ ) (Table 1). There was no significant difference in SGR among fish fed with any of the dietary treatments ( $P > 0.05$ ).

Food conversion ratio of fed control group diet was significantly higher than other groups and LC300 and LC600 values were similar. The cost of diets was reduced with L-carnitine addition (Table 2). The economic conversion ratio (ECR) of the control diet was the highest (2.71 US.\$  $\text{kg}^{-1}$ ), and ECR of the LC300 was the lowest (2.21 US.\$  $\text{kg}^{-1}$ ). However, there was no significant difference between L-carnitine supplemented groups according to ECR. Likewise the highest economic profit index (EPI) was obtained in fish fed with 300 mg  $\text{kg}^{-1}$  L-carnitine supplemented feed (0.464 US.\$  $\text{fish}^{-1}$ ) and the lowest

**Table 1.** Growth performance of rainbow trout

	Diets		
	Control	LC300	LC600
Initial Weight (g)	68.77 $\pm$ 4.5	71.39 $\pm$ 11.95	70.67 $\pm$ 6.09
Final weight (g)	216.97 $\pm$ 40.03 <sup>a</sup>	238.79 $\pm$ 35.72 <sup>b</sup>	257.27 $\pm$ 36.39 <sup>c</sup>
SGR (% bw $\text{day}^{-1}$ )	1.80 $\pm$ 0.19	1.89 $\pm$ 0.03	2.03 $\pm$ 0.09
Average weight gain (g $\text{fish}^{-1}$ )	148.20 $\pm$ 9.41 <sup>a</sup>	167.40 $\pm$ 3.69 <sup>ab</sup>	186.60 $\pm$ 8.9 <sup>b</sup>
Total net biomass gain (kg $\text{m}^{-3}$ )	7.41 $\pm$ 0.47 <sup>a</sup>	8.37 $\pm$ 0.18 <sup>ab</sup>	9.33 $\pm$ 0.44 <sup>b</sup>
Total yield (kg $\text{m}^{-3}$ )	10.85 $\pm$ 0.49 <sup>a</sup>	11.93 $\pm$ 0.07 <sup>ab</sup>	12.86 $\pm$ 0.46 <sup>b</sup>
Total feed (kg $\text{cage}^{-1}$ )	14.82 $\pm$ 0.94	13.40 $\pm$ 0.29	15.49 $\pm$ 0.73
FCR	2.00 $\pm$ 0.05 <sup>b</sup>	1.60 $\pm$ 0.05 <sup>a</sup>	1.66 $\pm$ 0.09 <sup>a</sup>

**Table 2.** Assumptions for bio-economical analysis for the production of 1 kg reared rainbow trout

Parameters	Control	LC300	LC600
FCR	2.00 $\pm$ 0.05 <sup>b</sup>	1.60 $\pm$ 0.05 <sup>a</sup>	1.66 $\pm$ 0.09 <sup>a</sup>
Feed cost (US\$ $\text{kg}^{-1}$ )	1.35	1.35	1.35
Supplemented L-carnitine Cost (US.\$)	0	0.034	0.068
ECR	2.71 $\pm$ 0.06 <sup>a</sup>	2.21 $\pm$ 0.07 <sup>b</sup>	2.35 $\pm$ 0.12 <sup>b</sup>
EPI	0.358 $\pm$ 0.1 <sup>a</sup>	0.464 $\pm$ 0.08 <sup>b</sup>	0.460 $\pm$ 0.09 <sup>b</sup>

in fish fed with control diet (0.358 US.\$ fish<sup>-1</sup>). As shown in Figure 1, the following equations describe the relationships of the above parameters (ECR and EPI) and supplemented L-carnitine.

$$\text{L-carnitine} = 0.000004\text{ECR}^2 - 0.002\text{ECR} + 2.71, \\ R^2 = 0.99 \quad (1)$$

$$\text{L-carnitine} = -0.0000006\text{EPI}^2 + 0.0001\text{EPI} + 0.14, \\ R^2 = 0.99 \quad (2)$$

In accordance with equations (1) and (2), optimum supplementary L-carnitine inclusion for maximum ECR and EPI was calculated as 337.5 mg kg<sup>-1</sup> and 333.3 mg kg<sup>-1</sup>, respectively.

Relative composition of the fish muscle changed significantly over the course of the study (Table 3).

The protein content of LC300 and LC600 was significantly higher than the control group ( $P < 0.05$ ). However, no significant differences in lipid content were observed among the treatment within a time point ( $P > 0.05$ ). Furthermore, the ash content was the highest in control groups compared to LC300 and LC600. There were no significant differences within the moisture content of the groups ( $P > 0.05$ ).

Fatty acid class provides one of the clearest illustrations of the differences between the relative fatty acid compositions among the treatments. It was determined that the percentage of these acids in total fatty acids is 81% (Table 4).

In this respect, a significant increase in the absolute level of saturated fatty acids (SFA) in fish muscle samples from LC300 and LC600 was

**Table 3.** Effect of dietary L-carnitine on muscle proximate composition of rainbow trout

Proximate composition (%)	Control	LC300	LC600
Protein	20.75±0.18 <sup>a</sup>	21.88±0.19 <sup>b</sup>	21.13±0.21 <sup>b</sup>
Lipid	7.70±0.10	7.41±0.11	7.43±0.12
Ash	1.51±0.02 <sup>b</sup>	1.49±0.03 <sup>a</sup>	1.49±0.03 <sup>a</sup>
Moisture	70.04±1.49	69.22±1.28	69.95±1.59

**Table 4.** Effects of dietary L-carnitine on muscle fatty acid composition of the rainbow trout (% total fatty acids)

Fatty Acids	Control	LC300	LC600
C12:0	0.06±0.00	0.07±0.00	0.07±0.00
C13:0	0.04±0.00	0.04±0.00	0.04±0.00
C14:0	3.84±0.01 <sup>a</sup>	4.41±0.07 <sup>b</sup>	4.48±0.00 <sup>b</sup>
C15:0	0.55±0.00 <sup>a</sup>	0.62±0.01 <sup>b</sup>	0.63±0.00 <sup>b</sup>
C16:0	14.26±0.06 <sup>a</sup>	15.73±0.22 <sup>b</sup>	16.73±0.02 <sup>c</sup>
C17:0	0.75±0.07	0.81±0.01	0.79±0.00
C18:0	3.41±0.04 <sup>a</sup>	3.70±0.04 <sup>b</sup>	3.81±0.03 <sup>b</sup>
C20:0	0.74±0.00 <sup>b</sup>	0.46±0.00 <sup>a</sup>	0.48±0.04 <sup>a</sup>
C21:0	0.50±0.04 <sup>b</sup>	0.22±0.00 <sup>a</sup>	0.21±0.00 <sup>a</sup>
C22:0	0.39±0.03 <sup>b</sup>	0.17±0.00 <sup>a</sup>	0.16±0.00 <sup>a</sup>
C23:0	0.28±0.02 <sup>b</sup>	0.12±0.00 <sup>a</sup>	0.13±0.03 <sup>a</sup>
C24:0	0.30±0.01 <sup>b</sup>	0.09±0.00 <sup>a</sup>	0.08±0.00 <sup>a</sup>
C14:1	0.02±0.00	0.03±0.00	0.03±0.00
C15:1	0.13±0.00 <sup>a</sup>	0.14±0.00 <sup>b</sup>	0.14±0.00 <sup>b</sup>
C16:1	6.14±0.00 <sup>b</sup>	5.97±0.08 <sup>b</sup>	5.33±0.03 <sup>a</sup>
C17:1	0.54±0.01	0.56±0.01	0.56±0.01
C18:1(n-9t)	0.23±0.02 <sup>b</sup>	0.15±0.00 <sup>a</sup>	0.13±0.01 <sup>a</sup>
C18:1(n-9c)	16.49±0.22 <sup>a</sup>	18.36±0.21 <sup>b</sup>	18.60±0.05 <sup>b</sup>
C18:2(n-6t)	0.32±0.02 <sup>b</sup>	0.14±0.00 <sup>a</sup>	0.11±0.01 <sup>a</sup>
C18:2(n-6c)	6.29±0.06 <sup>a</sup>	6.69±0.08 <sup>b</sup>	6.67±0.02 <sup>b</sup>
C18:3(n-6)	0.46±0.02 <sup>b</sup>	0.12±0.00 <sup>a</sup>	0.12±0.00 <sup>a</sup>
C18:3(n-3)	1.57±0.10 <sup>b</sup>	1.22±0.01 <sup>a</sup>	1.25±0.01 <sup>a</sup>
C20:1(n-9)	1.55±0.10 <sup>b</sup>	1.24±0.01 <sup>a</sup>	1.28±0.01 <sup>a</sup>
C22:1(n-9)	0.30±0.01 <sup>b</sup>	0.22±0.00 <sup>a</sup>	0.22±0.00 <sup>a</sup>
C24:1(n-9)	0.59±0.03 <sup>b</sup>	0.46±0.01 <sup>a</sup>	0.49±0.00 <sup>a</sup>
C20:2	0.82±0.00 <sup>b</sup>	0.40±0.00 <sup>a</sup>	0.41±0.06 <sup>a</sup>
C22:2	1.47±0.22 <sup>b</sup>	1.01±0.02 <sup>a</sup>	1.02±0.00 <sup>a</sup>
C20:3(n-3)	0.47±0.01 <sup>b</sup>	0.19±0.01 <sup>a</sup>	0.19±0.00 <sup>a</sup>
C20:5(n-3)	5.06±0.07	5.35±0.04	5.35±0.66
C22:6(n-3)	13.51±0.27 <sup>a</sup>	15.54±0.14 <sup>b</sup>	15.46±0.00 <sup>b</sup>
Σ SFA	25.12±1.12 <sup>a</sup>	26.44±0.96 <sup>b</sup>	27.61±1.11 <sup>b</sup>
Σ MUFA	25.99±0.89 <sup>a</sup>	27.13±1.56 <sup>b</sup>	26.78±1.24 <sup>b</sup>
Σ PUFA	29.97±1.55	30.66±1.69	30.58±1.88

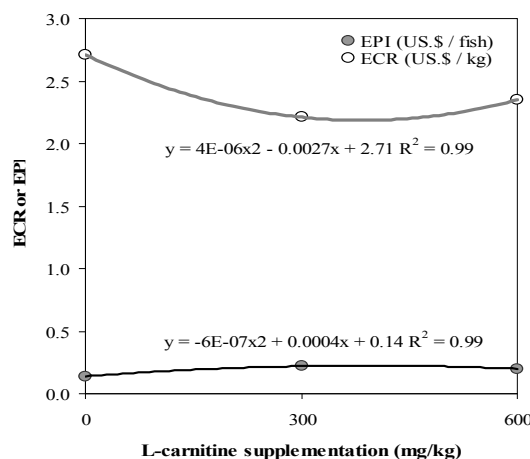


Figure 1. Second-order polynomial fitting of economic parameters.

observed over the period of the study. The highest levels of SFA were in fish from the LC300 and LC600 ( $P < 0.05$ ). The SFA in all samples were dominated by myristic (C14:0), palmitic (C16:0) and stearic acid (C18:0). All other SFA were presented at minor levels (Table 4).

The absolute levels of monounsaturated fatty acids (MUFA) significantly increased with increasing levels of L-carnitine. The MUFA were dominated by palmitoleic (C16:1), oleic (C18:1) in the control. However, supplementation of L-carnitine increased the proportion of gondoic acid (C18:1(n-9c)) and pentadecenoic acid (C15:1) compare with the control group. In terms of polyunsaturated fatty acids, there were no significant differences between groups apart from for docosahexaenoic acid. However, docosahexaenoic acid rates of the group fed with L-carnitine supplemented diet were found to be significantly higher than the control group.

## Discussion

The final weight of the trout was about three-fold higher than initial weight. Hence, the experimental period was sufficiently long to investigate possible effects of supplemental L-carnitine. The weight gain was found to be 7.73% higher in the group fed with L-carnitine supplemented diet than in the control group. The results obtained from this study clearly point out that L-carnitine has a positive effect on growth of rainbow trout in cage culture conditions. Fish receiving L-carnitine level of 600 mg kg<sup>-1</sup> in their diet showed the highest increase in growth rate. Even at the level of 300 mg kg<sup>-1</sup> carnitine positively affected the growth of fish. The same conclusion was reached by Santulli and D'Amelio, (1986) who demonstrated that L-carnitine administered to the level of 250 mg kg<sup>-1</sup> of body weight per day had a growth enhancing effect on European sea bass. Further investigations by Torreelle *et al.* (1993) working with the African catfish extended the above observations. Chatzifotis *et al.*

(1995) noticed that at the level of 2,088 mg kg<sup>-1</sup>, L-carnitine supplementation had a positive effect on growth of red sea bream. On the other hand, increased growth in the present study might be of secondary effect. Santulli and D'Amelio (1986) and Chatzifotis *et al.* (1995) found L-carnitine supplementation of diet promotes not only growth but also lipid metabolism in hatchery-reared sea bass and red sea bream. In the current study, dietary L-carnitine may have indirectly increased carnitine synthesis and consequently promote lipolysis activity. Thus, promoted lipolysis activity may have an effect on protein accumulation in our tested fish. However, it was important for the objectives of the present trail to note that there was an obvious effect of L-carnitine on the growth and fatty acid composition. More details of the effects of dietary ascorbate (indirectly L-carnitine metabolism) on the lipolysis and lipogenesis are given elsewhere (Ji *et al.*, 2009).

A significantly higher feed conversion appeared in control group for fish fed with control diet (2.00) compared with LC300 (1.60) and LC600 (1.66). By the side of a result of this study, supplementation with L-carnitine (300 mg kg<sup>-1</sup>) provides the fish culturist to save 0.40 kg feed per kg body weight gain, which is 25% less than the feed required for the control group. This aspect is of practical importance for the fish culturist both from an ecological and economic point of view. These findings have also been supported by Becker *et al.* (1999). Becker *et al.* (1999) observed that low level supplementation of L-carnitine (150 mg kg<sup>-1</sup>) for tilapia is effective but not the next higher supplementation level (300 mg kg<sup>-1</sup>). Supplemental dietary L-carnitine showed positive effects in terms of growth rate and food conversion ratio of red sea bream (Chatzifotis *et al.*, 1995; 1996), tilapia (Jayaprakas *et al.*, 1996; Dikel *et al.*, 2003), European sea bass (Santulli and D'Amelio, 1986) and African catfish (Torreelle *et al.*, 1993), hybrid striped bass (Twibell and Brown, 2000). In contrast dietary carnitine did not affect weight gain of the channel catfish (Burtle and Liu, 1994), rainbow trout

(Rodehutschord, 1995) or Atlantic salmon (Ji *et al.*, 1996). However, in the present study, growth rate and FCR of rainbow trout have been positively affected by carnitine administration. It is difficult to explain these conflicts. Because the mechanisms by which supplemental dietary L-carnitine may exert such beneficial effects in terms of growth performance are still little understood in fish. For these reasons, L-carnitine supplementations in fish have shown conflicting results even with the same species, like hybrid striped bass which reported by Twibell and Brown (2000) and Gaylord and Gatlin (2000). Rodehutschord (1995) noticed that L-carnitine supplementation did not effected on growth rate of rainbow trout. A possible explanation for this apparent difference in showed by Rodehutschord (1995) in trout and results didn not show any significant effect of L-carnitine administration; this might be due to lower supplementation level (230 mg kg<sup>-1</sup>) and different culture conditions under which their experiments were conducted. But investigations in many fish species have shown that; adequate supplementation levels of L-carnitine for carnivorous species such as rainbow trout and salmons were 500-1000 mg per kg feed (Anonymous, 2003).

As shown in Table 3, L-carnitine had no significant effect on proximate composition except for protein composition in rainbow trout. Although there were no statistical differences in total lipid content of fillets, carnitine group contained lower lipid than in control group. These results indicate that L-carnitine supplementation of diet promotes lipid metabolism, as observed by Nakagawa *et al.* (2000), activated lipid mobilization may resulted in depression of lipid accumulation in fish fed supplemented L-carnitine. Similar results were reported for rainbow trout by others (Rodehutschord, 1995; Chatzifotis and Takeuchi, 1997). Besides the effects on lipid accumulation, dietary L-carnitine supplementation affects fatty acid metabolism by shifting the energy from C14-C18 to C20-C22 fatty acids (Chatzifotis *et al.*, 1995; 1996). Although there are similarities among the distribution of fatty acids in total lipid in general, some differences were found to exist regarding certain fatty acids. Chatzifotis and Takeuchi (1997) reported that L-carnitine supplementation prior to starvation did not show any effect on the proximate and fatty acid composition in dorsal muscle of red sea bream. Also, Chatzifotis *et al.* (1996) showed that red sea bream reduced values of C20-24 fatty acids when fed with L-carnitine-supplemented diet. This is consistent with data from our fatty acid profile which showed that C14-18 increased in fed diets with dietary L-carnitine whereas C20-22 decreased with elevated L-carnitine supplementation. This might happen to be the indication of an increased rate of oxidation of these fatty acids. In addition, Ozório *et al.* (2003) showed decreased levels of Lc-fatty acids (EPA and DHA) content in African catfish fed with diets high in L-carnitine. In contrast to what was

previously observed in their work, present data showed that EPA and DHA in fish fed with carnitine function as part of the carnitine acyl-transferase enzyme for transporting fatty acids into the mitochondria for  $\beta$ -oxidation. Presumably, this enzyme little affected in trout evidenced by a lack of response in fatty acid composition in muscle with dietary L-carnitine supplementation in the present study. The result of our study revealed that most abundant PUFAs were DHA and increased depending on L-carnitine administration in diets.

One of the aims of this study was also to evaluate the possible effect of L-carnitine supplementation on cost analysis of production. The low FCR that we obtained with L-carnitine supplemented groups would be ultimately lower final cost of production than control. Calculated ECR in trout fed with 300 mg kg<sup>-1</sup> L-carnitine supplemented feed (2.27 US.\$ kg<sup>-1</sup>) would represent a saving of 0.44 US.\$ kg<sup>-1</sup> (16.3%) compared to the control (2.71 US.\$ kg<sup>-1</sup>). Between 40-50% of the variable cost of rainbow trout production is attributed to feed (Vandenberg and Moccia, 1998); therefore a 16.3% reduction in the feed price per kg would represent a 6.5-8.1% savings per year.

Overall, dietary supplemental L-carnitine could improve growth of rainbow trout (70 g) with 600 mg kg<sup>-1</sup> supplementation level. Even though there were generally no significant differences between fatty acid compositions among the groups, it is surmised that L-carnitine treatment has a positive effect on particularly DHA polyunsaturated fatty acid contents. On the other hand, economic profit view point, optimum L-carnitine inclusion for minimum ECR and maximum EPI was calculated as 337.5 mg kg<sup>-1</sup> and 333.3 mg kg<sup>-1</sup> respectively.

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## References

- Anonymous 2003. What is L-carnitine? Agro Food Industry Hi-Tech, prepared by Dr. Oresta Piccolo ve Lonza Ltd., January-February). ([http://www.teknoscienze.com/agro/ins\\_agro/top/sp\\_hglts/l\\_carnitine.pdf](http://www.teknoscienze.com/agro/ins_agro/top/sp_hglts/l_carnitine.pdf))24.12. 2003
- AOAC. 1984. In Williams, S. (Ed.). Official Methods of Analysis, (14<sup>th</sup> Ed. Methods 32.027, 24.003, and 47.021. Association of Official Analytical Chemists, Washington, DC.
- Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37: 911-917.
- Becker, K. and Focken, U. 1995. Effect of feed supplementation with L-carnitine on growth,

- metabolism and body composition of carp (*Cyprinus capio* L.). *Aquaculture*, 129: 34–43.
- Becker, K., Schreiber, S., Angoni, C. and Blum, R. 1999. growth performance and feed utilization response of *Oreochromis niloticus* x *Oreochromis aureus* hybrids to L-carnitine measured over a full fattening cycle under commercial conditions. *Aquaculture*, 174: 313–322.
- Bremer, J. 1983. Carnitine metabolism and functions. *Physiol. Rev.*, 63: 1420–1480.
- Burtle, J.G. and Liu, Q. 1994. Dietary carnitine and lysine affect channel catfish lipid and protein composition. *J. World Aquacult. Soc.*, 25: 169–174.
- Chatzifotis, S., Takeuchi, T. and Seikai, T. 1995. The effect of dietary L-carnitine on growth performance and lipid composition in red sea bream fingerlings. *Fish. Sci.*, 61: 1004–1008.
- Chatzifotis, S., Takeuchi, T. and Seikai, T. 1996. The effect of dietary supplementation L-carnitine on growth of red sea bream *Pagrus major* fingerlings at two levels of dietary lysine. *Aquaculture*, 147: 235–248.
- Chatzifotis, S. and Takeuchi, T. 1997. Effect of supplemental carnitine on body weight loss, proximate and lipid compositions and carnitine content of Red Sea bream (*Pagrus major*) during starvation. *Aquaculture*, 158: 129–140.
- Chatzifotis, S., Takeuchi, T., Watanabe, T. and Sato, S. 1997. The effect of dietary carnitine on growth rainbow trout fingerlings. *Fish. Sci.*, 63(2): 321–322.
- Dikel, S., Alev, M.V., Kiris, G.A. and Celik, M. 2003. Effects of supplemental dietary L-carnitine on the growth of Nile tilapia (*Oreochromis niloticus*) in cage conditions. *Turk. J. Vet. Anim. Sci.*, 27: 663–669.
- Dzikowski, R., Hulata, G., Karplus, I. and Harpaz, S. 2001. Effect of temperature and dietary L-carnitine supplementation on reproductive performance of female guppy (*Poecilia reticulata*). *Aquaculture*, 199: 323–332.
- Duncan, D.B. 1995. Multiple range and multiple F tests. *Biometrics*, 11: 1–42
- Focken, U., Becker, K. and Lawrence, P. 1997. A note on the effects of L-carnitine on the energy metabolism of individually reared carp, *Cyprinus carpio* L. *Aquacult. Nutr.*, 3: 261–264.
- 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.
- Harpaz, S., Becker, K. and Blum, R. 1999. The effect of dietary L-carnitine supplementation on cold tolerance and growth of the ornamental cichlid fish (*Pelvicachromis pulcher*) preliminary results. *Journal of Thermal Biology*, 24: 57–62.
- Harpaz, S. 2005. L-Carnitine and Its attributed functions in fish culture and nutrition- a review. *Aquaculture*, 249: 3–21.
- Hausenblasz, J., Acs, M., Petri, A. and Mezes, M. 1996. Effect of L-carnitine on some metabolic parameters of foals. *Allattenyesztes es takarmanyozas*, 45: 397–403.
- IUPAC, 1979. Standarts methods for analysis of oils, In: C. Paquot (Ed.), *Fats and Derivatives 6<sup>th</sup> Ed.*, Pergaman Press, Oxford: 59–66
- Jayaprakas, V. and Sambhu, C. 1996. Growth response of white prawn, *Penaeus indicus*, to dietary L-carnitine. *Asian Fisheries Science*, 9: 209–219.
- Jayaprakas, V., Sambhu, C. and Kumar, S. 1996. Effect of dietary L-carnitine on growth and reproductive performance of male *Oreochromis mossambicus* (Peters). *Fish. Technol.*, 33: 84–90.
- Ji, H., Bradley, T.M. and Tremblay, G.C. 1996. Atlantic salmon *Salmo salar* fed L-carnitine exhibit altered intermediary metabolism and reduced tissue lipid, but no change in growth rate. *J. Nutr.*, 126: 1937–1950.
- Ji, H., Om, A.D., Yoshimatsu, T., Umino, T., Nakagawa, H. and Sakamoto, S. 2009. Effect of dietary ascorbate on lipogenesis and lipolysis activities in black sea bream, *Acanthopagrus schlegelii*. *Fish. Physiol. Biochem.*, DOI 10.1007/s 10695-009-9349-z.
- Keshavanath, P. and Renuka, P. 1998. Effect of dietary L-carnitine supplements on growth and body composition of fingerling rohu, *Labeo rohita* Hamilton. *Aquacult. Nutr.*, 4: 83–87.
- Martínez-Llorens, S., Moñino, A.V., Tomás, A., Pla, M. and Jover, M. 2007. Soybean meal as partial dietary replacement for fish meal in gilthead sea bream (*Sparus aurata*) diets: effects on growth, nutritive efficiency and body composition. *Aquac. Res.*, 38: 82–90.
- Nakagawa, H., Mustafa, M.G., Takii, K., Umino, T. and Kumai, H. 2000. Effect of dietary catechin and Spirulina on vitamin C metabolism in red sea bream. *Fish Sci.*, 66: 321–326.
- Ozório, R.O.A. 2001. Dietary L-carnitine and energy and lipid metabolism in African catfish (*Clarias gariepinus*) juveniles. PhD thesis. Holland: Wageningen University.
- Ozório, R.O.A. 2009. Dietary L-carnitine supplementation to cultivated fish: A Mini-Review. *Current Nutrition and Food Science*, 5: 40–48.
- Rabie, M.H., Szilagy, M. and Gippert, T. 1997. Effects of dietary L-carnitine supplementation and protein level on performance and degree of meatiness and fatness of broilers. *Acta Biologica Hungarica*, 48: 221–239.
- Rebouche, C.J. 1991. Ascorbic acid and carnitine biosynthesis. *Amer. J. Clin. Nutr.*, 54: 1147–1152.
- Rodehutsord, M. 1995. Effects of supplemental dietary L-carnitine on growth and body composition of rainbow trout *Oncorhynchus mykiss* Fed High-Fat Diets. *J. Anim. Physiol. Anim. Nutr.*, 73: 276–279.
- Santulli, A. and D'Amelio, V. 1986. Effects of Supplemental Dietary Carnitine on Growth and Lipid Metabolism of Hatchery-Reared Sea Bass *Dicentrarchus labrax* L. *Aquaculture*, 59: 177–186.
- Schlechtriem, C., Bresler, V., Fishelson, L., Rosenfeld, M. and Becker, K. 2004. Protective effects of dietary L-carnitine on tilapia hybrids (*Oreochromis niloticus* x *Oreochromis aureus*) reared under intensive pond-culture conditions. *Aquaculture Nutrition*, 10: 55–63.
- Schreiber, S., Becker, K., Bresler, V. and Fishelson, L. 1997. Dietary L-carnitine protects the gills and skin of guppies (*Poecilia reticulata*) against anionic xenobiotics. *Comparative Biochemistry and Physiology*, 117: 99–102.
- Torrelee, E., Van Der Sluizen, A. and Verreth, J. 1993. The Effect of Dietary L-carnitine on the Growth Performance in Fingerlings of the African Catfish *Clarias gariepinus* in Relation to Dietary lipid. *Br. J. Nutr.*, 69: 289–299.
- Twibell, R.G. and Brown, P.B. 2000. Effects of Dietary Carnitine on Growth Rates and Body Composition of Hybrid Striped Bass (*Morone saxatilis* male x *M.*

- chrysops* female). Aquaculture, 187: 153-161.
- Vandenberg, G.W. and Moccia, R.D. 1998. Growth performance and carcass composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed the  $\beta$ -agonist ractopamine, Aquaculture Research, 29(7): 469-479.
- Weeden, T.L., Nelssen, J.L., Hansen, J.A., Fitzner, G.E. and Goodband, R.D. 1991. The effect of L-carnitine on starter pig performance and carcass composition. Journal of Animal Science, 69: 105 Abstract.