EFFECT OF FEED SUPPLEMENTATION WITH L-CARNITINE ON GROWTH AND COLD TOLERANCE OF NILE TILAPIA AND COMMON CARP

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ABSTRACT

The effect of dietary L-carnitine and lipid on growth performance, feed utilization and body composition of Nile tilapia, *Oreochromis niloticus* and common carp, *Cyprinus carpio* were evaluated in two separate 2×5 factorial experiments as well as the effect of dietary L-carnitine on cold tolerance of Nile tilapia was evaluated in a third experiment of the present study. The effect of L-carnitine was evaluated in five levels, 0, 300, 600, 900 and 1200 mg/kg diet at each of two lipid levels (5 and 10%), therefore ten isonitrogenous (30% CP) and isocaloric (3000 kcal ME/kg) diets were formulated and tested in two replicates with Nile tilapia (First experiment) and common carp (Second experiment). Another five isonitrogenous (30% CP), isocaloric (3000 kcal ME/kg diet) and isolipidic (5%) diets were formulated to contain increasing levels of L-carnitine, 0, 300, 600, 900 and 1200 mg/kg diet to study the effect of L-carnitine on cold tolerance of Nile tilapia (Third experiment). Results of the present study indicated that:

Dietary L-carnitine at any level significantly increased all growth parameters (body weight, body length, weight gain, specific growth rate) and improved feed conversion and protein efficiency ratio of Nile tilapia (first experiment) and the same trend was also observed with common carp (second experiment).

Compared to control group dietary L-carnitine supplementation significantly ($P<0.05$) increased the percentage of dress-out and flesh and decreased the percentage of by-products of Nile tilapia and these results are relatively similar to those obtained for common carp. Also, increased dietary L-carnitine level decreased ($P<0.05$) fat content and increased ($P<0.05$) protein content of Nile tilapia and common carp.

Results of the third experiment of the present study clearly showed that, the addition of L-carnitine to the diet of tilapia fish can substantially reduce their mortality when reared in cold water during winter season. The fish fed the diet supplemented with 900 or 1200 mg/kg exhibited the best tolerance to cold water.

In conclusion, dietary L-carnitine supplementation in low or high levels (300 to 1200 mg/kg diet) improved growth and feed utilization, decrease tissue fat and increased tissue protein of Nile tilapia and common carp and also increased survival rate of tilapia fry reared during cold season.

INTRODUCTION

L-carnitine (γ- trimethyl-amino-β-hydroxybutyrate) is synthesized in vivo from lysine and methionine and is essential for the transport of long-chain fatty acids from the cytosol into the mitochondria where the β-oxidation of these fatty acids occurs (Dunn, 1981). Fish biologists first became interested in L-carnitine when Bilinski and Jonas (1970) observed that addition of L-carnitine to their incubation media enhanced transport and oxidation of long chain fatty acids in isolated trout mitochondria. The improved energy production in mitochondria through B-oxidation of fatty acids may be suggest that exogenous administration of L-carnitine could enhance the performance of fish by improving energy utilization efficiency from lipid oxidation (Torreele et al. 1993; Chatzifotis et al. 1995).

It has also been found that there is an increased tolerance of ammonia (Tremblay and Bradley 1992) that can not be directly explained by the effect of L-carnitine. It also increases the rate of protein synthesis (Santulli *et al.* 1990) and enhancing the generation of metabolic energy. This could stimulate some specific cell functions and may influence several biochemical and physiological process, i.e., cell protection against xenobiotics (Torreele et al. 1993, Chatzifotis et al. 1995).

Lipid nutrition of fish produced in aquaculture has attracted considerable interest both historically from the standpoint of satisfying essential fatty acid requirements (NRC, 1993) and more recently for the protein-sparing capability. Most warmwater species are typically fed diets with less than 10% lipid due to their ability to utilize higher levels of carbohydrates as energy source, the high cost of lipid relative to carbohydrates, and unwanted accretion of lipid depots. To improve utilization of dietary lipids by fish, L-carnitine has shown promise by improving growth and feed efficiency and reducing lipid deposition in some fish species (Torreele *et al.*, 1993; Chatzifotis *et al.*, 1995 and Ji *et al.*, 1996)

Chichlids such as tilapia, are amongst the most popular and promising fish for warm water aquaculture and/or ornamental fish production. One of the most serious drawbacks to growing this specie in temperate zones is their sensitivity to low ambient temperature, leading in extreme cold temperature to mass mortality (Chervinski and Lahav, 1976). Overwintering therefore presents a serious problem. Therefore, the purpose of the present study was to determine if Nile tilapia and common carp could utilize elevated levels of dietary lipid, and if supplemental L-carnitine could enhance utilization of dietary lipid. Also the effect of dietary L-carnitine on cold tolerance of Nile tilapia was evaluated.

MATERIALS AND METHODS

The experimental work of the present study was carried out at Fish Nutrition Laboratory, Faculty of Agriculture, Moshtohor, Zagazig University (Benha branch).

Growth performance experiments:

Fish: Nile tilapia, *O. niloticus* and common carp, *Cyprinus carpio* fingerlings were obtained from Abbassa hatchery, Sharkia Governorate. The experimental fish were transported in a 50-liter plastic bags filled with water and oxygen to the fish laboratory. Fish were adapted for two weeks and then distributed randomly into 40 glass aquaria (100×40×50 cm) (20 tank for each specie). Each fish was taken out by a net and weighed then transferred randomly to the experimental aquaria. 20 tanks were randomly stocked with 30 tilapia fish for each tank (first experiment) and the other 20 tanks were randomly stocked with 25 carp fish for each tank (second experiment).

Fish grouping: For each experiment fish were grouped into control group (0 L- carnitine) and four dietary L-carnitine (Arab Company For Pharmaceuticals & Medical Plants – MEPACO - Egypt) at concentrations of 300, 600, 900 and 1200 mg/kg diet in two dietary lipid levels, 5 and 10%. Therefore, the experimental diets designated as F5C0, F5C300, F5C600, F5C900, F5C1200, F10C0, F10C300, F10C600, F10C900 and F10C1200, respectively. All these fish groups were arranged in two replicates.

Feed and feeding: Composition and proximate analysis of basal diets used in the two experiments are presented in Table 1. Two basal diets were formulated to contain 5 or 10% dietary lipid. Each basal diet was divided into 5 diets and L-carnitine was added with five levels, 0, 300, 600, 900 and 1200 mg/kg diet, therefore 10 isonitrogenous (30% CP) and isocaloric (3000 kcal ME/kg) diets were formulated. Fish were given the diets at a daily rate 5% of total biomass. Fish were fed twice daily at 9:00 am and 2:00 pm. Records of live body weight (g) and body length (cm) of individual fish were measured at the start and the end of each experiment. Growth performance and feed utilization parameters were measured by using the following equations:

$$\text{Specific growth rate (SGR)} = \frac{\text{LnW2} - \text{LnW1}}{t} \times 100$$

Where:- Ln = the natural log, W1 = initial fish weight; W2 = the final fish weight in "grams" and t = period in days.

Weight gain (WG) = final weight (g) – initial weight (g)

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 fish were randomly sampled from each aquarium and slaughtered. The weight of head, viscera, flesh, carcass and total by-products were recorded. All carcass components were measured according to Lovell (1981). Another three fish were also chosen at random and exposed to the proximate analysis of whole fish body according to the methods of AOAC (1990).

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

Ingredient	5% lipid	10% lipid
Fish meal	28.0	28.0
Soybean meal	18.0	18.0
Yellow corn	24.0	16.5
Wheat flour	13.0	9.0
Wheat bran	9.0	15.5
Corn oil	4.0	9.0
Vit.&Min. mix.[1]	4.0	4.0
Sum	100.0	100.0
Proximate analysis (Dry matter basis)		
Dry matter	95.34	95.78
Protein	30.12	30.23
Lipid	5.32	10.11
Ash	7.96	8.11
Crude fiber	9.87	9.55
NFE[2]	46.73	42.00
ME (Kcal/kg diet)[3]	3019	3028
P/E ratio[4]	99.77	99.83

[1] Vitamin & mineral mixture/kg premix:: Vitamin D3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g , B1, 0.4 g; Riboflavin, 1.6 g; 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; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

[2] Nitrogen free extract (NFE) =100-(CP+EE+CF+Ash)

[3] Metabolizable energy was calculated from ingredients based on **NRC (1993)** values for tilapia.

[4] Protein to energy ratio : mg protein/kcal ME.

Cold tolerance experiment:

The third experiment of the present study aimed to study the effect of dietary L-carnitine on cold tolerance of Nile tilapia, therefore, 10 glass aquaria (100×40×50 cm) were stocked with 40 tilapia for each aquarium and the aquaria were divided into five groups (in two replicates). During the experimental period (from 1December to 1 March 2004) fish fed five isonitrogenous (30% CP) and isocaloric (3000 ME/kg diet) diets at a rate of 1% of body weight

(6 days/week). The experimental diets formulated to contains, 0, 300, 600, 900 and 1200 mg L-carnitine/kg diet. Temperature was weekly recorded in all aquaria.

Statistical analysis: The statistical analysis of growth performance experiments (first and the second experiments) were carried out by applying the computer program SAS (1996) by adopting the following model:-

$Y_{ijkl}=\mu+R_i+\alpha_j+B_k+(\alpha B)_{jk}+E_{ijkl}$,

Where: Y_{ijkl} = the observation on the ijklth fish eaten the diet contained the kth L-carnitine level and jth lipid level for the ith replicate; μ = overall mean, R_i = the effect of ith replicate; α_j = the effect of jth lipid level; B_k = the effect of kth L-carnitine level; $(\alpha B)_{jk}$ = the effect of interaction between jth lipid level and kth L-carnitine level and E_{ijkl} = random error.

Statistical analysis of cold tolerance experiment was carried out by the following model:-

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

Where: Y_{ij} = the observation on the ijth fish eaten the diet contained the ith L-carnitine level; μ = overall mean; α_i = the effect of ith L-carnitine level and E_{ij} = random error.

RESULTS AND DISCUSSION

First experiment

I-Effect of dietary lipid and L-carnitine on growth performance of Nile tilapia:

Table 2 showed the effect of L-carnitine on growth performance and feed utilization of Nile tilapia, it revealed that, at the experiment start body weight (BW) ranged between 5.78 to 6.13 g and body length (BL) ranged between 6.90 to 7.27 cm with no significant differences between fish groups in BW and BL indicating the random distribution of fish around the different treatments. At the end of the experimental period (90 days) results of Table 2 indicated that, dietary factors had dramatic effects on growth and feed utilization. Dietary lipid levels studied (5 and 10%) had no significant effect on all growth and feed utilization parameters while all dietary L-carnitine levels significantly ($P<0.05$) increased BW, BL, WG, SGR, feed intake (FI) and improved FCR and PER. The present study revealed that, L-carnitine caused a significant increase in BW, WG and SGR of Nile tilapia, *O. niloticus* and these results in accordance with those observed by Jayaprakas *et al*., (1996) and Azab *et al*., (2002), they found that, supplementation of L-carnitine to Nile tilapia diets significantly increased WG and improved FCR. In the same respect, Jayaprakas and Sambhu (1998) found a significant increase in growth of Pearlspot, *Etroplus suratensi* when treated with 750 mg carnitine/kg diet.

With regard to the interaction between dietary lipid and L-carnitine, results of Table 2 indicated that, with each lipid level the lower L-carnitine level (300 mg/kg diet) significantly improved BW, BL, WG, SGR, FI, FCR and PER but did not significantly different from the higher L-carnitine levels (600, 900 and 1200 mg/kg diet). The lack of a strong L-carnitine effect on tilapia growth at the higher levels (600, 900 or 1200 mg/kg diet) in the present experiment appears to be due to the ability of fish synthesize adequate quantities of L-carnitine for lipid metabolism. The diets were limiting in lysine or methionine which are precursors for L-carnitine synthesis. If a limited precursor pool was available for metabolism, the higher L-carnitine levels may have had more dramatic influences on growth and/or proximate composition of gain. Azab *et al*., (2002) found that, growth rate of Nile tilapia improved

Table (2): Least square means and standard error for the effect of dietary fat and L-carnitine on growth performance and feed utilization of Nile tilapia.

Item	No.	Body weight (g)		Body length (cm)		Weight gain (WG)	Specific growth rate (SGR)	Feed intake (g)/fish (FI)	Feed conversion ratio (FCR)	Protein efficiency ratio (PER)
		Initial	Final	Initial	Final					
Fat (F)										
5% (F5)	300	5.91±0.12	24.59±0.31	7.15±0.05	11.51±0.06	18.69±0.02	1.69±0.01	51.54±0.15	2.76±0.02	1.20±0.12
10% (F10)	300	6.04±0.12	25.19±0.31	7.12±0.05	11.71±0.06	19.15±0.02	1.70±0.01	52.59±0.15	2.75±0.02	1.21±0.12
L-carn. mg/kg diet (C)										
0 (C0)	120	5.81±0.21	19.83±0.54 b	7.11±0.09	10.64±0.11 b	14.02±0.03 e	1.47±0.02 b	43.64±0.25 b	3.12±0.03 a	1.07±0.20 b
300 (C300)	120	5.97±0.21	25.64±0.54 a	7.16±0.09	11.98±0.11 a	19.67±0.03 d	1.74±0.02 a	53.39±0.25 a	2.71±0.03 b	1.23±0.20 a
600 (C600)	120	5.98±0.21	25.73±0.54 a	6.95±0.09	11.75±0.11 a	19.76±0.03 d	1.74±0.02 a	53.54±0.25 a	2.71±0.03 b	1.23±0.20 a
900 (C900)	120	6.04±0.21	26.23±0.54 a	7.22±0.09	11.84±0.11 a	20.20±0.03 a	1.75±0.02 a	53.45±0.25 a	2.65±0.03 b	1.26±0.20 a
1200 (C1200)	120	6.01±0.21	25.89±0.54 a	7.15±0.09	11.66±0.11 a	19.88±0.03 c	1.75±0.02 a	54.12±0.25 a	2.72±0.03 b	1.22±0.20 a
Fat × L-carnitine										
F5 C0	60	5.84±0.29	19.60±0.77 b	7.08±0.13	10.75±0.15 c	13.76±0.05 g	1.45±0.03 b	43.32±0.36 b	3.15±0.05 a	1.05±0.29 b
F5 C300	60	5.88±0.29	25.48±0.77 a	7.17±0.13	11.62±0.15 b	19.66±0.05 d	1.75±0.03 a	52.33±0.36 a	2.66±0.05 b	1.25±0.29 a
F5 C600	60	5.91±0.29	25.23±0.77 a	6.99±0.13	11.69±0.15 b	19.32±0.05 e	1.73±0.03 a	52.74±0.36 a	2.73±0.05 b	1.22±0.29 a
F5 C900	60	5.95±0.29	26.03±0.77 a	7.27±0.13	11.73±0.15 b	20.08±0.05 bc	1.76±0.03 a	53.00±0.36 a	2.64±0.05 b	1.26±0.29 a
F5 C1200	60	5.90±0.29	25.31±0.77 a	7.20±0.13	11.53±0.15 b	19.41±0.05 e	1.74±0.03 a	53.89±0.36 a	2.78±0.05 b	1.19±0.29 a
F10 C0	60	5.78±0.29	20.05±0.77 b	7.15±0.13	10.54±0.15 c	14.27±0.05 f	1.49±0.03 b	43.96±0.36 b	3.08±0.05 a	1.08±0.29 b
F10 C300	60	6.07±0.29	25.81±0.77 a	7.15±0.13	12.35±0.15 a	19.74±0.05 d	1.73±0.03 a	54.45±0.36 a	2.76±0.05 b	1.21±0.29 a
F10 C600	60	6.04±0.29	26.23±0.77 a	6.90±0.13	11.81±0.15 b	20.20±0.05 ab	1.74±0.03 a	54.34±0.36 a	2.69±0.05 b	1.23±0.29 a
F10 C900	60	6.13±0.29	26.44±0.77 a	7.16±0.13	11.94±0.15 ab	20.31±0.05 a	1.74±0.03 a	53.89±0.36 a	2.65±0.05 b	1.25±0.29 a
F10 C1200	60	6.12±0.29	26.46±0.77 a	7.11±0.13	11.79±0.15 b	20.34±0.05 a	1.75±0.03 a	54.34±0.36 a	2.67±0.05 b	1.24±0.29 a

Means followed by the same letter in each column for each factor are not significantly different ($P<0.05$)

when L-carnitine was supplemented at a level of 900 mg/kg diet. However, Kheyyali and Tahari (1998) found that, L-carnitine supplementation to the level 250 mg/kg improved weight gain of rainbow trout, *Oncorhynchus mykiss* to 83% of the control and further supplementation of L-carnitine gave lower performance and 100 mg L-carnitine/kg diet resulted in the lowest gain with 61% of the control.

II- Effect of dietary lipid and L-carnitine on carcass traits and body composition of Nile tilapia:

Results of Table 3 indicated that, dietary lipids (5 and 10%) had no significant effect in carcass traits of Nile tilapia. Compared to control all L-carnitine levels significantly increase the percentage of dress-out and flesh while the percentage of by-products significantly (P<0.05) decreased. With regard to the effect of interaction between dietary lipid and L-carnitine, results of Table 3 revealed that, the diet contained 10% lipid and 0 L-carnitine (F10 C0) recorded the lowest dress-out and flesh and the higher by-products percentages.

Table (3): Least square means and standard error for the effect of dietary fat and L-carnitine on carcass analysis of Nile tilapia.

Item	No.	Dress-out %	Flesh %	By-products %
Fat (F)				
5% (F5)	30	49.95±0.42	34.71±0.48	57.44±0.48
10% (F10)	30	49.06±0.42	34.77±0.48	56.31±0.48
L-carnitine mg/kg diet (C)				
0 (C0)	6	47.54±0.73 b	32.95±0.83 b	59.16±0.84 a
300 (C300)	6	49.84±0.73 a	36.22±0.83 a	55.53±0.84 b
600 (C600)	6	49.45±0.73 a	35.96±0.83 a	55.37±0.84 b
900 (C900)	6	49.64±0.73 a	33.91±0.83 a	56.96±0.84 b
1200 (C1200)	6	51.06±0.73 a	35.27±0.83 a	56.82±0.84 b
Fat × L-carnitine				
F5 C0	3	49.33±1.04 a	34.96±1.17 ab	57.31±1.19 abc
F5 C300	3	50.29±1.04 a	34.65±1.17 ab	57.57±1.19 abc
F5 C600	3	48.84±1.04 a	35.68±1.17 ab	57.34±1.19 abc
F5 C900	3	49.50±1.04 a	34.95±1.17 ab	57.48±1.19 abc
F5 C1200	3	51.43±1.04 a	35.18±1.17 ab	56.26±1.19 bcd
F10 C0	3	45.75±1.04 b	30.95±1.17 c	61.02±1.19 a
F10 C300	3	49.38±1.04 a	37.78±1.17 a	53.49±1.19 d
F10 C600	3	50.06±1.04 a	36.24±1.17 ab	53.39±1.19 d
F10 C900	3	49.79±1.04 a	32.86±1.17 bc	58.45±1.19 ab
F10 C1200	3	50.70±1.04 a	35.36±1.17 ab	57.38±1.19 abc

Means followed by the same letter in each column for each factor are not significantly different (P<0.05)

Proximate analysis of tilapia fish as affected by dietary lipid and L-carnitine outlined in Table 4. As described in this table, the lower lipid level (5%) released the higher (P<0.05) moisture and ash and the lower fat content. Also it was found that, as dietary L-carnitine increased fat content of tilapia fish significantly (P<0.05) decreased and protein content significantly increased while moisture and ash content did not significantly affected. The higher protein content and lower fat content were recorded with fish fed the diet contained the higher L-carnitine level (1200 mg/kg diet).

Table (4): Least square means and standard error for the effect of dietary fat and L-carnitine on proximate analysis of tilapia fish.

Item	No.	Moisture %	Protein %	Fat %	Ash %
Fat (F)					
5% (F5)	30	76.18±0.25 a	69.79±0.20	12.92±0.06 b	17.04±0.06 a
10% (F10)	30	74.40±0.25 b	69.23±0.20	14.47±0.06 a	13.83±0.06 b
L-carnitine mg/kg diet (C)					
0 (C0)	6	75.09±0.44	68.21±0.35 b	14.78±0.10 a	15.20±0.11
300 (C300)	6	75.92±0.44	69.06±0.35 ab	14.37±0.10 a	15.80±0.11
600 (C600)	6	75.27±0.44	68.91±0.35 b	13.92±0.10 ab	15.28±0.11
900 (C900)	6	74.68±0.44	69.74±0.35 ab	13.50±0.10 ab	15.31±0.11
1200 (C1200)	6	75.60±0.44	70.31±0.35 a	12.89±0.10 b	15.76±0.11
Fat × L-carnitine					
F5 C0	3	76.12±0.62 ab	67.71±0.50 e	13.57±1.14 d	17.40±0.15 a
F5 C300	3	76.84±0.62 a	69.10±0.50 bcde	13.13±1.14 e	17.82±0.15 a
F5 C600	3	75.87±0.62 ab	69.75±0.50 bc	12.88±1.14 e	16.88±0.15 b
F5 C900	3	75.29±0.62 abc	69.96±0.50 bc	12.68±1.14 e	16.61±0.15 b
F5 C1200	3	75.99±0.62 ab	70.62±0.50 ab	12.68±1.14 e	16.64±0.15 b
F10 C0	3	74.06±0.62 bc	68.71±0.50 cde	15.99±1.14 a	13.00±0.15 e
F10 C300	3	75.01±0.62 abc	69.03±0.50 bcde	15.62±1.14 a	13.70±0.15 d
F10 C600	3	74.67±0.62 bc	68.06±0.50 de	14.95±1.14 b	13.67±0.15 d
F10 C900	3	74.07±0.62 bc	69.52±0.50 bcd	14.31±1.14 c	14.01±0.15 d
F10 C1200	3	75.20±0.62 abc	70.00±0.50 bc	13.11±1.14 e	14.88±0.15 c

Means followed by the same letter in each column for each factor are not significantly different (P<0.05)

With regard to the effect of interaction between dietary lipid and L-carnitine, results of Table 4 show that, within each dietary lipid level, increased L-carnitine level from 0 to 1200 mg/kg diet significantly decreased fat and relatively increased protein content of tilapia fish while moisture and ash had no clear trend. These results similar to those obtained by Jayaprakas and Sambhu (1998), they found that, body protein content increased while lipid decreased (P<0.01) with carnitine administration to pearlspot, *Ertoplus suratensis*. In contrast with our results, some authors found that, dietary L-carnitine did not alter tissue composition of hybrid tilapia (Becker *et al*., 1999) and Nile tilapia, *O. niloticus* (Azab *et al*., 2002).

From these results, it can be concluded that, L-carnitine at concentrations ranged from 300 to 1200 mg/kg diet in dietary lipid 5 or 10% improve growth performance, feed efficiency, carcass traits and increased tissue protein and decreased tissue fat percentages of Nile tilapia.

Second experiment

I-Effect of dietary lipid and L-carnitine on growth performance of common carp:

Table 5 showed the effect of dietary lipid and L-carnitine on growth performance and feed utilization of common carp. Results of this Table indicated that, the increase in dietary lipid from 5 to 10% significantly (P<0.05) improved final BW, WG, SGR and FI but had no significant effect on BL, FCR and PER. Several studies have showed that, providing adequate energy with dietary lipids can minimize the use of more protein as energy source (Ringrose, 1971; Lee and Putnam, 1973 and Watanabe, 1977). Shiau and Huang (1990) reported that, in general the growth of fish increased as the lipid content increased in the diet. However, when lipid content exceeded 15% in the 21% protein diets no further growth enhancement was observed. Takeuchi *et al.*, (1978) also reported that, when the lipid content exceeded 18% in a 35% protein in the diet, no further growth was observed in rainbow trout.

Compared to control group, results of Table 5 show that, all dietary L-carnitine levels (300, 600, 900 and 1200) significantly increased final BW, BL, WG, SGR and FI and significantly improved FCR and PER.

With respect to the interaction between dietary lipid and L-carnitine, results of Table 5 revealed that, within the two dietary lipid tested (5 and 10%) the diets contained the lower L-carnitine level (300 mg/kg diet) released the best growth and feed utilization parameters (BW, BL, WG, SGR, FCR and PER) and this indicated that, dietary L-carnitine at the lower level is effective in improvement of growth and feed utilization in carp fish.

The present experiment revealed that, L-carnitine caused a significant increase in BW, WG and SGR of common carp, *Cyprinus carpio*. Dietary L-carnitine has also been shown to an increase growth rates in juvenile hybrid striped bass fed L-carnitine (Twibell and Brown 2000), rohu (Keshavanath and Renuka, 1998), carp (Focken *et al*., 1997), red sea bream (Chatzifotis *et al*., 1995, 1996 and 1997), European sea bass (Santulli and D'Amelio, 1986) and African catfish (Torreele *et al*., 1993). In contrast, dietary carnitine did not affect WG of channel catfish (Burtle and Liu 1994), rainbow trout (Rodehutscord, 1995), Atlantic Salmon (Ji *et al*., 1996), hybrid striped bass, *Morone chrysops* × *M. saxtilis* (Gaylord and Gatlin III (2000) or European seabass, *Dicentrarchus labrax* (Dias el al., 2001). This variation in the effect of L-carnitine in different species of fish as recorded by several authors is not attributed to the concentration in the L-carnitine in the diet, because low carnitine concentrations (150 mg/ kg diet) caused an increase in the WG of tilapia (Jayaprakas *et al*., 1996), while high concentrations of 3700, 1000 and 230 mg/kg diet had no significant effect on growth rates of Atlantic salmon (Ji *et al*., 1996), channel catfish (Burtle and Liu, 1994) or rainbow trout (Rodehutscord 1995) respectively. Moreover, Harpaz *et al*., (1999) observed that, L-carnitine in the level of 500 mg/kg diet caused a better growth rate in *Ornamental Cichlid*, while L-carnitine at the level of 1000 and 2000 mg/kg diet reduced growth performance.

The obtained results show a significant improvement in FCR in all groups fed L-carnitine. Similarly, several researchers have speculated that, increased growth rates of fish fed supplemental carnitine were due to improved feed conversion via increased fatty acid oxidation and increased utilization of dietary energy as observed by Becker *et al*., (1999) in tilapia, Becker and Focken (1995) and Torreele *et al*., (1993) in carp and African catfish. In contrast, other researchers observed a significant increases in feed consumption and growth rates without significant improvement in feed efficiency (Twibell and Brown 2000) in hybrid striped bass and in red sea bream, *Pagrus major*.

II- Effect of dietary lipid L-carnitine on carcass traits and body composition of common carp:

Results of Table 6 indicated that, increasing dietary lipids from 5 to 10% significantly decreased dress-out percentage but the percentages of flesh and by-products did not significantly changed. Results of the same table showed that, increasing dietary L-carnitine significantly ($P<0.05$) increased flesh and decreased by-products but had no clear trend on dress-out.

With regard to the effect of dietary lipid and L-carnitine on proximate analysis of carp fish, results of Table 7 show that, increasing dietary lipids from 5 to 10% significantly decreased moisture, protein and ash content of whole fish and the opposite trend was observed for fat content.

Table (5): Least square means and standard error for the effect of dietary fat and L-carnitine on growth performance and feed utilization of common carp.

Item	No.	Body weight (g)		Body length (cm)		Weight gain (WG)	Specific growth rate (SGR)	Feed intake (g)/fish (FI)	Feed conversion ratio (FCR)	Protein efficiency ratio (PER)
		Initial	Final	Initial	Final					
Fat (F)										
5% (F5)	150	12.45±0.35	40.74±0.77 b	8.76±0.10	13.30±0.13	28.29±0.18 b	1.31±0.03 b	64.81±0.11 b	2.30±0.02	1.45±0.01
10% (F10)	150	12.30±0.35	44.46±0.77 a	8.69±0.10	13.57±0.13	32.16±0.18 a	1.43±0.03 a	72.06±0.11 a	2.24±0.02	1.49±0.01
L-carnitine mg/kg diet (C)										
0 (C0)	60	12.31±0.55	37.85±1.22 b	8.88±0.17	12.78±0.21 b	25.54±0.28 d	1.25±0.04 c	65.02±0.17 d	2.55±0.04 a	1.31±0.01 b
300 (C300)	60	12.05±0.55	45.96±1.22 a	8.81±0.17	13.82±0.21 a	33.92±0.28 a	1.49±0.04 a	73.76±0.17 a	2.18±0.04 b	1.54±0.01 a
600 (C600)	60	12.40±0.55	42.29±1.22 a	8.82±0.17	13.51±0.21 a	29.90±0.28 c	1.37±0.04 ab	66.93±0.17 c	2.24±0.04 b	1.49±0.01 a
900 (C900)	60	12.69±0.55	42.84±1.22 a	8.72±0.17	13.55±0.21 a	30.15±0.28 c	1.35±0.04 b	66.90±0.17 c	2.22±0.04 b	1.50±0.01 a
1200 (C1200)	60	12.44±0.55	44.07±1.22 a	8.50±0.17	13.54±0.21 a	31.64±0.28 b	1.41±0.04 ab	69.59±0.17 b	2.20±0.04 b	1.52±0.01 a
Fat × L-carnitine										
F5 C0	30	12.51±0.78	36.62±1.72 d	8.90±0.23	12.80±0.30 b	24.11±0.40 g	1.19±0.06 c	63.17±0.25 g	2.62±0.05 a	1.27±0.02 d
F5 C300	30	11.97±0.78	44.07±1.72 abc	8.69±0.23	13.62±0.30 ab	32.10±0.40 cd	1.45±0.06 ab	69.99±0.25 c	2.18±0.05 b	1.53±0.02 a
F5 C600	30	12.94±0.78	41.74±1.72 bcd	9.01±0.23	13.49±0.30 ab	28.80±0.40 e	1.30±0.06 bc	65.66±0.25 f	2.28±0.05 b	1.46±0.02 b
F5 C900	30	12.42±0.78	39.43±1.72 cd	8.69±0.23	13.32±0.30 ab	27.01±0.40 f	1.28±0.06 bc	60.23±0.25 h	2.23±0.05 b	1.49±0.02 ab
F5 C1200	30	12.44±0.78	41.86±1.72 bcd	8.50±0.23	13.29±0.30 ab	29.42±0.40 e	1.35±0.06 abc	65.02±0.25 f	2.21±0.05 b	1.51±0.02 ab
F10 C0	30	12.11±0.78	39.07±1.72 cd	8.86±0.23	12.75±0.30 b	26.96±0.40 f	1.30±0.06 bc	66.86±0.25 e	2.48±0.05 a	1.34±0.02 c
F10 C300	30	12.12±0.78	47.85±1.72 a	8.72±0.23	14.01±0.30 a	35.73±0.40 a	1.53±0.06 a	77.53±0.25 a	2.17±0.05 b	1.54±0.02 a
F10 C600	30	11.85±0.78	42.85±1.72 abc	8.64±0.23	13.52±0.30 ab	31.00±0.40 d	1.43±0.06 ab	68.20±0.25 d	2.20±0.05 b	1.52±0.02 a
F10 C900	30	12.96±0.78	46.25±1.72 ab	8.75±0.23	13.77±0.30 a	24.11±0.40 g	1.19±0.06 c	73.57±0.25 b	2.21±0.05 b	1.51±0.02 ab
F10 C1200	30	12.43±0.78	46.29±1.72 ab	8.50±0.23	13.79±0.30 a	33.86±0.40 cd	1.45±0.06 ab	74.15±0.25 b	2.19±0.05 b	1.52±0.02 a

Means followed by the same letter in each column for each factor are not significantly different (P<0.05)

Compared to control group, all L-carnitine levels significantly increased protein content and decreased fat content of whole fish but had no significant effect on ash content. The low L-carnitine doses (300 or 600 mg/kg diet) did not significantly affected moisture content while the high doses (900 or 1200 mg/kg diet) significantly decreased moisture content of common carp bodies. In the same aspect, dietary L-carnitine reduced tissue lipid concentrations in channel catfish (Burtle and Liu 1994), tilapia (Jayaprakas *et al*., 1996), Atlantic Salmon (Ji *et al*., 1996) and rohu (Keshavanath and Renuka 1998). In contrast, several authors found that, dietary carnitine did not alter tissue composition of rainbow trout (Rodehutscord 1995), hybrid striped bass (Twibell and Brown, 2000) or European seabass, *Dicentrarchus labrax* (Dias *et al*., 2001).

The interaction between dietary lipid and L-carnitine doses had a significant effect on protein and fat and had no clear trend on the moisture and ash content of common carp bodies (Table 7). Within each dietary lipid level (5 or 10%) the graded levels of L-carnitine in the diets significantly increased protein and reduced fat content of common carp bodies.

Table (6): Least square means and standard error for the effect of dietary fat and L-carnitine on carcass analysis of common carp.

Item	No.	Dress-out %	Flesh %	By-products %
Fat (F)				
5% (F5)	30	62.26±0.37 a	45.82±0.19	52.90±0.14
10% (F10)	30	60.15±0.42 b	46.27±0.15	52.16±0.14
L-carnitine mg/kg diet (C)				
0 (C0)	6	61.72±0.58 a	44.13±0.29 c	55.31±0.22 a
300 (C300)	6	62.17±0.58 a	46.07±0.29 b	52.68±0.22 b
600 (C600)	6	60.41±0.58 ab	46.56±0.29 b	51.72±0.22 c
900 (C900)	6	59.83±0.58 b	46.04±0.29 b	51.50±0.22 c
1200 (C1200)	6	61.88±0.58 a	47.43±0.29 a	51.43±0.22 c
Fat × L-carnitine				
F5 C0	3	64.47±0.82 a	41.48±0.41 e	56.98±0.30 a
F5 C300	3	62.99±0.82 abc	44.96±0.41 d	53.47±0.30 b
F5 C600	3	60.06±0.82 d	46.51±0.41 c	51.79±0.30 c
F5 C900	3	60.47±0.82 cd	47.99±0.41 ab	51.17±0.30 c
F5 C1200	3	63.30±0.82 ab	48.15±0.41 a	51.09±0.30 c
F10 C0	3	58.97±0.82 d	46.78±0.41 bc	53.63±0.30 b
F10 C300	3	61.36±0.82 bcd	47.17±0.41 abc	51.89±0.30 c
F10 C600	3	60.77±0.82 bcd	46.61±0.41 c	51.65±0.30 c
F10 C900	3	59.20±0.82 d	44.08±0.41 d	51.83±0.30 c
F10 C1200	3	60.46±0.82 cd	46.72±0.41 bc	51.77±0.30 c

Means followed by the same letter in each column for each factor are not significantly different (P<0.05)

Table (7): Least square means and standard error for the effect of dietary fat and L-carnitine on proximate analysis of carp fish.

Item	No.	Moisture %	Protein %	Fat %	Ash %
Fat (F)					
5% (F5)	30	74.34±0.20 a	59.04±0.30 a	24.91±0.22 b	9.29±0.13 a
10% (F10)	30	72.22±0.20 b	55.77±0.30 b	33.70±0.22 a	7.57±0.13 b
L-carnitine mg/kg diet (C)					
0 (C0)	6	73.44±0.32 a	52.51±0.47 b	33.16±0.35 a	8.29±0.21
300 (C300)	6	73.75±0.32 a	59.39±0.47 a	27.49±0.35 c	8.44±0.21
600 (C600)	6	73.94±0.32 a	58.59±0.47 a	28.06±0.35 c	8.41±0.21
900 (C900)	6	72.35±0.32 b	58.45±0.47 a	29.33±0.35 b	8.43±0.21
1200 (C1200)	6	72.92±0.32 b	58.06±0.47 a	28.48±0.35 b	8.60±0.21
Fat × L-carnitine					
F5 C0	3	74.98±0.46 a	52.18±0.66 f	31.19±0.49 c	8.84±0.29 ab
F5 C300	3	75.40±0.46 a	60.10±0.66 bc	21.71±0.49 f	9.59±0.29 a
F5 C600	3	74.77±0.46 a	60.85±0.66 ab	23.24±0.49 e	8.60±0.29 bc
F5 C900	3	73.09±0.46 bc	59.78±0.66 bc	25.25±0.49 d	9.73±0.29 a
F5 C1200	3	73.46±0.46 b	62.27±0.66 a	23.18±0.49 e	9.71±0.29 a
F10 C0	3	71.91±0.46 cd	52.85±0.66 f	35.14±0.49 a	7.73±0.29 cde
F10 C300	3	72.11±0.46 bcd	58.67±0.66 cd	33.28±0.49 b	7.28±0.29 e
F10 C600	3	73.12±0.46 bc	56.33±0.66 e	32.88±0.49 b	8.22±0.29 bcd
F10 C900	3	71.60±0.46 d	57.13±0.66 de	33.42±0.49 b	7.13±0.29 e
F10 C1200	3	72.37±0.46 bcd	53.85±0.66 f	33.78±0.49 b	7.50±0.29 de

Means followed by the same letter in each column for each factor are not significantly different ($P<0.05$)

Third experiment

During the period between December to March 2004 water temperature in the experimental aquaria ranged between 9.35 and 11.50°C (Table 8). Final BW and BL of tilapia fish did not significantly affected by dietary L-carnitine. Results of cold tolerance challenge showed a significant advantage of all treatment groups which received the diets supplemented with L-carnitine compared to control group (had no L-carnitine supplementation). This was evident by the high survival rate for all L-carnitine supplemented groups. Fish group received L-carnitine at a level of 900 or 1200 mg/kg diet exhibited the best survival rate. Similar results were obtained by Harpaz *et al*., (1999). They found that, addition of L-carnitine to diets of *Pelvicachromis pulcher* at a level of 900 or 1000 mg/kg diet seems to yield the best protection against exposure to cold.

CONCLUSION

From the obtained results, it could be concluded that, L-carnitine at low level (300 mg/kg diet) in dietary lipid 5 or 10% is adequate as supplement for tilapia and common carp. The benefit of L-carnitine came in improvement of growth, feed utilization, increase protein content of fish and reduction of fat deposition in Nile tilapia and common carp as well as its effect in improvement of survival rate of tilapia fry during cold season taking in consideration the economic cost of L-carnitine supplementation to fish diets. Therefore, economic cost of L-carnitine supplementation to fish diets and growth performance and cold tolerance under commercial condition in Egypt needs further studies.

Table (8): Effect of L-carnitine levels in diets on cold tolerance of Nile tilapia, *O. niloticus* fry.

	L-carnitine level				
	Control (0 mg/kg)	300 mg/kg	600 mg/kg	900 mg/kg	1200 mg/kg
Initial	3.63±0.17	3.70±0.17	3.72±0.17	3.71±0.17	3.73±0.17
Final	7.51±0.76	8.01±0.67	8.36±0.67	8.96±0.67	9.01±0.65
Initial	6.58±0.08	6.45±0.08	6.37±0.08	6.52±0.08	6.58±0.08
Final	8.18±1.77	8.01±1.57	7.88±1.57	7.81±1.51	8.21±1.65
Survival rate	64.52±0.26 d	75.81±0.26 c	82.26±0.26 b	87.10±0.26 a	87.32±0.26 a
Temperature °C					
December	11.38	11.20	11.28	11.40	11.45
January	10.42	10.51	11.50	10.91	11.30
February	10.25	9.35	9.40	9.75	9.90
Average °C	10.70	10.40	10.60	10.70	10.90

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تأثير إضافة الكارنيتين إلى العلف على النمو وتكوين الجسم وتحمل البرودة فى أسماك البلطى النيلى والمبروك العادى

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أجريت هذه الدراسة لتقييم تأثير الكارنيتين على كفاءة النمو وكفاءة الغذاء وتكوين الجسم فى أسماك البلطى النيلى والمبروك العادى. ولتقييم تأثير إضافة الكارنيتين إلى العلف على كفاءة النمو وكفاءة الغذاء فى أسماك البلطى النيلى والمبروك العادى أجريت تجربتان عامليتان (٢ x ٥) كما أجريت تجربة ثالثة فى هذه الدراسة وذلك لتقييم تأثير إضافة الكارنيتين إلى العلف على زيادة مقاومة أسماك البلطى على تحمل البرودة. وقد تم إستخدام الكارنيتين بمستويات صفر، ٣٠٠، ٦٠٠ ، ٩٠٠ ، ١٢٠٠ مجم/علف وفى كل مستوى تم إختبار مستويين من الدهن (٥ ، ١٠%) وبذلك تم تكوين ١٠ علائق متساوية فى محتواها من البروتين (٣٠% بروتين) ومتساوية فى محتواها من الطاقة (٣٠٠٠ كيلو كالورى طاقة ممثلة/كجم علف) وقد تم إختبار هذه العلائق (فى مكررين) على أسماك البلطى (التجربة الأولى) وكذلك أسماك المبروك العادى (التجربة الثانية). كما تم تكوين ٥ علائق أخرى متساوية فى محتواها من البروتين (٣٠% بروتين) ومتساوية فى محتواها من الطاقة (٣٠٠٠ كيلو كالورى/كجم علف) ومتساوية كذلك فى محتواها من الدهن (٥%) كما أضيف إليها نفس مستويات الكارنيتين السابقة وذلك لدراسة تأثير إضافة الكارنيتين إلى العلف على زيادة مقاومة أسماك البلطى على تحمل البرودة وقد أستغرقت كل تجربة من التجارب الثلاثة ثلاثة أشهر وكان من أهم النتائج المتحصل عليها مايلى:

أدت إضافة الكارنيتين إلى علائق الأسماك إلى زيادة معنوية فى كل مقاييس النمو (وزن الجسم ، طول الجسم ، الزيادة فى وزن الجسم ، معدل النمو) كما أدت إلى زيادة معدل تحويل الغذاء وكفاءة البروتين فى أسماك البلطى (التجربة الأولى) وقد ظهر نفس الإتجاه فى أسماك المبروك (التجربة الثانية).

مقارنة بمجموعة المقارنة أدت إضافة الكارنيتين إلى علائق أسماك البلطى والمبروك العادى إلى زيادة نسبة الذبيحة واللحم ونقص نسبة المخلفات فى الذبيحة كما أدت زيادة مستوى الكارنيتين إلى نقص معنوى فى محتوى الجسم من الدهن وزيادة محتواه من البروتين فى البلطى والمبروك.

وقد أظهرت نتائج التجربة الثالثة أن إضافة الكارنيتين إلى علائق أسماك البلطى أدت إلى زيادة معنوية فى نسبة حيوية الأسماك أثناء شهور الشتاء الباردة وقد أظهرت الأسماك التى تغذت على العلائق المحتوية على ٩٠٠ أو ١٢٠٠ مجم كارنيتين أعلى نسبة حيوية.

وبصفة عامة يمكن إستنتاج أن إضافة الكارنيتين بمستويات مرتفعة أو منخفضة (من ٣٠٠ إلى ١٢٠٠ مجم/كجم علف) قد أدى إلى تحسين كفاءة النمو والإستفادة من الغذاء وكذلك إنخفاض فى محتوى الجسم من الدهن وزيادة محتواه من البروتين وذلك فى أسماك البلطى والمبروك كما أدت إضافة الكارنيتين إلى علائق البلطى إلى زيادة نسبة الحيوية لأسماك البلطى النيلى فى درجات الحرارة المنخفضة اثناء شهور الشتاء.