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Effect of L-Carnitine and Amino Acids on Growth and Feed Utilization of Nile Tilapia, *Oreochromis niloticus*

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Abstract: This experiment aimed to evaluate the effect of dietary supplementation of L-carnitine and amino acids (methionine+lysine) for Nile tilapia (*Oreochromis niloticus*), the basal diet (300 g protein and 19 MJ Gross energy kg^{-1} dry matter) was formulated and served as control (D1). The based diet was supplemented with 300 mg L-carnitine (D2), 5g methionine+10g lysine (D3), 7g methionine+15g lysine (D4), 5g methionine+10g lysine+300 mg L-carnitine (D5), or 7g methionine+15g lysine+300 mg L-carnitine (D6). A total number of 300 apparent healthy Nile tilapia fry (1.63±0.07g) were randomly distributed into 18 rectangular aquaria (160 liter for each) and each aquarium holding 25 fry was then randomly assigned to one of three replicates of six diets. Fish group fed D6 retained the highest significant body weight (BW), body length (BL), weight gain (WG), specific growth rate (SGR) and significantly ($P<0.05$) improved feed intake, feed conversion ratio (FCR) while protein efficiency ratio was not significantly affected. Also, fish group fed D6 gained the highest significant ($P<0.05$) protein and lowest insignificant ($P>0.05$) dry matter, fat and ash while fish fed the control diet (D1) released the lowest significant protein and the highest insignificant fat content. All treatments significantly altered protein content while dry matter, fat and ash content of fish flesh was not significantly affected. It is concluded that, dietary supplementation of *O. niloticus* with 7g methionine+15 g lysine+300 mg L-carnitine kg^{-1} significantly improved growth and feed utilization, increases tissue protein and decrease tissue fat of Nile tilapia flesh.

Key words: L-carnitine · Oreochromis niloticus · Growth performance · Feed utilization

INTRODUCTION

As the largest aquaculture industry of finfish in Egypt, the improvement of growth performance for Nile tilapia (*Oreochromis niloticus)* is highly concerned by the feed companies and nutritional scientists [1-4]. 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 [5, 6]. Tilapia has become one of the most important fish species for fresh water culture [7]. L-Carnitine is synthesized from the essential amino acids lysine and methionine with the assistance of vitamin C and other secondary compounds produced in the body [8]. As for carnitine biosynthesis, it was shown that the methyl groups of carnitine come from methionine (but not from choline) and that g-butyrobetaine (but not g-aminobutyric acid or g-dimethylaminobutyrate) is converted to carnitine. It was also shown that lysine is converted to carnitine with 6-N-trimethyllysine as an intermediate [9]. The endogenous formation of carnitine in vertebrates occurs primarily in the liver, as well as in the kidneys and the brain, as a result of the occurrence of the required enzyme 4-butyrobetaine hydroxylase. The process requires two essential amino acids (lysine and methionine), iron (Fe^{2+}), vitamin C, vitamin B_6 and niacin in the form of nicotinamide adenine dinucleotide (NAD). One of the earliest symptoms of vitamin C deficiency is fatigue, thought to be related to decreased synthesis of L-carnitine [10].

The growth-promoting effects of dietary L-carnitine have generally been explained by increasing utilization of dietary energy resulting from increased oxidation of fatty acids [11]. The improved energy production in

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mitochondria through β-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 [12]. The ability of L-carnitine to increase growth rate and reduce tissue lipid concentrations has been evaluated in several fish species with conflicting results. Dietary L-carnitine supplementation showed improved growth performances and feed utilization in red sea bream, *Pagrus major* [13]; common carp, *Cyprinus carpio* [14]; hybrid striped bass, *Morone saxatilis*×*Morone chrysops* [15], Nile tilapia [16]; beluga, *Huso huso* [17]. It has also been found that there is an increased tolerance of ammonia that cannot be directly explained by the effect of L-carnitine [18]. It also increases the rate of protein synthesis and enhancing the generation of metabolic energy [19]. This could stimulate some specific cell functions and may influence several biochemical and physiological process, i.e., cell protection against xenobiotics [12]. Also, supplementation of dietary l-carnitine significantly improved survival of Nile tilapia during overwintering [20].

The objective of this experiment was to evaluate the effects of dietary L-carnitine or its precursor (methionine+lysine) or the combination of L-carnitine and its precursors on growth performance, feed utilization and proximate composition of Nile tilapia, *O. niloticus*.

MATERIALS AND METHODS

Experimental Diets: This study was carried out at Fish Nutrition Lab, Faculty of Agriculture, Benha University, Egypt. The basal diet (300 g protein and 19.5 MJ gross energy kg^{-1} dry matter) was formulated and divided into 6 parts (diets). L-carnitine and amino acids (L-lysine sulphate and methionine hydroxy analog-Ca (MHA-Ca) were obtained from Arab Company For Pharmaceutical & Medical Plants - MEPACO – Egypt, L-lysine sulphate and MHA-Ca with markedly lower water solubility than their traditional products were selected, which in some extent diminished the influence of amino acid leaching on fish growth and achieved significant difference in results [21]. L-carnitine and amino acids were supplemented to the basal diets as follows:

D1: basal diet (kept as control group).
D2: basal diet supplemented with 300 mg L-carnitine kg^{-1} diet.
D3: basal diet incorporated with 5 g methionine+10 g lysine kg^{-1} diet.
D4: basal diet supplemented with 7 g methionine+15 g lysine kg^{-1} diet.
D5: basal diet supplemented with 5 g methionine+10 g lysine+300 mg L-carnitine kg^{-1} diet.
D6: basal diet supplemented with 7 g methionine+15 g lysine+300 mg L-carnitine kg^{-1} diet.

All dry ingredients of the fish meal, soybean meal, yellow corn, wheat bran, ascorbic acid and vitamin and mineral mixture were blended for 5 min and thoroughly mixed with corn oil. The ingredients were mixed well and made into dry pellets using a laboratory pellet mill (California Pellet Mill, San Francisco, CA, USA). The pellets (1-mm die) were dried for 4 h at 60°C and stored at -20°C until use.

Experimental Fish and Facilities: Nile tilapia, *Oreochromis niloticus* fry were obtained from Abbassa hatchery, Sharkia Governorate, Egypt. Fingerlings were transferred in a 50-liter plastic bags filled with water and oxygen to Fish Lab. Prior to the beginning of the experiment, fish were acclimatized to the experimental conditions and fed commercial diet (300 g protein kg^{-1}) twice daily to apparent satiation by hand for 15 days. After acclimatization, fry (1.63±0.07g) were stocked into eighteen glass aquaria (160 L). Three replicate aquaria were randomly assigned to each treatment and each aquarium was stocked with 25 fish. The glass aquaria were supplied with de-chlorinated tap water and were continuously supplied with compressed air. About one-third of water volume in each aquarium was daily replaced by new aerated fresh water after cleaning and removing the accumulated excreta. A photoperiod of 12 h light, 12h dark (08.00 to 20.00 h) was used. Fluorescent ceiling lights has supplied the illumination. Fish were fed their respective diets by hand one of six experimental diets for 84 days. Fish were given the diets at a daily rate 5% of total biomass. The daily ration was divided into two equal amounts and offered two times a day (9:30 and 14.00). All fish in each aquarium were weighed biweekly and the amount of daily allowance feed was adjusted accordingly.

Water temperature, dissolved oxygen, pH and total ammonia were monitored during the study, to maintain water quality at optimal range for common carp. Water temperature was recorded daily at 13.00 using a mercuric thermometer suspended at 30 cm depth. Dissolved oxygen (DO) was measured daily at 08.00 using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA) and pH was recorded daily at 09.00 h using a pH meter (Orion pH meter, Abilene, Texas, USA). Total ammonia was measured two times a week according to APHA [22]. During the period of the feeding trial, the water-quality parameters were averaged (±SD):

Table 1: Formulation and proximate composition of the experiment diets

	Experimental diets					
Ingredients	D1	D2	D3	D4	D5	D6
Fish meal	18	18	18	18	18	18
Soybean meal	35	35	35	35	35	35
Yellow corn	32	32	32	32	32	32
Wheat bran	8.3	8.0	6.8	6.1	6.5	5.8
Corn oil	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin &Minerals[1]	2.4	2.4	2.4	2.4	2.4	2.4
Ascorbic acid	0.3	0.3	0.3	0.3	0.3	0.3
L-carnitine (g kg-1)	0.0	0.3	0.0	0.0	0.3	0.3
lysine	0.0	0.0	1.0	1.5	1.0	1.5
methionine	0.0	0.0	0.5	0.7	0.5	0.7
Proximate analysis (% Dry matter basis)						
Dry matter	91.00	91.22	90.11	89.98	90.92	90.67
Crude protein	30.18	30.57	30.19	30.45	30.15	30.38
Ether extract	6.55	6.44	6.55	6.34	6.44	6.33
Ash	5.64	5.71	5.81	5.52	5.61	5.66
Total carbohydrate[2]	57.63	57.28	57.45	57.69	57.80	57.63
Gross energy (Mj kg^{-1})[3]	19.60	19.59	19.58	19.59	19.55	19.56

[1]Vitamin and mineral mixture kg^{-1} of mixture contains: 4800 I.U. Vit A, 2400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B_{12}, 4.0 g Vit B2, 6 g Vit B6, 4.0 g Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 65mg; manganese sulfate ($MnSO_4$, 36% Mn), 89 mg; zinc sulfate ($ZnSO_4.7H_2O$, 40% Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I),

[2]Total carbohydrate =100-(CP + EE+ Ash).

[3] Gross energy calculated using gross calorific values of 0.2363, 0.3952 and 0.1715 MJ/g for protein, fat and carbohydrate, respectively according to Brett [28].

Water temperature was 28.23±0.7°C: dissolved oxygen, 6.3±0.5 mg/L: pH 8.35±0.2 and total ammonia, 0.17±0.10 mg/L. All tested water quality criteria were suitable and within the acceptable limits for rearing *O. niloticus* [23].

Growth Indices: Records of all fish body weight length individually measured for each aquarium at the initiation and the termination of the feeding trail. Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated using the following equations:

WG (g/fish)=*FBW–IBW*; SGR% = [*lnFBW* - *ln* IBW]/t×100, where FBW is final body weight (g); IBW is initial body weight (g); ln= natural logarithmic; t=time in days. FCR=*FI /WG*, where FI is feed intake (g); PER=*WG*/*protein intake* (g).

Proximate Analysis of Fish and Experimental Diets: At the experiment termination, five fish were randomly sampled from each aquarium and exposed to the proximate analysis of whole fish body according to the methods of AOAC [24]. Fish and diet samples were oven-dried 105°C for 24 h, ground and stored at -20°C for subsequent analysis. Dry matter was determined after drying the samples in an oven (105°C) for 24 h. Ash by incineration at 550°C for 12 h. Crude protein was determined by micro-Kjeldhal method, N×6.25 (using Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by Soxhlet extraction with diethyl ether (40–60°C). Crude fiber content of diets was determined using the method of Van Soest *et al.,* [25]. Nitrogen-free extract was computed by taking the sum of values for crude protein, crude lipid, crude fiber and ash then subtracting this sum from 100.

Statistical Analysis: Statistical analysis of the obtained data was analyzed according to SAS [26]. Differences between means were tested for significance according to Duncan's multiple rang test as described by Duncan [27].

RESULTS

Biological Performance of Fish: The growth performance of Nile tilapia fed the experimental diets were presented in Table 2. The final mean weight, final length, WG and SGR of the fish fed diet with supplemental L-carnitine or methionine+lysine were significantly higher in comparison with the reference group. Average initial body

Table 2: Growth indices of *O. niloticus* fed diets supplemented with L-carnitine and amino acids

	BW (g)		BL(cm)			
Diets	Initial	Final	Initial	Final	WG (g/fish)	SGR(%)
D1	1.63	19.28^{c}	4.41	10.24^{c}	18.05^{b}	2.76^{b}
D2	1.62	22.92^{b}	4.49	10.64^{bc}	21.30^{a}	2.93^{a}
D3	1.60	22.14^{b}	4.40	10.57^{bc}	20.54^{ab}	2.91^{a}
D4	1.61	24.41^{b}	4.49	11.30^{b}	22.81^{ab}	3.02^{ab}
D5	1.64	24.40^{b}	4.41	11.04^{b}	22.75^{a}	2.99^{a}
D6	1.66	27.94^{a}	4.47	11.54^{a}	26.28^{a}	3.12^{a}
Standard error	0.07	0.94	0.07	0.16	1.17	0.04

Means followed by the different letters in each column are significantly (P<0.05) different.

Table 3: Feed Intake (FI) Feed conversion ratio (FCR) and protein efficiency ratio (PER) of *O. niloticus* fed diets supplemented with L-carnitine and amino acids

Diets	FI	FCR	PER
D1	47.15^{b}	1.73^{d}	1.67
D2	48.66^{b}	1.54^{c}	1.70
D3	49.95^{b}	1.62^{c}	1.71
D4	49.15^{b}	1.47^{b}	1.74
D5	51.15^{a}	1.33^{b}	1.73
D6	51.50^{a}	1.21^{b}	1.75
Standard error	0.06	0.04	0.02

Means followed by the different letters in each column are significantly (P<0.05) different

Table 4: Proximate analysis of *O. niloticus* fed diets supplemented with L-carnitine and amino acids

Diets	Dry matter %	Protein %	Fat %	Ash %
D1	32.07	62.04^{b}	26.62	10.72
D2	32.40	62.41^{b}	26.04	10.78
D3	32.78	62.84^{b}	26.18	10.06
D4	31.88	63.96^{b}	25.65	9.28
D5	31.66	65.19^{a}	25.20	9.16
D6	31.31	67.34^{a}	25.12	9.14
Standard error	0.33	0.63	0.57	0.77

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

weight (IBW) and initial body length (IBL) of Nile tilapia *O. niloticus* ranged from 1.60 to 1.66 g for BW and 4.40 to 4.49 cm for BL and the differences among fish groups for IBW and IBL were insignificant (Table 2). After 84 days from the experiment start, BW ranged between 19.28 and 27.94 and BL ranged between 10.24 to 11.54 cm. Weight gain (WG) values ranged from 18.05 g to 26.28 g/fish and specific growth rate ranged between 2.76 and 3.12% day^{-1} and the differences in BW, BL, WG and SGR among the different treatments were significant (P<0.05). Compared to control group (D1), all treatments showed a significant improvement of growth indices and fish group fed D6(7 g methionine+15 g lysine+300 mg L-carnitine kg^{-1} dry matter) resulted the highest BW (27.94 g), BL (11.54 cm), WG (26.28 g/fish) and SGR 3.12%) while fish group fed the control diet (D1) showed the significant (P<0.05) lowest BW (19.28 g), BL (10.24 cm), WG (19.28 g) and SGR (2.76%).

Feed intake (FI) for the different fish groups ranged from 47.15 to 51.51 g and lie in two clusters. The first one included D1, D2, D3 and D4 and the second cluster included D5 and D6. The differences among the two clusters was significant (Table 3) while the differences among means within each cluster were insignificant. Compared to control group all treatments significantly (P<0.05) improved feed conversion ratio (FCR) and fish group fed D6 showed the best FCR. The obtained results (Table 3) also showed that L-carnitine or its precursor did not significantly altered PER.

Proximate Composition of Fish: Dry matter, protein, fat and ash content of whole body ranged between 31.10-32.78 for dry matter, 62.04-66.34 for protein, 4.12-26.62% for fat and 9.14-10.78% for ash (Table 4). The obtained results showed that, the combination of L-carnitine and amino acids (methionine+lysine) in low or high levels

(D5 and D6) released the highest significant protein content of whole body while dry matter, fat and ash content did not significantly affected by dietary L-carnitine or amino acids (methionine+lysine).

DISCUSSION

Biological Performance of Fish: Dietary supplementation of L-carnitine or its precursors (methionine+lysine) in low or high levels significantly improved growth indices (BW, BL, WG and SGR) specially fish group fed D6 indicating that the dietary methionine and lysine used in vivo to synthenyze L-carnitine, therefore, the dietary and synthenized L-carnitine in fish group fed D6 significantly increased growth indices of *O. niloticus* in comparison to the other diets. A growth-promoting effect of supplemental dietary L-carnitine has been reported in all L-carnitine sources or levels. Such advances were also observed i by [21, 29, 30] whom reported that supplementing growing Nile tilapia diets with both methionine and lysine to reach 30% over the recommended levels significantly improved final weights, weight gain protein. On the other hand, Chatzifotis *et al.* [13] 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. Several studies reported that L-carnitine could induce an improved growth performance, probably caused by increased lipid oxidation in several marine and freshwater species [11-13, 16, 17, 31, 32]. In contrast, dietary carnitine did not affect growth of channel catfish [33], rainbow trout [34], Atlantic salmon [35], African catfish [36], European seabass, *Dicentrarchus labrax* [37], or tilapia [38].

The obtained results showed that dietary supplementation of methionine and lysine have the same effect of dietary L-carnitine on improving feed utilization of *O. niloticus* and this effect was clearly noticed when dietary L-carnitine combined with dietary amino acids (methionine+lysine). 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 [39]. Yang *et al.* [21] demonstrated that, protein retention (PR) of grass carp, *Ctenopharyngodon idella* fed diet with supplemental lysine and methionine were significantly higher, while the feed conversion ratio (FCR) were significantly lower in comparison with the reference group.

The obtained results are in agreement with the finding of Soltan *et al.* [40] who found that, L-carnitine levels 300 to 1500 mg/kg diet increased feed intake and improved FCR but had no significant effect on PER of *O. niloticus*. Similarly, several researchers have speculated that increasing growth rates of fish fed supplemental L-carnitine may due to its role in improving FCR via increasing fatty acid oxidation and increasing utilization of dietary energy as observed by Azab *et al.* [16], Hamackova *et al.* [41] in common carp, Ozorio *et al.* [36] in catfish, Schuhmacher and Gropp [30] in trout and finally [12] in red sea bream. In this respect, Ozorio *et al.* [42] with African catfish suggest that dietary L-carnitine supplementation may increase fatty acid oxidation and possibly decrease amino acid consumption for energy.

Proximate Analysis of Fish: Our results show that, all treatments had no significant effect on dry matter, fat and ash content of whole fish bodies. On the other hand, L-carnitine (D2) or its precursor (methionine+lysine) in low (D3) or high (D4) levels did not significantly alter protein content while the combinations of L-carnitine and methionine+lysine in low (D5) or high (D6) levels significantly ($P<0.05$) increased protein content of whole fish body. Yang *et al*. [21] demonstrated that whole body moisture and muscle protein of grass carp were significantly increased, while lipid level of whole body was significantly decreased ($P<0.05$) with the supplementation of lysine and methionine.

The ability of L-carnitine to increase protein and reduce tissue lipid content has been illustrated in several fish species with conflicting results. Azab *et al*. [16] revealed that, L-carnitine in concentration of 600 or 900 mg kg^{-1} diet at 15% dietary lipid increase tissue protein and did not alter fat content of Nile tilapia, *O. niloticus*. On the other hand, fat content of several tissues, such as liver, muscle and viscera, were reduced as the L-carnitine supplemented to the diets of channel catfish [33], tilapia [31], Atlantic salmon [35], rohu [43], juvenile black sea bream [32] and beluga, *Huso huso* [17]. In contrast, the absence of a lipotropic action of carnitine feeding had been previously reported in red seabream, rainbow trout, hybrid tilapia, hybrid striped bass and European seabass, *Dicentrarchus labrax* [15, 37]. Whether such variability in terms of the body lipid lowering effect of supplemental L-carnitine is associated with dietary lipid level (and indirectly to the energy density of the diet) remains unclear. Whereas Rodehutscord [34] reported that supplemental dietary L-carnitine was ineffective for

reducing body fat in rainbow trout fed high-fat diets (26%), Ji *et al.* [35], in Atlantic salmon fed a 10% lipid diet, found that carnitine-fed fish exhibited a decrease in the lipid content in white muscle and viscera by as much as 73 and 43%, respectively. L-Carnitine plays an important role in promoting the transport of long-chain fatty acids across the inner mitochondrial membrane, resulting in extra energy from β-oxidation. Therefore, dietary L-carnitine supplements should enhance the oxidation of these fatty acids, thereby decreasing their availability or esterification to triacylglycerols and deposition in the various lipid storage tissues [37].

REFERENCES

1. Hassaan, M.S. and M.A. Soltan, 2016. Evaluation of Essential Oil of Fennel and Garlic Separately or Combined with *Bacillus licheniformis* on the Growth, Feeding Behaviour, Hemato-biochemical Indices of *Oreochromis niloticus* (L.) Fry. Journal of Aquaculture, Research & Development, 7: 3. http://dx.doi.org/10.4172/2155-9546.1000422.
2. Hassaan, M.S., M.A. Soltan and M.M.R. Ghonemy, 2014. Effect of synbiotics between *Bacillus licheniformis* and yeast extract on growth, hematological and biochemical indices of the Nile tilapia *Oreochromis niloticus*. The Egyptian Journal of Aquatic Research, 40: 199-208.
3. Hassaan, M.S., M.A. Wafa, M.A. Soltan, A.S. Goda and N.M.A. Mogheeth, 2014. Effect of Dietary Organic Salts on Growth, Nutrient Digestibility, Mineral Absorption and Some Biochemical Indices of Nile Tilapia; *Oreochromis niloticus* L. Fingerlings. World Applied Science Journal, 29(1): 47-55.
4. Soltan, M.A. and S.M.M. El-Laithy, 2008. Effect of probiotics and some spices as feed additives on the performance and behaviour of the Nile tilapia, *Oreochromis niloticus*. Egypt. J. Aquat. Biol. & Fish., 12(2): 63-80.
5. Abdel-Hakim, N.F., M.S. Hussein, M.N. Bakeer and M.A. Soltan, 2001. Effect of protein level and stocking density on growth performance of Nile tilapia (*Oreochromis niloticus)* cultured in tanks. Egyptian J. Nutrition and Feeds, 4: 763-780.
6. Ibrahim, M.K., Fatma, A. Hafez and M.A. Soltan, 2000. Effect of organic fertilization, supplementary feeding and stocking rate on growth performance of Nile tilapia and silver carp. Egypt. J. Agric., Res., 78(4): 1775-1799.
7. Yi, Y., Lin, C.K. and J.S. Diana, 1996. Influence of Nile tilapia (*Oreochromis niloticus*) stocking density in cages on their growth and yield in conges and in ponds containing the cages. Aquaculture, 146: 205-215.
8. Harpaz, S., 2005. L-Carnitine and its attributed functions in fish culture and nutrition-a review. Aquaculture, 249: 3-21.
9. Bremer, J., 1983. Carnitine-metabolism and functions. Physiol. Rev., 63: 1420-1480.
10. Lohninger, A., E. Kaiser, E. Legenstein and H. Staniek, 1987. Carnitine, metabolism and function. In: Kaiser, E., Lohninger, A. (Eds.), Carnitine. Its Role in Lung and Heart Disorders. Karger Press, Basel, pp: 1-25.
11. Becker, K., S. Scheiber, C. Angoni and R. Blum, 1999. Growth performance and feed utilization responded of *Oreochromis niloticus*×*O. aureus* hybrids to L-carnitine measured over a full fattening cycle under commercial conditions. Aquaculture, 174: 313-322.
12. Chatzifotis, S., T. Takeuchi and T. Seikai, 1995. The effect of dietary L-carnitine on growth performance and lipid composition in red sea bream fingerlings. Fish. Sci., 61: 1004-1008.
13. Chatzifotis, S., T. Takeuchi and T. Seikai, 1996. The effect of dietary carnitine supplementation on growth of red sea bream (*Pagrus major*) fingerlings at low levels of dietary lysine. Aquaculture, 147: 235-248.
14. Focken, U., K. Becker and P. Lawrence, 1997. A note on the effect of L-carnitine on the energy metabolism of individually reared carp, *Cyprinus carpio* L. Aquaculture Nutrition, 3: 261-264.
15. Twibell, R.G. and P.B. Brown, 2000. Effect of dietary carnitine on growth rates and body composition of hybrid striped bass (*Morone saxatilis* male × *M.Chrysops* female). Aquaculture, 187: 153-161.
16. Azab, M.E., S.I. Fathalla and M.A. Soltan, 2002. Effect of feed supplementation with L-carnitine on growth performance and body composition of Nile tilapia (*Oreochromis niloticus*). Suez Canal Vet. Med. J., 5(1): 201-214.
17. Mohseni, M. and R.O.A. Ozorio, 2014. Effects of dietary L-carnitine level on growth and body composition and antioxidant status in in beluga (*Huso huso* L. 1758). A quaculture Nutrition, 20: 477-485
18. Tremblay, G.C. and T.M. Bradley, 1992. L-carnitine protect fish against acute ammonia toxicity. Comp. Biochem. Physiol., 101C: 349-351.

19. Santulli. A., E. Puccia and V. D'Amelio, 1990. Preliminary study on the effect of short-term carnitine treatment on nucleic acid and protein metabolism in sea bass (*Dicentrachus labrax*) fry. Aquaculture, 87: 85-89.
20. Soltan, M.A., M.M. Abdella, G.A. El- Sayaad and M.H. Abu-El Wafa, 2015. Effect of some over-wintering regimes on survival and growth performance of Nile tilapia *Oreochromis niloticus*. Biological and Chemical Research, 2: 362-374.
21. Yang, H.J., Y.J. Liu, L.X. Tian, G.Y. Liang and H.R.L. Provincial, 2010. Effects of Supplemental Lysine and Methionine on Growth Performance and Body Composition for Grass Carp (*Ctenopharyngodon idella*). American Journal of Agricultural and Biological Sciences, 5(2): 222-227.
22. APHA, 1992. Standard Methods for the Examination of Water and Waste Waters, 18th ed. American Public Health Association, Washington, DC, pp: 1268.
23. Boyd, C.E., 1990. Water Quality in Ponds for Aquaculture. Auburn University Agriculture Experimental Station, Auburn, Alabama, USA.
24. AOAC, 1995. In: Cunni, P.A. (Ed.), Official Methods of Analysis of the Association Official Analytical Chemists, vol. 1, 16th ed. AOAC International, Arlington, USA, pp: 1298.
25. Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for Dietary Fiber, Neutral Detergent Fiber and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci., 74: 3583-3597.
26. SAS, 1996. SAS Procedure Guide "version 6.12 Ed". SAS Institute Inc., Cary, NC, USA.
27. Duncan, D.B., 1955. Multiple range and Multiple test. Biometerics, 11: 1-42.
28. Brett, J.R., 1973. Energy expenditure of Sockeye salmon *Oncorhynchus nerka* during sustained performance. J. Fish. Res. Board Can., 30: 1799-1809.
29. Abdel-Hakim, N.F., K.H. El-Kholy, A.A. Azab and A.A. Abdel-Warith, 2006. Effect of dietary high lysine and methionine levels on growth performance of Nile Tilapia (*O. niloticus*) fed on diet at different frequencies. Egyptian J. Nutrition and Feeds., 9(1): 55-70.
30. Schuhmacher, A. and J.P. Gropp, 1998. Carnitine a vitamin for rainbow trout. Journal of Applied Ichthyology, 14: 1-2, 87-90.
31. Jayaprakas, V., C. Sambhu and S.S. Kumar, 1996. Effect of dietary L-carnitine on growth and reproductive performance of male *Oreochromis mossambicus* (peters). Fish. Techonol., 33: 84-90.
32. Ma, J.J., Z.R. Xu, Q.J. Shao, J.Z. Xu, S.S.O. Hung, W.L. Hu and L.Y. Zhuo, 2008. Effect of dietary supplemental L-carnitine on growth performance, body composition and antioxidant status in juvenile black sea bream, *Sparus macrocephalus*. Aquaculture Nutrition, 14: 464-471.
33. Burtle, G.J. and O.H. Liu, 1994. Dietary carnitine and lysine affect on channel catfish lipid and protein composition. Journal of the world Aquaculture Society, 25(2): 169-174.
34. Rodehutscord, M., 1995. Effects of supplemental dietary L-carnitine on the growth and body composition of rainbow trout (*Oncorhynckus mykiss*) fed high-fat diets. J. Anim. Physiol. Anim. Nutr., 73: 276-279.
35. Ji, H., T.M. Bradiley and G.C. Tremblay, 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.
36. Ozorio, R.A., T.B. Van-Erkeren, E.A. Huisman and J.J. Verreth, 2001. Effect of dietary carnitine and protein energy: non protein energy ratios on growth, ammonia excertion and respiratory quatient in African carfish, *Clarias gariepinus* (Burche LL) Juveniles. Aquaculture Research, 32: 406-414.
37. Dias, J., J. Arzel, G. Corraze and J. Kaushik, 2001. Effect of dietary L-carnitine supplementation on growth and lipid metabolism in European seabass (*Dicentrarchus labrax*). Aquaculture Research, 32: 206-215.
38. Schlechtriem, C., V. Bresler, L. Fishelson, M. Rosenfeld and K. Becker, 2004. Protective effects of dietary L-Carnitine on tilapia hybrids (*Oreochromis niloticus* × *Oreochromis aureus)* reared under intensive pond-culture conditions. Aquaculture Nutrition, 10: 55-63.
39. Tanphaichitr, V., D.W. Horne and H. Broquist, 1971. Lysine a precursor of carnitine in the rat. J. Biol. Chem., 246: 6363-6366.
40. Soltan, M.A., H.M. Agouz, A.I. El-Sayed and A.A. El-Qobatti, 2016. Effect of feed supplementation with L-carnitine on growth and cold tolerance of Nile tilapia, *Oreochromis niloticus*. Egypt. J. Aquat., Biol., & Fish. (In press).
41. Hamackova, J., P. Kozak, J. Kouril and Z. Adamek, 1998. The effect of L-carnitine supplementation into feed mixtures upon production and over wintering results in common carp (*Cyprinus caprio*) culture. Krmiva. 1998, 40: 4, 175-181; 9 ref.

42. Ozorio, R.A., G.R. Booms, E.A. Huisman and J.J. Verreth, 2002. Changes in amino acid composition in the tissues of African catfish (*Clarias gariepinus*), as a consequence of dietary L-carnitine supplements. J. Appl. Ichthyol; Z-Angew-Ichthyol, 18: 140-147.

43. Keshavanath, P. and P. Renuka, 1998. Effect of dietary L-carnitine supplements on growth and body composition of fingerlings rohu, *lebeo rohita* (*Hmiltoin*). Aquaculture Nutrition, 4: 83-87.