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Short Communication

DL-carnitine as supplementary levocarnitine source in plant-based diets to Nile tilapia (*Oreochromis niloticus*) fingerlings

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ABSTRACT. The objective was to evaluate the effect of DL-carnitine levels on the growth and whole body and muscle composition of Nile tilapia fingerlings. A basal plant-based diet was supplemented with 0, 500, 1000, 1500, 2000, 3000 and 4000 mg kg^{-1} of DL-carnitine hydrochloride in a completely randomized design (n = 3). Fish (initial weight 0.8 ± 0.01 g) was hand-feed daily into three meals until apparent satiety for 70 days. The growth parameters were not influenced ($P > 0.05$) by dietary DL-carnitine levels. Muscle lipid decreased ($P < 0.05$) in fish fed with diets supplemented with 3000 and 4000 mg kg^{-1} of DL-carnitine, but there was no effect ($P > 0.05$) on whole body lipid. These results with DL-carnitine is similar to other studies using pure L-carnitine. Thus, the use of DL-carnitine as a source of levocarnitine in Nile tilapia diets require future studies.

Keywords: Nile tilapia, feed additive, fish nutrition, lipid metabolism.

The inclusion of plant ingredients in aquafeeds has increased in recent years, which is necessary to reduce dependence on fishmeal as a protein source and improve aquaculture sustainability. However, plant protein sources are admittedly deficient in some nutrients that need dietary supplementation (Yang *et al*., 2012). L-carnitine is a quaternary amine, endogenously synthesized from lysine and methionine, together with vitamin C and other secondary compounds as cofactors.

The main function of L-carnitine is the transport of long-chain fatty acids through the cell membrane for oxidation in mitochondria. It is present in high concentrations in animal feeds (16-1200 mg kg^{-1}). However, plant ingredients are poor (<0.5 mg kg^{-1}) in L-carnitine (Harpaz, 2005).

In farming conditions, stressful situations (*e.g.*, thermal stress, the presence of xenobiotics) are frequent, and therefore, the endogenous demand for L-carnitine increase. Fish in the initial growth stage possess a lower ability to produce endogenous L-carnitine, although at this age there is a high-energy demand and a lower resistance to stress factors. Under such circumstances, dietary L-carnitine supplementation may be necessary, as the concentration present in high-fat diets and rich in plant protein sources may not be enough to address fish dietary requirements (Becker *et al*., 1999; Yang *et al*., 2012). Additionally, farmed fish have lower concentrations of endogenous L-carnitine when compared to wildlife fish (Santulli & D'Amelio, 1986).

To date, a cost-effective optimum level of dietary L-carnitine supplementation to promote fish growth has not yet been identified (Mohseni & Ozorio, 2014). In fact, results have been inconsistent, even in studies with the same species (Harpaz, 2005). For instance, growth promotion in tilapias was reported in concentrations that ranged from 150 to 900 mg of levocarnitine per kg of diet (Jayaprakas *et al*., 1996; Becker *et al*., 1999). These levels are three to eighteen times higher than the ones recommended for poultry (Arslan, 2006). Becker *et al*. (1999) suggested that supplementation of up to 150 mg kg^{-1} of L-carnitine in diets for hybrid tilapias (*O. niloticus* × *O. aureus*) would be cost-effective. Nonetheless, due to the high cost of pure levocarnitine supplements, an analysis of cost-effectiveness is always necessary when including these in fish diets (Harpaz, 2005). By contrast, DL-carnitine has a lower cost (Dąbrowska & Starek, 2014) concerning the pure levorotatory form. Probably, due to this fact, it has been

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Table 1. Ingredients and chemical composition (wet basis) of basal diet. a) Assurance levels (kg^{-1} product): vit. A, 1.000.000 UI; vit. D3, 312.500 UI; vit. E, 18.750 UI; vit. K3, 1.250 mg; vit. B, 2.500 mg; vit. B2, 2.500 mg; vit. B6, 1.875 mg; vit. B12, 4 mg; Vitamin C, 31.250 mg; Nicotínic acid. 12.500 mg; Calcium pantothenate 6.250 mg; Biotin 125 mg; Folic acid 750 mg; Choline 50.000 mg; Inositol 12.500 mg; Iron sulphate 6.250 mg; Copper sulfate 625 mg; Zinc sulfate 6.250 mg; Manganese sulfate 1875 mg; Sodium selenite 13 mg; Calcium iodate 63 mg; Cobalt sulphate 13 mg, b) DL-carnitine hydrochloride (50% L-carnitine) - Sigma Aldrich® (C9 500), c) calculate from Furuya *et al*. (2010).

Ingredients	g kg^{-1}
Soybean meal	600.0
Wheat bran	150.0
Corn	105.8
Soybean oil	60.0
Cornstarch	50.0
Vitamin and mineral mix[a]	10.0
Dicalcium phosphate	10.0
DL-Methionine	4.0
Inert	10.0
DL-Carnitine[b]	0.0
BHT	0.2
Chemical composition	
Dry matter (g kg^{-1})	953.1
Crude protein (g kg^{-1})	322.9
Crude lipid (g kg^{-1})	67.5
Ash (g kg^{-1})	60.0
Digestible energy (kcal kg^{-1})[c]	3,594

used in several pharmaceutical formulations and supplement manufacturers in different countries (Lopes *et al.*, 2004; Sánchez-Hernández *et al.*, 2010b). Thus, this study evaluated the supplementation of DL-carnitine in diets of Nile tilapia fingerlings.

A basal diet (Table 1) was formulated without animal protein sources to minimize the presence of endogenous carnitine and supply the nutritional requirements of Nile tilapia (NRC, 2011). DL-carnitine was supplied as chloridrate of DL-carnitine (Sigma Aldrich®). DL-carnitine (DLC) was included in supplemented diets decreasing the inert content in the same proportion (0.5 DLC+9.5 INE; 1.0 DLC+9.0 INE; 1.5 DLC+8.5 INE; 2.0 DLC+8.0 INE; 3.0 DLC+ 7.0 INE and 4.0 DLC+6.0 INE g kg^{-1} of diet). All other ingredients were supplied in the same concentrations described for basal diet.

Each experimental ingredient was ground using a hammer mill (0.8 mm sieve). Then, the ingredients were mixed, moistened (30%) with distilled water at 40ºC and pelletized in a mincer. Diets were dried at in a forced ventilation oven (30ºC by 24 h) and stored at 4ºC until used.

Nile tilapia fingerlings (initial weight of 0.8 ± 0.01 g) were stocked in a set of 60 L glass aquariums (15 fish/aquarium) within a recirculation water system, with biological, mechanical and ultraviolet filters, supplemental aeration and temperature control. Every two days, the aquariums were drained at 30% of water volume to remove feces and diet waste. Water temperature (28.5 ± 0.8ºC) and dissolved oxygen (4.3 ± 0.6 mg L^{-1}) were monitored daily. Additional water quality parameters were checked weekly, such as ammonia (<0.01 mg L^{-1}), nitrite (0.6 ± 0.3 mg L^{-1}), hardness (106 ± 25 mg $CaCO_3$ L^{-1}), alkalinity (36 ± 16 mg $CaCO_3$ L^{-1}) and pH (6.8 ± 0.4). A constant photoperiod of 12 h light and 12 h dark was maintained.

Experimental diets were singly assigned to four groups, in a randomized experimental design. Fish were hand-fed carefully three times per day until apparent satiation (08:30, 12:30 and 17:30 h). Feed consumption was recorded weekly for each aquarium.

A sample of fish (n = 30) from the same population that started the experiment fasted for 24 h, killed by benzocaine overdoses (500 mg L^{-1}) for the determination of whole-body chemical composition. After 70 days, fish have fasted for 24 h, anesthetized (benzocaine, 50 mg L^{-1}) and the number and weight of the fish in each replicate was measured. Five fish from each aquarium were killed, as previously described, to obtain a pooled sample to determine whole-body chemical composition. Another five fish were randomly collected, killed, individually weighted, dissected to obtain liver and mesenteric fat weight and muscle samples. All biological samples were kept frozen (20ºC) prior to the analysis. The weight gain, feed intake, feed conversion and nutrient retention were calculated according to NRC (2011).

The chemical analyses of diets and fish samples were performed according to the methods of the Association of Official Analytical Chemists (AOAC, 2000). Crude protein content was determined by the Kjeldahl method (N×6.25). Crude lipid content was determined by the Soxhlet method. Moisture content was determined after drying in an oven at 105°C until constant weight. Ash content was determined after combustion in a muffle furnace at 550°C for 6 h.

Data were checked for normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett test) before statistical analysis. One way ANOVA followed by Tukey's Studentized Range Test were used to determine the significant effect of treatments. The significance of differences was tested at $P < 0.05$ level.

The global survival rate of fish during the trial was 98.1% and was not influenced ($P > 0.05$) by treatments.

The before fish well accepted all experimental diets. Growth parameters of fish fed on DL-carnitine supplemented diets did not differ ($P > 0.05$) from that of fish fed on control diet (Table 2). Dietary DL-carnitine content did not affect ($P > 0.05$) the whole body dry matter, protein, and ash (Table 3). A similar result ($P > 0.05$) was obtained for dry matter and protein from muscle. Muscular lipids decreased ($P < 0.05$) in fish fed with 3000 and 4000 mg kg^{-1} DL-carnitine-supplemented diets (Fig. 1), but no additional reduction ($P > 0.05$) in lipids was observed increasing dietary DL-carnitine from 3000 to 4000 mg kg^{-1}. However, there was no effect on dietary DL-carnitine supplementation in whole body lipid content in tilapia fingerlings.

Usually, the positive results of the supplementation of L-carnitine in aquafeeds are attributed to the protein sparing effect as a result of increased energy from lipid oxidation (Mohseni & Ozorio, 2014). However, when there is no growth promoting effect, this result is attributed to differences in the physiology of the species, developmental stage of fish, farming conditions, formulation of experimental diets, in addition to other reasons (Harpaz, 2005). The physiologic mechanisms which promote fish growth when dietary L-carnitine is supplemented were not adequately defined (Ma *et al*., 2008). Some studies registered a linear dose-response relationship of L-carnitine in growth (Torreele *et al*., 1993; Jayaprakas *et al*., 1996). On the other hand, other studies have reported a null response (Becker *et al*., 1999) or a decrease in growth (Mohseni & Ozorio, 2014) at concentrations of L-carnitine above the recommended for effectiveness.

DL-carnitine hydrochloride comprises 50% of L-carnitine and 50% of D-carnitine. Consequently, we estimated daily intake of approximately 0.2 (diet with 500 mg kg^{-1}) to 1.5 mg (diet with 4000 mg kg^{-1}) of D-carnitine per g of live weight at the end of the experiment, without a negative effect on fish performance. On the other hand, Santulli & D'Amelio (1986) registered a decrease in growth of juvenile sea bass *Dicentrachus labrax* ranged from 13 to 25% when fed daily with 0.025 mg of pure D-carnitine per gram of live weight. Despite the high intake of D-carnitine in the presented study, the intestinal absorption of levocarnitine occurs 1.6 to 2.0 times faster than the D-form (Gross & Henderson, 1984), which has possibly hindered any adverse effect on the performance of tilapia fingerlings. Indeed, also no difference was recorded in the weight gain, hematological parameters and serum concentration of free carnitine between Winstar rats fed diets supplemented with L- or DL-carnitine (Bazotte & Lopes-Bertolini, 2012).

Generally, the dietary carnitine supplementation is expected to decrease the lipid deposition and increase the protein deposition in fish, due to its action in lipid metabolism (Harpaz, 2005; Mohseni & Ozorio, 2014). However, akin to what happens regarding growth, the action of L-carnitine on the lipid compo-sition of fish is also inconsistent. Several authors have not reported any effect on the body and/or muscle lipid composition of several species (Gaylord & Gatlin, 2000; Twibell & Brown, 2000; Dias *et al*., 2001; Yilmaz *et al*., 2004), including tilapias (Becker *et al*., 1999; Yang *et al*., 2009; Erdogan *et al*., 2015). It is possible that the location of adipocytes in body tissues are responsible for the interspecific differences in body chemical composition of fish when dietary L-carnitine is supplemented (Yang *et al*., 2012).

In the present study, the dietary DL-carnitine supplementation decreased the muscle lipid deposition, but it did not decrease the whole-body lipid or the liposomal fat deposition of fingerlings of the Nile tilapia. This result is similar to the one reported by Ma *et al*. (2008) for juvenile black sea bream *Sparus microcephalus*. Yang *et al*. (2012) also report an ambiguous result on L-carnitine in body lipids of juvenile silver perch *Bidyanus bidyanus*. These authors registered a decrease in whole-body lipid, but not in muscle lipid concentration of fish feeding supplemented diets with 400 mg kg^{-1} of L-carnitine.

Tilapias deposit lipids primarily in the form of intraperitoneal fat due to excess dietary energy (Han *et al*., 2011). However, fish were at an early growth stage, when high-energy demand results in minimal fat deposition (Shearer, 1994). The concentration of muscle carnitine is directly associated with dietary supplementation of L-carnitine (Gaylord & Gatlin, 2000; Yang *et al*., 2012), since, in the body of animals, the muscle is the primary accumulation site (Harpaz, 2005). Hence, the decrease of body lipids of tilapia fingerlings that stems from the dietary supplementation of 3000 mg kg^{-1} of DL-carnitine is likely related to the accumulation of L-carnitine in muscle, as hypothesized by Ma *et al*. (2008).

Comparative evaluations of results drawn for different species under varying infrastructural and animal management conditions are a rather complex task, especially because many studies do not report origin, level of purity and/or the chemical form of the L-carnitine source which was used. Several pharmaceutical formulations and supplements are manufactured with racemate (DL-carnitine), although only the levorotatory form is biologically active (Sánchez-Hernández *et al*., 2010a, 2010b; Dąbrowska & Starek, 2014). Therefore, considering that no effect on growth promotion and decreasing muscle lipid contents has already been reported for different species' fed diets supplemented with L-carnitine (Harpaz, 2005), it is fair to infer that, in some cases, manufacturers

Table 2. Growth performance of Nile tilapia fingerlings fed with graded levels of DL-carnitine in free-animal protein diets after 70 days. FW: final weight (g), WG: weight gain [WG (%) = 100×(final weight-initial weight/initial weight)], SGR: specific growth rate [SGR (% live weight per day) = (ln final weigh-in initial weight)/experimental period in days], DFI: daily feed intake index [DFI (% live weight per day) = 100×feed intake/(final weight+initial weight)/2/experimental period in days], FCR: feed conversion ratio (FCR = feed intake/weight gain), PPV: protein productive value [PPV (%) = (final weight × final whole body protein)-(initial weight × initial whole-body protein)/crude protein intake].

Parameters	DL-carnitine levels (mg kg^{-1})						
	0	500	1000	1500	2000	3000	4000
FW	21.2 ± 2.3	23.0 ± 0.7	21.0 ± 1.9	23.7 ± 0.9	20.8 ± 1.7	21.4 ± 1.9	21.5 ± 2.4
WG	2481 ± 312	2725 ± 101	2466 ± 230	2799 ± 10	2424 ± 169	2544 ± 231	2508 ± 267
SGR	4.6 ± 0.2	4.8 ± 0.1	4.6 ± 0.1	4.8 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.7 ± 0.1
DFI	3.5 ± 0.1	3.4 ± 0.1	3.6 ± 0.1	3.4 ± 0.1	3.7 ± 0.3	3.6 ± 0.3	3.7 ± 0.2
FCR	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
PPV	33.1 ± 1.5	35.2 ± 1.0	32.6 ± 2.0	33.9 ± 1.4	31.0 ± 2.0	32.8 ± 2.9	33.4 ± 3.1

Table 3. Chemical composition (wet basis) of Nile tilapia fingerlings fed with graded levels of DL-carnitine in free-animal protein diets after 70 days.

Parameters	DL-carnitine levels (mg kg^{-1})						
	0	500	1000	1500	2000	3000	4000
Whole body							
Dry matter (%)	26.5 ± 0.2	25.1 ± 0.8	26.0 ± 1.2	25.3 ± 0.6	25.6 ± 2.4	25.5 ± 1.3	26.0 ± 0.8
Ash (%)	2.5 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.6 ± 0.1
Protein (%)	14.3 ± 0.3	13.9 ± 0.4	14.3 ± 0.6	14.1 ± 0.3	14.2 ± 1.0	14.2 ± 0.4	14.4 ± 0.5
Muscle							
Dry matter (%)	21.8 ± 0.2	21.7 ± 0.5	22.3 ± 0.2	21.5 ± 0.3	22.0 ± 0.1	21.8 ± 0.3	22.1 ± 0.1
Protein (%)	18.1 ± 0.3	18.1 ± 0.4	18. 5 ± 0.2	17.7 ± 0.2	18.3 ± 0.3	18.1 ± 0.1	18.4 ± 0.2

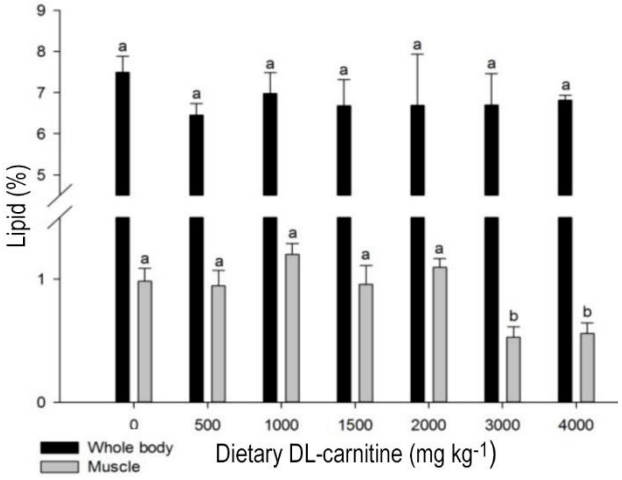

Figure 1. Lipid content of Nile tilapia fingerlings fed with graded levels of DL-carnitine in free-animal protein diets after 70 days. Different letters denote differences by Tukey's Studentized Range Test ($P < 0.05$).

of supplements may have used DL-carnitine. This can be a possible but yet partial, explanation for the conflicting results reported in studies on the supplementation of L-carnitine to fish diets.

Therefore, albeit the fact that dietary DL-carnitine supplementation did not promote an increase in Nile tilapia performance, there was a decrease in the deposition of muscle lipids of tilapia fingerlings. Despite the similarity of these results with other previous studies on the use of pure L-carnitine in fish, and considering to this supplement's low cost, we find it premature to rule out the eventual use of this source of supplementary L-carnitine in aquafeeds.

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