Effects of L-carnitine on growth performance of Nile tilapia (*Oreochromis niloticus)* fingerlings fed basal diet or diets containing decreasing protein levels

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Abstract: The effects of L-carnitine on growth rate, feed utilization efficiency and protein sparing of Nile tilapia (*Oreochromis niloticus* L.) fingerlings were investigated in two consecutive experiments. In experiment 1, triplicate groups of 10 fingerlings (4.16 ±0.07) each were stocked in 85 L glass aquaria, filled with 70 L dechlorinated tap water. Five levels of L-carnitine (0, 75, 150, 300, 450 mg/kg) were separately added to the basal diet (30% crude protein and 18.74 Mj GE/kg). The fish were fed the diets, at a daily rate of 5% BW, twice a day for 70 days. The results revealed that fish growth rates, feed utilization and whole body protein and lipid levels were increased with increasing L-carnitine levels. In experiment 2, Nile tilapia fingerlings (4.3±0.1 g) were fed diets containing decreasing levels of protein (30, 25, and 20%) and supplemented with 450 mg L-carnitine/kg diet, for 84 days. Fish performance was not significantly affected with decreasing dietary protein levels up to 20%. These results suggest that dietary inclusion of L-carnitine in Nile tilapia diets may significantly reduce dietary protein requirements and may facilitate the use of fatty acids for obtaining energy and consequently, can spare dietary protein for somatic growth. [Journal of American Science 2010;6(5):165-172]. (ISSN: 1545-1003).

Keywords: L-carnitine, Nile tilapia, performance, protein sparing.

1. Introduction

Tilapia culture has been growing at an outstanding rate during the past two decades. As a result, the production of farmed tilapia has witness a 6-fold increase during the past 15 years, jumping from 383,654 mt in 1990 to 2,348,656 mt in 2006 (FAO, 2008). In addition, there has been a gradual shift in tilapia culture from traditional semi-intensive to more intensive farming systems. This has created an increasing demand for artificial feed.

The replacement of fishmeal with other plant- or animal-based protein sources in tilapia feeds is well documented (El-Sayed, 1990; 1992; 1998; 1999; Ebrahim and Abou-Seif, 2008). However, most of these protein sources contain different antinutrients and are deficient in certain essential amino acids, and may therefore lead to retarded performance (Francis *et al*., 2001). Supplementing these sources with certain compounds may improve their nutritive values for fish and accordingly reduce the feed costs.

L-carnitine (l-h-hydroxy-g-N,N,N-trimethylaminobutyric acid) is one of those compounds that may play a significant role in fish nutrition. It is a lysine derivative, hygroscopic, water soluble organic compound (Harpaz, 2005). It is also non essential since can be synthesized from lysine and methionine in animal and human liver, brain and kidney (Emaus and Bieber, 1983). L-carnitine acts as a cofactor for the transport and oxidation of long chain fatty acids by the mitochondria by facilitating the use of fatty acids for obtaining energy, and thus sparing dietary protein for anabolic processes (Bilinski and Jonas, 1970; Emaus and Bieber, 1983).

The use of L-carnitine as a growth promoter in fish is controversial. Many studies revealed significant effects of dietary L-carnitine on the performance of European sea bass (Santulli and D'Amelio, 1986); African catfish (Torreele *et al*., 1993); red sea bream (Chatzifotis *et al*., 1995); Indiana major carp rohu (Keshavanath and Renuka, 1998); hybrid striped bass (Twibell and Brown, 2000); Beluge sturgeon

(Mohsni *et al.*, 2008); Mossambique tilapia (Jayaprakas *et al*. 1996) and Hybrid tilapia *Oreochromis niloticus* × *O. aureus* (Becker *et al*., 1999) and Nile tilapia (Yang *et al*, 2009)..

In contrast, no effects of L-carnitine supplementation was reported in European sea bass (Dias *et al*., 2001), channel catfish (Burtle and Liu, 1994), Atlantic salmon (Ji *et al*., 1996), Rainbow trout (Rodehutscord, 1995; Chatzifotis *et al*. 1997) , Hybrid striped bass (Gaylord and Gatlin, 2000 a,b), African catfish (Ozorio *et al*. 2001 a,b) and Hybrid tilapia *O. niloticus* × *O. aureus* (Schlechtriem *et al*., 2004).

It appears from this discrepancy that the response of fish to supplemental L-carnitine is species specific. This response may also be affected by other dietary components being, carnitine levels, culture conditions and water quality. Therefore, more work is urgently needed to verify the effects of L-carnitine supplementation on fish performance and health.

The present study was carried out, in two consecutive experiments. The first experiment aimed at investigating the effects of L-carnitine supplementation on the performance and body composition of Nile tilapia (*Oreochromis niloticus)* fingerlings fed basal diets. The second experiment investigated the effect of L-carnitine supplementation on protein sparing effect in Nile tilapia fingerlings fed decreasing dietary protein levels.

2. Material and Methods

Fish and culture facilities

The present study was carried out at fish research unit, Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. Mono sex Nile tilapia *Oreochromis niloticus* fingerlings used in the present study were obtained from a private hatchery at Kafr El-Sheikh Governorate. The fish were adapted to the lab conditions for 7 days, during which they were fed the test diets assigned to each treatment. At the ends of the conditioning period, the fish were netted, reweighed collectively, and the average initial weight was recorded. Culture aquaria were continuously provided with aeration through an air compressor. Tanks were cleaned every morning, before the first feeding, and the faeces were removed then, water was replaced by 10% of new fresh, dechlorinated water. Natural photoperiod was adopted throughout the study. Water quality parameters, including temperature (T), pH, dissolved oxygen (DO), total ammonia (NH_3), and nitrites (NO_2-N) were monitored weekly. The average values of these parameters throughout the study were; T 27± 0.5° C, pH 7.03± 0.38, DO 6.8± 0.63 mg^{-1}, NH_3 0.4± 0.1 mg^{-1} and NO_2 0.2±0.02 mg^{-1} .

Experimental Design

Experiment 1

The first experiment was designed to study the effects of supplemental L-carnitine on growth performance, feed conversion efficiency and body composition of Nile tilapia (*Oreochromis niloticus)* fingerlings fed basal diet contain 30% crude protein and 18.74 MJ GE/kg. Five levels of L-carnitine (0, 75, 150, 300, 450 mg/kg) were separately added to the basal diet to formulate 5 experimental diets (Table 1). Proximate analysis of the basal diet was performed according to AOAC (1998). Fish with an initial average weight of 4.16 ± 0.07 g were stocked in triplicates into 85 L glass aquaria, filled with 70 L dechlorinated tap water, at a density of 10 fish/aquarium. The fish were fed the diets, at a daily rate of 5% BW, twice a day for 70 days. The fish were weighed collectively at 15-day intervals, their average weights recorded and daily feeds were rations readjusted accordingly. At the ends of the experiment, fish were netted, weighed individually and the average final weight of each replicate was recorded.

Experiment 2

In experiment 2, triplicate groups of Nile tilapia fingerlings (4.3±0.1 g) were fed diets containing decreasing levels of protein (30, 25, and 20%) and supplemented with 450 mg L-carnitine/kg diet, for 84 days (Table 2). Lysine and methionine were added to the diets to adjust the amino acids required by Nile tilapia according to NRC (1993) proximate analysis of the experimental diets was done according to AOAC (1998). The fish were exposed to the same culture conditions and feeding regime adopted in experiment 1.

Growth rates and feed efficiency

Growth rates and feed conversion efficiency were calculated as follows:

Average daily gain (ADG) = Weight gain (g)/time of experiment (days),

Specific growth rate (% SGR) = 100 (ln average final weight–ln average initial weight)/time (days),

Feed conversion ratio (FCR) = g dry feed given/g wet weight gain.

Protein efficiency ratio (PER) = g wet weight gain/g protein fed.

Net protein utilization (NPU) = 100×(final body protein –initial body protein)/protein fed.

Body composition

Initial body composition of fish was analyzed from samples of 50 fish which were frozen (at -20ºC) prior to the study. At the end of the study, the fish in each aquarium were netted, their total weight (g) were recorded and frozen for final chemical analysis. Chemical analyses of fish and test diets were performed according to standard AOAC (1998) methods.

Statistical analysis

Simple linear and non-linear regressions were performed to correlate the obtained results. A one-way analysis of variance (ANOVA) was conducted to test the effect of dietary treatments on the performance of Nile tilapia fingerlings, using the computer program SPSS (SPSS Version11.0.0, 2003). Least significant difference was used to compare between means at P=0.05, as described by (Gill, 1981).

3. Results

Experiment 1

Fish performance in Experiment 1 was significantly affected by dietary treatments (P<0.05). Growth rates were significantly increased (P<0.05) with increasing L-carnitine (Table 3). Feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) were also improved with increasing L-carnitine levels in the diets (P<0.05). However, fish fed diets containing 300 and 450 mg/kg L-carnitine showed similar gain and SGR (P>0.05).

Body composition of Nile tilapia fed the experimental diets in Experiment 1 was significantly affected by L-carnitine supplementation (Table 4). Body moisture and ash contents were negatively correlated with L-carnitine, while crude protein and lipid contents significantly increased (P<0.05) with increasing L-carnitine in the diets.

Experiment 2

Fish performance in Experiment 2 was significantly affected by decreasing dietary protein levels and supplementation of L-carnitine (Table 5). Growth rates of fish fed diets containing 20-30% CP were not significantly different (P>0.05). Similarly, FCR was not significantly affected by dietary treatments (P>0.05). In contrast, PER and NPU were improved (P<0.05) with decreasing dietary protein levels.

Body composition was also significantly affected by decreasing dietary protein levels and supplementation of L-carnitine (Table 6). Body water and ash contents slightly increased with decreasing dietary protein levels (P>0.05), while body protein and lipids content were negatively correlated (P>0.05) with decreasing dietary protein level.

4. Discussions

It has been reported that carnitine is closely associated with lipid metabolism, through promotion of fatty acid oxidation. Therefore, it is expected that carnitine administration in fish diets may improve energy utilization and spare protein for somatic growth. Several studies have investigated this assumption in recent years. However, controversial results have been reported, depending on fish species and size, carnitine levels, dietary composition and culture conditions.

In the present study, we investigated the response of Nile tilapia fingerlings to diets supplemented with L-carnitine levels. The results clearly revealed that fish growth rates, feed utilization and whole body protein and lipid content were significantly increased with increasing dietary L-carnitine levels.

Similarly, studies on other tilapias including Mozambique tilapia (*Oreochromis mossambicus*) (Jayaprakas *et al*., 1996), hybrid tilapia *Oreochromis niloticus* × *O. aureus* (Becker *et al* ., 1999) and Nile tilapia (Abou-Seif, 2006) revealed that dietary L-carnitine supplementation resulted in improved growth rates and feed efficiency. Similar results have also been reported with several other species reared on diets supplemented with L-carnitine, including European sea bass (Santulli and D'Amelio, 1986), African catfish (*Clarias gariepinus*) (Torreele *et al* ., 1993; Ozorio *et al*., 2001b), red sea bream (Chatzifotis, *et al* ., 1995), Indian major carp rohu (Keshavanath and Renuka, 1998); hybrid striped bass (Twibell and Brown, 2000).

However, the scale of improvement in growth rates in the present study (18.2, 29.3, 45.5 and 54.5% at 75, 150, 300 and 450 mg carnitine kg^{-1}, Respectively) was much higher than those previously reported on *O. mossambicus* (16%) (Jayaprakas *et al*., 1996) and *O. niloticus* × *O. aureus* (18.5%) (Becker *et al*., 1999).

The significant differences of FCR in experiment 1, may suggest that the improved growth was related to feed consumption and better fed utilization efficiency. Therefore, the improved performance of Nile tilapia in experiment 1 with L-carnitine supplementation is presumably due to the enhancement, caused by carnitine, of energy

utilization from fatty acid oxidation (through β-oxidation) (Torreele *et al*., 1993; Chatzifotis *et al.*, 1995). It has also been reported that L-carnitine facilitates the removal of short chain organic acids from the mitochondria, leading to freeing coenzyme A to participate in β-oxidation and tricarboxylic acid cycle pathways (Harpaz, 2005).

In contrast, no effects of L-carnitine supplementation was reported in European sea bass (Dias *et al*., 2001), Channel catfish (Burtle and Liu, 1994), Atlantic salmon (Ji *et al* ., 1996), Rainbow trout (Rodehutscord 1995; Chatzifotis *et al* ., 1997), hybrid striped bass (Gaylord and Gatlin 2000 a,b), African catfish (Ozorio *et al* ., 2001 a,b) and hybrid tilapia *O. niloticus* × *O. aureus* (Schlechtriem *et al* . 2004). The discrepancies in these results may have been due to the differences in culture conditions, diets composition and fish species and sizes.

Dietary protein requirement of fish is affected by dietary energy. In other words, fish growth is sustained from the energy supplied from dietary protein or energy sources. Therefore, the relationship between dietary protein and energy in fish feeds should be considered if cost-effective and environmentally friendly diets are formulated. Many studies have indicated that at inadequate energy level, dietary protein may be used as an energy source, whereas at high protein level, a proportion of this protein will be deaminated and the carbon skeleton used as an energy source (Garling and Wilson, 1976). At adequate energy level, dietary protein can be spared for anabolic functions (Garling and Wilson, 1976; El-Sayed, 1987; El-Sayed and Kawanna, 2008). Thus, the design of practical fish diets is a compromise between a protein level that will permit good growth with little conversion to energy, and an energy level concomitant with a high rate of protein synthesis, without excessive deposition of carcass lipid.

In experiment 2 of the present study, the inclusion of L-carnitine in the diets has significantly enhanced the growth, PER and NPU of Nile tilapia despite the reduction of dietary protein from 30% to 20%. Once again, these results suggest that carnitine enhanced energy utilization through promotion of fatty acid oxidation and accordingly, sparing dietary protein for somatic growth. This may explain the increase of PER and NPU at lower dietary protein levels. The results of experiment 2 are in agreement with the result of Ozorio *et al.* (2001b), who found that dietary carnitine supplementation in African catfish diets improved growth rates and FCR when protein energy (PE) – to – non protein energy (NPE) was low (i.e., when dietary protein was in shortage), leading to increasing body protein : fat ratio. Those authors suggested that low dietary PE:NPE may lead to higher enzymatic activity and elevated availability of free carnitine in fish tissues, which, in turn, leads to improved utilization of dietary lipid for energy fuel and spare protein for growth.

5. Conclusion

The present study indicated that a significant improvement in fish performance was observed when the diets were supplemented with 450 mg L-carnitine/kg. The study revealed also that the inclusion of L-carnitine in tilapia diets may significantly reduce dietary protein requirements. Moreover, the results suggest that dietary inclusion of L-carnitine in Nile tilapia diets may facilitate the use of fatty acids for energy and consequently, spare dietary protein for somatic growth.

Table 1. Formulation and proximate composition (%) of the basal diet used in Experiment 1.

Ingredients	%
Fish Meal (72%)	24.00
Soybean meal (44%)	21.00
Yellow corn	51.00
Corn oil	2.50
Vit & Min mix[1]	1.50
Total	100
Proximate composition	
Crude protein	29.62
EE	7.90
Ash	6.92
CF	5.11
NFE[2]	50.45
GE[3]	18.74

[1]Contains per kg: vitamin A, 4.8 m. I.U; vit D3 , 0.8 m.I.U; vit E, 4.0 g; vit. K, 0.8 g; vit B1, 0.49, vit. B2, 1.6 g; vit. B6, 0.6 g; vit. B12, 4 mg; Pantothenic acid, 4 g; Nicotinc acid, 8 g; Folic acid, 400 mg; Biotin, 20 mg; Choline chloride, 200 mg; Copper, 4.0 g ; Iodine, 0.4g ; Iron, 12 mg ; Manganese, 22 g; Zinc, 22 g and Selenium 0.04 g.

[2]Nitrogen free extract, determined by difference.

[3]Gross energy (MJ/kg), calculated based on 0.17, 0.237, 0.398 MJ/g for carbohydrate, protein and lipid, respectively (Jobling, 1983).

Table 2. Formulation and proximate composition (%) of the diets used in Experiment 2.

	Diets		
Ingredients	1	2	3
Fish meal (72%)	8	8	8
Soybean meal (44%)	46	36	22
Yellow corn	39.14	49.34	56.34
Corn oil	2	2	3
Vit & Min Mix[1]	2	2	2
Wheat bran	0.5	0.5	7
L-Lysine HCL	1.86	1.60	1.26
DL-Methionine	0.50	0.46	0.40
Total	100	100	100
Proximate composition			
CP	29.54	24.62	20.48
EE	11.42	11.52	11.36
CF	4.20	3.70	10.30
Ash	5.59	5.43	5.52
NFE[1]	49.25	54.73	52.34
GE (MJ/kg)[1]	19.92	19.72	18.28
P/E ratio[2]	14.82	12.48	11.20
PE/NPE[3]	0.54	0.47	0.39

[1]Gross energy (MJ/kg), calculated based on 0.17, 0.237, 0.398 MJ/g for carbohydrate, protein and lipid, respectively. [2]Protein-to-energy ratio (g protein/MJ). [3]Protein energy/non protein energy

Table 3. Performance (mean ± SE) of Nile tilapia fingerlings fed different levels of L–carnitine(Experiment 1).

Carnitine (mg/kg)	IW	FW	SGR	% gain	FCR	PER	NPU
0	4.16 ^{a} ±0.01	14.08 ^{c} ±0.55	1.74 ^{d} ±0.05	239 ^{d} ±13	2.66 ^{e} ±0.04	1.27 ^{e} ±0.02	19.82 ^{e} ±0.51
75	4.23 ^{a} ±0.02	16.64 ^{b} ±0.36	1.96 ^{c} ±0.03	293 ^{c} ±8	2.41 ^{d} ±0.02	1.40 ^{d} ±0.01	22.58 ^{d} ±0.44
150	4.09 ^{a} ±0.02	18.20 ^{b} ±0.23	2.13 ^{b} ±0.02	345 ^{b} ±6	2.22 ^{c} ±0.02	1.52 ^{c} ±0.01	24.53 ^{c} ±0.61
300	4.19 ^{a} ±0.01	20.48 ^{a} ±0.99	2.27 ^{ab} ±0.08	389 ^{ab} ±25	2.01 ^{b} ±0.01	1.68 ^{b} ±0.01	28.05 ^{b} ±0.32
450	4.11 ^{a} ±0.02	21.75 ^{a} ±0.47	2.38 ^{a} ±0.03	429 ^{a} ±12	1.82 ^{a} ±0.06	1.86 ^{a} ±0.07	31.38 ^{a} ±0.92

Values in the same column with different superscripts are significantly different ($p< 0.05$).

Table 4. Effect of L-carnitine Levels on body composition (% dry weight) of Nile tilapia fingerlings (Experiment 1).

Carnitine (mg/kg)	Moisture	Protein	Lipid	Ash
0	72.55 ^{a} ±0.30	53.79 ^{d} ±0.19	18.92 ^{d} ±0.20	27.07 ^{a} ±0.12
75	72.02 ^{a} ±0.18	54.53 ^{cd} ±0.29	20.66 ^{c} ±0.31	24.59 ^{b} ±0.47
150	72.24 ^{a} ±0.36	55.58 ^{bc} ±0.33	22.93 ^{b} ±0.33	21.16 ^{c} ±0.42
300	71.62 ^{a} ±0.28	56.04 ^{b} ±0.38	23.04 ^{b} ±0.17	20.43 ^{c} ±0.34
450	71.78 ^{a} ±0.30	57.14 ^{a} ±0.46	24.45 ^{a} ±0.30	18.00 ^{d} ±0.80
Initial	74.89 ±0.38	51.21 ±1.11	24.98 ±0.34	23.50 ±0.62

Values in the same column with different superscripts are significantly different ($p< 0.05$).

Table 5. The Effect of L-carnitine supplementation (450 mg/kg) on growth rates and feed utilization efficiency of Nile tilapia fingerlings fed decreasing dietary protein levels (Experiment 2).

Item	Dietary protein level (%)		
	30	25	20
IW	4.29 ±0.02	4.30 ±0.01	4.29 ±0.01
FW	50.00 [a] ±0.40	49.54 [a] ±1.03	48.69 [a] ±0.70
SGR	2.92 [a] ±0.14	2.91 [a] ±0.19	2.89 [a] ±0.21
ADG	0.54 [a] ±0.06	0.54 [a] ±0.05	0.53 [a] ±0.01
FCR	1.99 [a] ±0.13	1.96 [a] ±0.11	1.95 [a] ±0.13
PER	1.70 [c] ±0.17	2.07 [b] ±0.19	2.34 [a] ±0.23
NPU	28.87 [c] ±1.57	33.52 [b] ±1.19	36.86 [a] ±0.41

Values in the same column with different superscripts are significantly different ($p < 0.05$).

Table 6. The Effect of L-carnitine supplementation (450 mg/kg) on body composition (% dry weight) of Nile tilapia fingerlings fed decreasing dietary protein levels in (Experiment 2.)

Dietary protein (%)	Moisture	Protein	Lipid	Ash
30	74.61 [a] ±0.24	65.03 [a] ±0.27	22.86 [a] ±0.34	10.91 [a] ±0.32
25	75.42 [a] ±0.14	64.24 [a] ±0.25	22.75 [a] ±0.42	11.49 [a] ±0.17
20	75.92 [a] ±0.23	63.94 [a] ±0.14	22.16 [a] ±0.19	12.55 [a] ±0.15
Initial	78.18 ±0.89	58.42 ±0.58	21.90 ±0.18	17.26 ±0.43

Values in the same column with different superscripts are significantly different ($p < 0.05$).

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