Effect of L-carnitine or its precursor on growth, feed utilization and body composition of Nile tilapia, *(Oreochromis niloticus)*

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Abstract

The experiment aimed to evaluate the effect of different L-carnitine levels and amino acids in tilapia diets (control diet, 300 mg L-carnitine, 10 g lysine+5 g methionine, 15 g lysine+7 g methionine, 10 g lysine+5 g methionine+300 mg L-carnitine and 15 g lysine+7 g methionine + 300 mg L-carnitine) on growth performance, feed utilization and body composition of Nile tilapia. The diet contained (15g lysine + 7g methionine+300 mg L-carnitine/kg basal diet) significantly increased final body weight (BW), body length (BL), weight gain (WG), specific growth rate (SGR) and improved (reduced) feed intake, feed conversion ratio (FCR) while protein efficiency ratio was not significantly affected. Proximate analysis of whole fish showed that, the diet contained of (15g lysine + 7g methionine + 300 mg L- carnitine) significantly increased protein and decreased fat and ash while dry matter content of fish flesh was not significantly affected. It is concluded that, dietary L-carnitine supplementation (15 g lysine + 7 g methionine + 300 mg L-carnitine/kg/diet) improved growth and feed utilization, decrease tissue fat and increased tissue protein of Nile tilapia.

Keywords: L-carnitine- Nile tilapia – growth performance

1. Introduction

Tilapia is an important food fish in many tropical and subtropical countries. More than 20 species of tilapia have been cultured in developing countries, where animal protein is lacking. The most important tilapia species are *Oreochromis niloticus, O. mossambicus, O. aureus* and *O. galilae*. Among all cultured tilapia species, Nile tilapia (*Oreochromis niloticus*) which has emerged as the single most important species. The attributes which make Nile tilapia so suitable for fish farming are its general resistance to elevated environmental conditions, ease of breeding, rapid growth rate, ability to convert efficiency organic wastes into high quality protein and good taste, its resistance to disease and parasites. Tilapia culture has become more popular because of the relative ease of culture in a variety of aquaculture systems and because of favorable attributes as food fishes. Tilapia has become one of the most important fish species for fresh water culture (Yi *et al.*, 1996).

The ability of dietary carnitine to increase growth rates and reduce tissue lipid concentrations has been evaluated in several species of fish, with contradictory results. Carnitine, synthesized in vivo from lysine and methionine, is required for transport of long-chain fatty acids into the mitochondria, which is the site of beta oxidation. Early research with fish indicated that carnitine increased fatty acid oxidation in tissues of rainbow trout (Bilinski and Jonas, 1970). Recently, Ji *et al.* (1996) reported increased fatty acid oxidation in liver of Atlantic salmon fed 3700 mg carnitine / kg diet, which was accompanied by reduced lipid levels in muscle and viscera. Dietary carnitine has also been shown to increase growth rates of some species of fish (Santalli and D'Amelio, 1986; Torreele *et al.,* 1993; Keshavanath and Renuka, 1998).

2. Materials and methods

The present study was carried out at the Laboratory of Fish nutrition, Faculty of Agriculture, Moshtohor, Benha University. The experiment was conducted to evaluate the effect of addition of L-carnitine and its precursors to the experimental diets of Nile tilapia,

Orcochromis niloticus in growth performance, feed utilization and proximate analysis of tilapia fish (second experiment).

Eighteen rectangular aquaria 100 x 40 x 50cm (180 liter for each) were used for the experiment. The aquaria represent 6 treatments (3 replicates), and each aquarium was stocked with 25 fish with an initial weight of 1.63 ± 0.07 g . Fish grouped into six diets with L-carnitine (Arab Company For Pharmaceutical & Medical Plants - MEPACO - Egypt).

A basal diet containing (Table 1) (30% CP) and (3000 kcal ME/kg) was formulated and tested with or without the supplements in the experiment. The basal diet divided into six parts and L-carnitine and its precursors were added to formulate 6 diets as follows**:**

Group (1): basal diet (kept as control group without L-carnitine).
Croup (2): basal diet supplemented with 300 mg L-carnitine/kg basal diet.
Group (3): basal diet incorporated with 10 g lysine + 5 g methionine/k.
Group (4): basal diet supplemented with 15 g lysine + 7 g methionine/kg.
Group (5): basal diet supplemented with 10g lysine+5g methionine+300 mg L-carnitine /Kg.
Group (6): basal diet supplemented with 15 g lysine+7 g methionine+300 mg L-carnitine /Kg.

Table (1): Composition and proximate analysis of basal diet.

Ingredient	**%**
Fish meal	28.0
Soybean meal	18.0
Yellow corn	24.0
Wheat flour.	13.0
Wheat bran.	9.0
Com oil	4.0
Vit. & Min. mix.[1]	4.0
Sum	100.0
Proximate analysis	
Dry matter	95.23
Protein	30.12
Lipid	5.32
Ash	8.45
ME (Kcal/kg diet)[2]	3019
P/E ratio	99.78

1. Vitamin & mineral mixture / kg premix: Vitamin D3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g, Bl. 0.4 g; Riboflavin. 1.6g: B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg, Mn, *22* g; Zn, *22* g; Fc, 12 g; Cu, 4 g; I, 0.4 g. Selenium. 0.4 g and Co. 4.8 mg..
2. Based on kilocaloric values of 4.50 g^{-1} protein, 8.5 I g^{-1} lipid and 3.49 g^{-1} NFE **(Jauncey, 1982).**

The experimental fish were obtained from El-Manzala hatchery, Al-Dakahlya Governorate. The experimental fish were transported in a 50-liter plastic bags filled with water and oxygen to the laboratory, and after arrival to the laboratory fish were adapted and distributed randomly into eighteen aquaria representing the six treatments studied (3 replicates for each treatment). The diets were prepared by thoroughly mixing of the ingredients, which composed of fish meal, soybean meal, yellow corn, wheat flour, corn oil and bran with different percentage (Table 1). L-carnitine and its precursor were added to the basal diets as described above. Water was added to

the ingredients of each diet for mixing these ingredients and then dried. After drying, the diets were broken up and sieved into the convenient pellet size.

Fish were given the experimental diets of each experiment at a daily rate of 3% of total biomass till the end of experimental period. Fish were fed on the experimental diets at the rates mentioned above 6 day/week (twice daily at 9.00 am and 3.00 pm). Every two weeks, fish were taken from each aquarium then weighed and the amount of feed was adjusted according to the changes in body weight throughout the experimental period.

Records of live body weight (g) and body length (cm) of individual fish were measured in all fish for each aquarium and registered every 14 day (two weeks) during the experimental period (90 days). Growth performance parameters were measured by using the following equations:

Weight gain = final weight - initial weight (g)

Specific growth rate (SGR) = (LnW2 – LnW1) / × 100 where Ln = the natural log ; W_1 = first fish weight; W_2 = the following fish weight in "grams" and t = period in days.

Feed conversion ratio (FCR) = Feed ingested (g) / Weight gain (g)

Protein efficiency ratio (PER) = Weight gain (g) / Protein ingested (g)

At the end of each experiment, three fishes were chosen at random from each aquarium and exposed to the chemical composition of whole fish body and the flesh as well as by products (from carcass analysis) samples were chemically analyzed according to the methods of AOAC (1990).

The statistical analysis of data was carried out by applying the computer program, SAS (1996) by adopting the following model:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where, Y_{ij} = the observation on the j^{th} fish eaten the i^{th} diet; μ = overall mean, α_i = the effect of i^{th} diet and e_{ij} = random error.

3. Results and discussion

3.1. Growth performance:

Average initial body weight (BW) of Tilapia fish *Oreochromis niloticus* ranged from 1.60 to 1.66 g with non significant differences between groups (Table 2). After 90 days of the experimental start (end of the experimental), BW ranged between 19.28±0.94 and 27.94±0.94 and the differences in BW among the different treatments were significant (P<0.001).

Results indicated that the dose 15 g lysine + 7 g methionine + 300 mg L-carnitine / kg basal diet (group 6) resulted the highest BW (27.94 g) compared to the control group (0 L-carnitine) and the difference was significant (P < 0.001) and the other doses caused also significant increase in body weight compared to the control group and the same trend was also observed for body length (BL).

The obtained results are in accordance with the finding of Soltan *et al.* (2004) who reported that all L-carnitine levels (300 to 1500 mg/kg diet at each of two lipid levels (5 and 10%) significantly (P < 0.001) increased the BW of Nile tilapia. Litz (1993) attributed the increased growth performance, (BW and BL) of the carnitine supplemented fish groups to the increased feed intake in the trout fish.

In this respect, results of other authors showed that dietary L-carnitine seemed to increase the final BW of Nile Tilapia (Kumar and Jayaprakas, 1996; Jayaprakas *et al.,* 1996; Becker *et al.*, 1999; Azab *et al.,* 2002 and Dikel *et al.,* 2003), Carp (Focken *et al.,* 1997 and Zhang *et al.,* 2002), trout (Rodahuscord, 1995), juvenile hybrid striped bass (Twibell and Brown, 2000), red seas bream (Chatzifotis *et al.,* 1995 and 1996), European sea bass (Santulli and D'Amelio, 1986) and African catfish (Torreele *et al.,* 1993 and Twibell and Brown, 2000).

Furthermore Abdel-Hakim *et al.* (2006) reported that supplementing growing Nile tilapia diets with both methionin and lysine to reach 30% over the recommended levels improved significantly final weights, weight gains protein productive value and energy utilization. However, it has insignificant effects on feed conversion ratio and specific growth rate.

Table (2): The effect of dietary L- carnitine levels on body weight (BW) (g) and body length (BL) (cm) of Nile Tilapia fish.

Treatment	No.*	Body weight (g) (BW)		Body length (cm) (BL)	
		Initial	Final	Initial	Final
Group 1	75	1.63 ± 0.07	19.28 ±0.94 ^{c}	4.41 ±0.07	10.24 ±0.16 ^{c}
Group 2	75	1.62 ± 0.07	22.92 ±0.94 ^{b}	4.49 ±0.07	10.64 ±0.16 ^{bc}
Group 3	75	1.60 ± 0.07	22.14 ±0.94 ^{b}	4.40 ±0.07	10.57 ± 0.16 ^{bc}
Group 4	75	1.61 ± 0.07	24.41 ±0.94 ^{b}	4.49 ±0.07	11.30 ± 0.16 ^{b}
Group 5	75	1.64 ± 0.07	24.40 ±0.94 ^{b}	4.41 ±0.07	11.04 ±0.16 ^{b}
Group 6	75	1.66 ± 0.07	27.94 ±0.94 ^{a}	4.47 ±0.07	11.54 ± 0.16 ^{a}

Means with the same letters in each column are not significantly different.
* Average of 3 replicates for each treatment.

On the other hand, Chatzifotis *et al.* (1996) found that carnitine supplementation at a level of 2 g/kg^{-1} diet increased red sea bream growth fed 14 g lysine / kg^{-1} diet but did not cause any effect on growth in fish fed the diet containing 10 g lysine / kg^{-1} diet.

The weight gain (WG) values ranged from 18.05 g to 26.28 g and the differences between weight gain (WG) values were significant as shown in Table (3). The highest WG value (26.28 g) was obtained with fish fed the diet contained 15 g lysine+7 g methionine+300 mg L-carnitine / kg basal diet and the lowest WG value (18.05 g) was obtained with fish fed the control diet whereas the other doses released higher weight gain compared with control diet. Similar results were also obtained by Azab *et al.* (2002) who found that WG of Nile tilapia, *O. niloticus* was significantly increased in fish groups fed diets contained L-carnitine at levels of 300, 600 or 900 mg / kg compared to the control. Also, Soltan *et al.* (2004) found that, all dietary L-carnitine levels studied (300 to 1500 mg/kg diet) significantly ($P < 0.001$) increased weight gain of Nile tilapia whereas the diet contained 900 mg/kg L-carnitine released the higher WG. These results are in agreement with the finding of Twibell and Brown (2000) who found that feed intake and weight gain were significantly increased when hybrid striped bass fish fed on diets containing 369.7 mg L-carnitine/kg diet, compared to fish fed the basal diet containing 2.1 mg carnitine/kg diet.

On the other hand, Rodahutscord (1995) found that dietary treatment of L-carnitine had no significant effect on weight gain. In this respect, Hamackova *et al.* (1998) reported that the use of L-carnitine 250 mg/kg for feeding carp, did not have a significant influence on growth parameters although total and daily relative weight gains rates were better in comparison with the control group by 21.4 and 21.5%, respectively.

On contrast, dietary L-carnitine did not affect WG of channel catfish (Burtle and Liu 1994), rainbow trout (Rodahutscord, 1995) or Atlantic salmon (Ji *et al.,* 1996). This variation in the effect of L-carnitine in different fish species as recorded by several authors do not attributed to the concentration in L-carnitine in the diet, because low carnitine concentration (150 mg/kg diet) caused an increase in WG of tilapia (Jayaprakas *et al*., 1996), while high concentration of 3700, 1000 and 230 mg/kg diet had no significant effect on growth rates of Atlantic salamon (Ji *et al.,* 1996), channel catfish (Burtle and Liu, 1994) or rainbow trout (Rodahutscord, 1995).

Table (3): The effect of dietary L- carnitine levels on weight gain (WG) (g) and specific growth rate (SGR) of Nile Tilapia fish.

Doses	*No.* *	Weight gain (g) (WG)	Specific growth rate (SGR)
Group 1	3	18.05 ± 1.17^{c}	2.76 ± 0.04^{c}
Group 2	3	21.30 ± 1.17^{bc}	2.93 ± 0.04^{b}
Group 3	3	20.54 ± 1.17^{bc}	2.91 ± 0.04^{b}
Group 4	3	22.81 ± 1.17^{ab}	3.02 ± 0.04^{ab}
Group 5	3	22.75 ± 1.17^{ab}	2.99 ± 0.04^{ab}
Group 6	3	26.28 ± 1.17^{a}	3.12 ± 0.04^{a}

Means with the same letters in each column are not significantly different.
* Average of 3 replicates for each treatment.

The specific growth rate (SGR) values ranged from 2.76 to 3.12 and the differences between specific SGR values were significant ($P < 0.01$). The highest SGR value (3.12) was obtained with fish fed the diet contained (15 g lysine + 7 g methionine + 300 mg L-carnitine / kg basal diet) and the lowest specific growth rate (SGR) value (2.76) was obtained with fish fed the control diet. Also the other doses (group 2, 3, 4 and 5) caused a significant increase in SGR compared with control group (group 1). These results confirmed the findings of Soltan *et al.* (2004) who stated that, all L-carnitine levels studied (300–1500 mg/kg diet) caused significant ($P<0.001$) improvement in specific growth rate (SGR) of Nile tilapia fish. Also, Azab *et al.* (2002) found that, L-carnitine caused a significant increase in SGR of Nile tilapia fed diets contained L-carnitine levels of 300, 600 or 900 mg/kg diet. It was found also by Dikel *et al.* (2003) that, addition of L-carnitine at a level of 500 mg/kg diet significantly increased growth rate of Nile tilapia fingerlings by 7.9% compared to the control.

Similar finding was obtained by Becker and Focken (1995) who showed better SGR for carp fish fed the diet containing L-carnitine at 400 and 600 mg / kg levels, respectively, and by Jayaprakas *et al.* (1996) who reported that, increasing dietary L-carnitine in male of *O. mossambicus* diets significantly enhanced growth performance.

In this respect, Schuhmacher and Gropp (1998) found that incorporation of L-carnitine 450 mg / kg diet with lysine 5.3 g and methionine 3.5 g, caused significant improvement in SGR in rainbow trout fingerlings.

On the other hand, Hamackova *et al.* (1998) found that feeding of common carp on diets containing 250 mg/kg L-carnitine did not have a significant influence on growth parameters although total and daily relative weight gains and specific growth rates were better in comparison with the control group by 21.4, 21.5 and 12.5% respectively.

Feed utilization:

With regard to the effect of L-carnitine levels on feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) of Nile tilapia, results of Table (4) indicated that, compared to the control, all L-carnitine levels significantly ($P < 0.001$) increased feed intake (FI) and improved ($P < 0.001$) feed conversion ratio (FCR) but had no significant effect on protein efficiency ratio (PER). As described in Table (4) the higher feed intake value (51.50 g / fish) was obtained with fish fed the diet contained (15 g lysine +7 g methionine + 300 mg L-carnitine / kg basal diet) compared with the control group and the best values (1.21), (1.75) of FCR and PER were recorded also by fish fed the same diet (15 g lysine + 7 g methionine + 300 mg L-carnitine / kg basal diet).

These results are in agreement with the finding of Soltan *et al.* (2004) who found that, all L-carnitine levels (300 to 1500 mg/kg diet) increased FI and improved FCR but had no significant effect on PER. Also, Becker *et al.* (1999) found that, the Tilapia fish feed on the diets contained 150 and 300 mg L-carnitine/kg diet showed increased growth, feed

conversion and protein efficiency ratio and did not alter whole body composition. Similarly, several researchers have speculated that increasing growth rates of fish fed supplemental carnitine may due to its role in improving FCR via increasing fatty acid oxidation and increasing utilization of dietary energy as observed by Jayaprakas *et al.* (1996), Kumar and Jayaprakas (1996), Becker *et al.* (1999), Azab *et al.* (2002) and Soltan *et al.* (2004) in tilapia, Becker and Focken (1995) and Hamackova *et al.* (1998) in carp, Torreele *et al.* (1993) and Ozorio *et al.* (2001) in catfish, Litz (1993) and Schuhmacher and Gropp (1998) in trout and finally Chatzifotis *et al.* (1995) in red sea bream. In this respect, Ozorio *et al.* (2002) with African catfish suggest that dietary L-carnitine supplementation may increase fatty acid oxidation and possibly decrease amino acid consumption for energy.

The results of the present study showed that the important role of L-carnitine on metabolic rate is derived both from endogenous synthesis and diet as carnitine is synthesized from lysine and methionine (Bremer, 1961 and Tanphaichitr *et al.,* 1971).

Table (4): The effect of dietary L- carnitine levels on feed utilization of Nile Tilapia fish.

Doses	No.*	Feed intake (g) / fish (FI)	Feed conversion ratio (FCR)	Protein efficiency ratio (PER)
Group 1	3	47.15 ± 0.06 ^{f}	1.73 ± 0.04^{a}	1.67 ± 0.02
Group 2	3	48.66 ± 0.06 ^{e}	1.54 ± 0.04^{b}	1.70 ± 0.02
Group 3	3	49.95 ± 0.06 ^{c}	1.62 ± 0.04^{b}	1.71 ± 0.02
Group 4	3	49.15 ± 0.06 ^{d}	1.47 ± 0.04^{c}	1.74 ± 0.02
Group 5	3	51.15 ± 0.06 ^{b}	1.33 ± 0.04^{b}	1.73 ± 0.02
Group 6	3	51.50 ± 0.06 ^{a}	1.21 ± 0.04^{b}	1.75 ± 0.02

Means with the same letters in each column are not significantly different.

* Average of 3 replicates for each treatment.

3.2Proximate analysis of whole fish of Nile tilapia

Results of proximate analysis of whole tilapia fish at the end of the experiment are illustrated in Table (5). As described in this Table, it was found that dietary L-carnitine significantly increased protein ($P<0.001$) and ash content ($P < 0.01$) of bodies flesh, while fat content was decreased ($P<0.01$) but there was no significant effect on the percentage of dry matter. The highest protein content (59.34%) and the lowest fat content (28.12%) were obtained with fish fed the diet contained (15 g lysine+7 g methionine+300 mg L-carnitine/kg basal diet) and diet contained (10 g lysine+15 g methionine+300 mg L-carnitine/kg basal diet), respectively compared with control group. Soltan *et al.* (2004) came to the same results with tilapia fish. They found that L-carnitine in tilapia diets significantly increased protein and ash contents of fish flesh and decreased fat content of fish flesh while moisture content showed no clear trend.

In this connection Zhang *et al.* (2002) reported that averages muscle protein contents of common carp increased by 3.13% and fat contents decreased by 11.45% compared to the control group when fed on diets containing L-carnitine at 15 mg/kg level supplemented in diets contained 28, 32 or 36% protein in levels.

Azab *et al.* (2002) found no significant effect of dietary L-carnitine on tissue composition at low fat level (10%), while in high level of dietary fat (15%), L-carnitine caused a significant increase in tissue protein. In the same respect, dietary L-carnitine reduced lipid content of rohu (Keshavanath and Renuka, 1998), tilapia (Jayaprakas *et al.,* 1996), channel catfish (Burtle and Liu, 1994) and Atlantic salmon (Ji *et al.,* 1996). In contrast, several authors found that, dietary carnitine did not alter tissue composition of hybrid striped bass (Twibell and Brown, 2000), rainbow trout (Rodahutscord, 1995) or hybrid tilapia (Becker *et al.,* 1999).

Table (5): The effect of dietary L- carnitine levels on chemical composition of whole Nile Tilapia fish body.

Doses	No.*	Dry matter %	Protein %	Fat %	Ash %
Group 1	9	32.07 ±0.33^{ab}	56.04 ±0.63^{bc}	30.62 ±0.57^{a}	10.72 ±0.77^{c}
Group 2	9	32.40±0.33^{b}	55.41±0.63^{c}	30.04±0.57^{a}	10.78±0.77^{b}
Group 3	9	32.78±0.33^{ab}	55.84±0.63^{bc}	30.18±0.57^{a}	11.06±0.77^{ab}
Group 4	9	31.88±0.33^{ab}	55.96±0.63^{bc}	29.65±0.57^{ab}	11.28±0.77^{a}
Group 5	9	31.66±0.33^{ab}	57.79±0.63^{ab}	29.20±0.57^{ab}	11.86±0.77^{b}
Group 6	9	31.31±0.33^{b}	59.34±0.63^{a}	28.12±0.57^{b}	11.93±0.77^{b}

Means with the same letters in each column are not significantly different.
*Average of 3 replicates for each treatment.

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