EFFECT OF FEED SUPPLEMENTATION WITH L-CARNITINE ON GROWTH PERFORMANCE AND BODY COMPOSITION OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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ABSTRACT

The effect of dietary L-carnitine and lipid on growth performance and body composition of Nile tilapia, *Oreochromis niloticus* was evaluated in this study. A 4×2 factorial design was utilized to test the efficiency of L-carnitine at a rate of 0, 300, 600 and 900 mg/kg diet at each of two lipid levels (10 and 15%). Therefore, eight isonitrogenous (30%) and isocaloric (3000 kcal ME /kg diet) were formulated and fed twice daily to two replicate groups of Nile tilapia. Fish of all groups were initially weighed and then every 2 weeks for 8 weeks. It was found that body weight (BW) was significantly increased in groups fed dietary L-carnitine 900 mg/kg at dietary lipid levels 10 and 15% compared with control groups. Specific growth rate (SGR) was significantly increased in groups fed dietary L-carnitine 600, 900 mg/kg at both dietary levels. Weight gain (WG) was significantly increased in groups fed L-carnitine 300, 600 and 900 mg/kg diet at dietary lipid level 10 and 15% compared with control groups. Feed conversion ratio (FCR) was significantly decreased in all groups supplemented with L-carnitine. Tissue protein percent was significantly increased in groups supplemented with L-carnitine at dietary lipid 15%. The diet contained 15% lipids caused significantly increase in SGR and WG compared with dietary lipid 10%. Results of this study revealed that using L-carnitine in concentration of 600 and 900 mg/kg diet at 15% dietary lipid could improve growth rate and increase tissue protein taking in consideration the economic cost of L-carnitine supplementation to fish diets.

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)***. The effect of dietary L-carnitine on growth rate and body composition has been reported in several species of fish with different results. Accordingly, this study aimed to evaluate the effect of dietary L-carnitine and lipid level on growth performance and body composition of Nile tilapia (*O. niloticus*).

MATERIALS AND METHODS

Fish: Nile tilapia, *O. niloticus* fingerlings were obtained from El-Manzalah hatchery, Al-Dakahlya 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 16 tanks. Each fish was taken out by a net and weighed to the nearest 0.01g then transferred randomly to the experimental aquaria. Each

aquarium was randomly stocked with fifteen fish .

Fish grouping: Fish were grouped into control group (0 L- carnitine) and three dietary carnitine (Arab Company For Pharmaceuticals & Medical Plants – MEPACO - Egypt) at concentrations of 300, 600, 900 mg/kg diet in two dietary lipid levels, 10 and 15%. All these fish groups were arranged in two replicates. The fish were divided into the following groups:

Group 1	0 mg L-carn./kg diet	10% dietary lipid
Group 2	0 mg L-carn./kg diet	15% dietary lipid
Group 3	300 mg L-carn./kg diet	10% dietary lipid
Group 4	300 mg L-carn./kg diet	15% dietary lipid
Group 5	600 mg L-carn./kg diet	10% dietary lipid
Group 6	600 mg L-carn./kg diet	15% dietary lipid
Group 7	900 mg L-carn./kg diet	10% dietary lipid
Group 8	900 mg L-carn./kg diet	15% dietary lipid

Aquarium system: Sixteen glass aquaria (100×40×50 cm) used in the present study. Each aquarium was filtered with bio-mechanical filter. Air was bubbled with 2 air stones connected to an air pump in each aquarium. And water temperature was kept at 28± 1°C.

Feed and feeding: Composition and proximate analysis of diets used in the two experiments are presented in Table 1. Fish were given the diets at a daily rate 5% of total biomass. Fish were fed twice daily at 9:00 am and 3:00 pm. All fish groups were initially and bi-weekly weighed for 8 weeks. The following parameters were recorded: live body weight, weight gain (WG) = (final weight - initial weight),

Specific growth rate (SGR) =

$$\frac{LnW2 - LnW1}{t} \times 100$$

where: Ln = the natural log, W_1= First fish weight, W_2= the following fish weight in " grams", t = Period in days.

Feed conversion ratio (FCR) = feed intake (g) / weight gain(g). At the end of experiment all fish groups were sampled for proximate analysis of body composition and gonadal weight.

Statistical analysis: The statistical analysis of data was 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-canitin level; $(\alpha B)_{jk}$ = the effect of interaction between jth lipid level and kth L-carnitine level and E_{ijkl} = random error assumed to be independently and randomly distributed (0, δ^2 e).

RESULTS

I-Effect of L-carnitine on growth performance:

Table 2 showed the effect of L-carnitine on BW, it revealed that a significant increase in BW was detected in fish groups fed L-carnitine at 900 mg/kg diet with dietary lipid levels of 10% and 15% after 2 and 6 weeks compared to control groups. However at 4 and 8 weeks no significant differences were detected. Table 3 revealed that SGR was significantly increased in groups fed L-carnitine 600 and 900 mg/kg diet in both two different dietary lipid levels. During the whole experimental period (0-8 weeks) WG was significantly increased in groups fed L-carnitine 300, 600 and 900 mg/kg diet at dietary lipid levels of 10% and 15% compared to control groups (Table 4), and fish group fed the diet contained 900 mg L-carnitine with 15% dietary lipid gained the higher WG whereas the fish group fed experimental diet without supplementation of L-carnitine (control) and 10% dietary lipid level gained the poorest one.

Table 5 showed that, during the whole experimental period (0-8 weeks) FCR was significantly improved in fish groups fed L-carnitine 300, 600, 900 mg/kg diet at dietary level 10 and 15% compared with control groups. Effect of dietary lipids on body growth revealed that, 15% dietary lipid caused non significant increase in the BW (Table 2). However, SGR and WG were significantly increased (Tables 3 and 4,).

II- Effect of L-carnitine on body composition:

Table 6 showed that L-carnitine caused no significant changes in moisture, fat and ash percentage, whereas,

protein percentage was significantly increased in groups fed L-carnitine 300, 600, 900 mg/kg diet at dietary lipid 15%.

III-Effect of L-carnitine on weight of gonads:

Table 7 showed that L-carnitine caused no significant changes in weight of gonads in both male and female groups compared with control groups.

DISCUSSION

The present study revealed that L-carnitine caused a significant increase in BW, WG SGR of Nile tilapia, *O. niloticus*. 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),*** tilapia ***(Jayaprakas et al. 1996),*** 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 ***(Rodehuts-cord, 1995)*** or Atlantic Salmon ***(Ji et al., 1996).*** 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 fish, 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 Fochen (1995)*** and ***Torreele et al., (1993)*** in carp and African catfish. In contrast, other researchers observed that 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*, ***Chatzifotis et al. (1996).***

No significant effect of dietary L- carnitine was detected on tissue composition in the present study at low dietary fat level, while in high level of dietary fat, carnitine caused a significant increase in tissue protein. In the same aspect, several authors found that, dietary carnitine did not alter tissue composition of hybrid striped bass ***(Twibell and Brown 2000)***, rainbow trout ***(Rodehutscord 1995),*** or hybrid tilapia ***(Becker et al., 1999).*** In contrast, dietary carnitine reduced tissue lipid concentrations in rohu ***(Keshavanath and Renuka 1998),*** tilapia ***(Jayaporakas et al., 1996),*** channel catfish ***(Burtle and Liu 1994)*** and Atlantic Salmon ***(Ji et al., 1996).*** The observation that L-carnitine can improve growth rate without effect on lipid content of the carcass would suggest that it is involved in the metabolism of other compounds besides lipids. Indeed, such several functions have been described in mammals including man, but in most cases the mechanisms of L-carnitine action are not known ***(Borum 1987)***. Moreover, ***Focken et al., (1997)*** reported that L-carnitine treatment are associated with a decrease in energy expenditure and the highest level of supplementation caused an increase in energy retention. Tissue protein in fish fed dietary carnitine at high level of dietary fat was increased in the present study. Similarly, ***Burtle and Liu (1994)*** and ***Ji et al. (1996)*** observed that a significant increases in tissue protein in both channel catfish and Atlantic salmon respectively. Moreover, 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).***

The present study revealed that dietary lipid at level of 15% caused a significant increase in SGR and WG. Similarly, ***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.

From these results, it can be concluded that L-carnitine at concentrations 600 and 900 mg/kg diet in dietary lipid 15% would improve growth performance, feed efficiency and tissue protein percent, 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 under commercial condition in Egypt needs further studies.

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

Ingredient	10% lipid				15% 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	6.0				14.5			
Corn oil	7.0				10.0			
Vit.&Min. mix.[1]	4.0				4.0			
Sum	100.0				100.0			
Proximate analysis								
Protein	30.12				30.23			
Lipid	10.11				15.27			
ME (Kcal/kg diet)[2]	301.9				302.76			
P/E ratio	99.78				99.85			
L-carnitine mg/kg	0	300	600	900	0	300	600	900

[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.

[3] Based on kilocalorie values of 4.50 g^{-1} protein, 8.51 g^{-1} lipid and 3.49 g^{-1} NFE (Jauncy, 1982).

Table (2): Least square means and standard error for the effect of dietary fat and l-carnitine on body weight (g) of Nile tilapia, *O. niloticus*.

Fat	L-carnitine	No.+	At start	2 weeks	4 weeks	6 weeks	8 weeks
	0	30	12.55±0.52 a	14.96±0.68 b	20.84±1.02 a	26.17±1.25 c	32.24±1.58 a
10%	300 mg	30	12.58±0.52 a	14.94±0.68 b	21.02±1.02 a	26.06±1.25 c	32.99±1.58 a
	600 mg	30	12.44±0.52 a	14.88±0.68 b	21.16±1.02 a	27.69±1.25 bc	34.12±1.58 a
	900 mg	30	12.49±0.52 a	17.59±0.68 a	22.37±1.02 a	30.64±1.25 ab	35.05±1.58 a
	0	30	12.48±0.52 a	15.20±0.68 b	20.90±1.02 a	28.43±1.25 abc	33.82±1.58 a
15%	300 mg	30	12.47±0.52 a	15.39±0.68 b	22.04±1.02 a	28.46±1.25 abc	33.99±1.58 a
	600 mg	30	12.46±0.52 a	15.52±0.68 b	21.81±1.02 a	29.22±1.25 abc	34.47±1.58 a
	900 mg	30	12.48±0.52 a	18.15±0.68 a	22.86±1.02 a	31.79±1.25 a	37.13±1.58 a

Table (3): Least square means and standard error for the effect of dietary fat and l-carnitine on specific growth rate of Nile tilapia, *O. niloticus*.

Fat	L-carnitine	No. +	0-2 weeks	2-4 weeks	4-6 weeks	6-8 weeks	0-8 weeks
	0	2	1.21±0.31 bc	2.21±0.03 cd	1.53±0.03 e	1.39±0.02 c	1.58±0.01 e
10%	300 mg	2	1.13±0.31 c	2.27±0.03 bc	1.44±0.03 f	1.59±0.02 a	1.61±0.01 e
	600 mg	2	1.22±0.31 bc	2.29±0.03 abc	1.74±0.03 d	1.52±0.02 b	1.69±0.01 c
	900 mg	2	2.32±0.31 ab	1.58±0.03 e	2.08±0.03 b	0.91±0.02 g	1.73±0.01 b
	0	2	2.25±0.31 abc	2.14±0.03 d	2.07±0.03 b	1.13±0.02 e	1.65±0.01 d
15%	300 mg	2	1.43±0.31 abc	2.40±0.03 a	1.66±0.03 d	1.24±0.02 d	1.68±0.01 cd
	600 mg	2	1.49±0.31 abc	2.34±0.03 ab	1.87±0.03 c	1.11±0.02 e	1.70±0.01 bc
	900 mg	2	2.53±0.31 a	1.53±0.03 e	2.20±0.03 a	1.03±0.02 f	1.82±0.01 a

Means with the same letters in each column are not significantly different (P<0.05).
+ Number of replicates

Table (4): Least square means and standard error for the effect of dietary fat and l-carnitine on weight gain (g)/pond of Nile tilapia, *O. niloticus*.

Fat	L-carnitine	No. +	0-2 weeks	2-4 weeks	4-6 weeks	6-8 weeks	0-8 weeks
	0	2	36.15±0.51 e	88.21±0.90 c	79.90±1.21 e	91.20±1.01 b	296.15±0.55 g
10%	300 mg	2	35.40±0.51 e	91.23±0.90 b	75.55±1.21 e	103.80±1.01 a	306.00±0.55 f
	600 mg	2	36.70±0.51 e	94.30±0.90 b	97.95±1.21 d	96.60±1.01 ab	327.50±0.55 cd
	900 mg	2	76.50±0.51 b	71.70±0.90 d	124.02±1.21 b	66.10±1.01 d	338.40±0.55 b
	0	2	40.80±0.51 d	85.52±0.90 c	112.95±1.21 c	80.95±1.01 c	320.25±0.55 e
15%	300 mg	2	43.70±0.51 c	99.75±0.90 a	96.30±1.21 d	82.90±1.01 c	322.75±0.55 d
	600 mg	2	45.90±0.51 c	94.35±0.90 b	111.15±1.21 c	78.80±1.01 c	330.80±0.55 c
	900 mg	2	85.02±0.51 a	70.70±0.90 d	133.92±1.21 a	80.20±1.01 c	369.80±0.55 a

Table (5): Least square means and standard error for the effect of dietary fat and l-carnitine on feed conversion ratio of Nile tilapia, *O. niloticus*.

Fat	L-carnitine	No. +	0-2 weeks	2-4 weeks	4-6 weeks	6-8 weeks	0-8 weeks
	0	2	3.82±0.05 a	1.91±0.02 d	2.95±0.13 ab	3.23±0.04 e	2.83±0.01 a
10%	300 mg	2	3.98±0.05 a	1.84±0.02 e	3.14±0.13 a	3.03±0.04 f	2.75±0.01 c
	600 mg	2	3.83±0.05 a	1.83±0.02 ef	2.49±0.13 c	2.99±0.04 f	2.61±0.01 e
	900 mg	2	1.84±0.05 d	2.76±0.02 a	2.03±0.13 d	3.22±0.04 a	2.77±0.01 b
	0	2	3.46±0.05 b	2.00±0.02 c	2.08±0.13 d	3.95±0.04 d	2.71±0.01 d
15%	300 mg	2	3.23±0.05 c	1.73±0.02 g	2.57±0.13 bc	3.86±0.04 d	2.73±0.01 c
	600 mg	2	3.07±0.05 c	1.77±0.02 fg	2.56±0.13 bc	4.16±0.04 c	2.69±0.01 d
	900 mg	2	1.66±0.05 e	2.46±0.02 b	1.93±0.13 d	4.45±0.04 b	2.60±0.01 e

Means with the same letters in each column are not significantly different (P<0.05) + Number of replicates

Table (6): Least square means and standard error for the effect of dietary fat and l-carnitine on chemical analysis of Nile tilapia, *O. niloticus*.

Fat	L-carnitine	No. +	Moisture	Protein	Fat	Ash
	0	3	78.24±4.18 a	50.83±2.26 b	25.57±1.12 a	14.27±0.77 a
10%	300 mg	3	79.43±4.18 a	52.21±2.26 b	25.63±1.12 a	14.80±0.77 a
	600 mg	3	76.93±4.18 a	52.45±2.26 b	24.67±1.12 a	16.19±0.77 a
	900 mg	3	79.65±4.18 a	52.09±2.26 b	26.00±1.12 a	14.92±0.77 a
	0	3	79.95±4.18 a	50.25±2.26 b	28.28±1.12 a	15.05±0.77 a
15%	300 mg	3	79.99±4.18 a	55.10±2.26 a	26.16±1.12 a	14.90±0.77 a
	600 mg	3	79.25±4.18 a	56.64±2.26 a	26.98±1.12 a	13.98±0.77 a
	900 mg	3	79.89±4.18 a	56.60±2.26 a	26.53±1.12 a	13.99±0.77 a

Table (7): Least square means and standard error for the effect of dietary fat and l-carnitine on weight of gonads (g) of Nile tilapia, *O. niloticus*.

Fat	L-carnitine	No. +	Males	Females
10%	0	3	1.63±0.34 a	4.96±1.27 a
	300 mg	3	1.41±0.34 a	3.56±1.27 a
	600 mg	3	1.21±0.34 a	4.05±1.27 a
	900 mg	3	1.52±0.34 a	6.14±1.27 a
15%	0	3	1.85±0.34 a	4.39±1.27 a
	300 mg	3	1.31±0.34 a	4.79±1.27 a
	600 mg	3	1.41±0.34 a	3.98±1.27 a
	900 mg	3	1.88±0.34 a	4.79±1.27 a

Means with the same letters are not significantly different (P<0.05) + Number of fish

تأثير إضافة الكارنيتين إلى العلف على كفاءة النمو وتكوين الجسم فى أسماك البلطى النيلى

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أجريت هذه الدراسة لتقييم تأثير الكارنيتين على كفاءة النمو وتكوين الجسم فى أسماك البلطى النيلى وقد تم إضافة الكارنيتين إلى الأعلاف بثلاثة مستويات هى ٣٠٠، ٦٠٠ ، ٩٠٠ ملليجرام/كجم علف مع استخدام نوعين من الأعلاف: الأول يحتوى على ١٠% دهن والثانى على ١٥% دهن. تم وزن الأسماك فى بداية التجربة ثم كل أسبوعين لمدة ٨ أسابيع ثم تم حساب معدل النمو النوعى والزيادة فى الوزن ومعدل تحويل الغذاء. وفى نهاية التجربة تم أخذ عينات من كل مجموعة لتحليل مكونات جسم الأسماك وقد أظهرت النتائج مايأتى:

زاد وزن الجسم زيادة معنوية فى المجموعات المضاف إليها الكارنيتين بنسبة ٩٠٠ ملليجرام/كيلو علف مقارنة بالمجموعات الضابطة بينما زاد معدل النمو النوعى فى المجموعات المضاف إليها الكارنيتين بنسبة ٦٠٠ ، ٩٠٠ ملليجرام/ كيلو علف. أما الزيادة فى الوزن فقد ازدادت فى المجموعات المضاف إليها الكارنيتين بنسبة ٣٠٠ ، ٦٠٠، ٩٠٠ ملليجرام/ كجم علف. تحسنت قيم معدل تحويل الغذاء مع كل المجموعات المضاف إليها الكارنيتين بالمقارنة بالمجوعات الضابطة. أما بالنسبة لتأثير الكارنيتين على تكوين الجسم فقد ازدادت نسبة البروتين فى المجموعات المضاف إليها الكارنيتين مكع مستوى الدهون ١٥% وذلك بالمقارنة بالمجموعات الضابطة. أدت زيادة مستوى الدهون فى الأعلاف إلى ١٥% إلى زيادة فى معدل النمو النوعى والزيادة فى الوزن بالمقارنة بالمجموعات الضابطة. ومن هذه النتائج يمكن استخلاص أن إضافة الكارنيتين إلى أعلاف أسماك البلطى النيلى بمعدل ٦٠٠، ٩٠٠ ملليجرام/كيلو علف قد يؤدى إلى زيادة وزن الجسم ومعدل النمو مع الأخذ فى الاعتبار التكلفة الاقتصادية لإستخدام الكارنيتين على مستوى الإنتاج التجارى فى المزارع وهذا يحتاج إلى أبحاث أخرى.