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Research Article

Crude fat, digestible protein and DL-carnitine levels in plant-based diets to Nile tilapia *Oreochromis niloticus* fingerlings

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ABSTRACT. The chemical composition of diets is appointed as one responsible by different results of L-carnitine supplementation in fish. This study determined the effects of digestible dietary protein (DP; 250 and 290 g kg^{-1}) and crude fat (CF; 100 and 150 g kg^{-1}) at levels of DL-carnitine supplementation (DLC; 0 and 1.0 g kg^{-1}) in plant based-diets on growth and whole-body composition of Nile tilapia *Oreochromis niloticus* fingerlings (1.55 ± 0.03 g). Fish were hand-fed for 74 days, three times per day until apparent satiation, in a completely randomized experimental design, 2×2×2 factorial scheme (n = 3). There was no effect ($P > 0.05$) of DP, CF, and DLC, or interactions, on growth performance. There was a significant interaction between DP×CF; DP×DLC and DLC×CF on feed efficiency. There was interaction ($P < 0.05$) between CF×DLC on feed intake (DFI). The DLC supplementation in 100 g kg^{-1} CF diets increased DFI 5.6% more than fish fed with the same fat level without DLC. Whole-body protein decreased significantly due to DLC supplementation in fish fed with 150 g kg^{-1} CF diets. The level of DLC supplementation in Nile tilapia diets is variable and should consider the dietary CF levels, but not DP.

Keywords: *Oreochromis niloticus*; fingerlings; feed additive; fish nutrition; growth; lipid metabolism; aquaculture

INTRODUCTION

Dietary protein and lipids are the primary nutrients in aquafeeds used as energy sources by fish. However, protein also is the costliest macro-nutrient required in fish diets, and when excessively provided, it increases the environmental and economic losses in aquaculture. Moreover, animal protein sources used in formulating fish feeds are more expensive than vegetable protein sources. Consequently, the latter ones are used in high proportion to formulate diets for omnivorous species. Additionally, increase dietary lipids levels to spare protein as an energy source is a usual strategy to reduce the cost of formulation of fish diets, even for omnivorous species as Nile tilapia *Oreochromis niloticus* (Stickney & Hardy, 1989; De Silva *et al.*, 1991; Lim *et al.*, 2011).

L-carnitine (LC) is a water-soluble quaternary amine synthesized endogenously from lysine and methionine and essential to mitochondrial β-oxidation of long-chain fatty acids (Bilinski & Jonas, 1970). Plant ingredients are poor sources of LC (usually 0.5 mg kg^{-1}) than animal protein sources that contain until 1.2 g kg^{-1} (Demarquoy *et al.*, 2004; Harpaz, 2005). Thus, in a future perspective of the manufacture of aquafeeds with high levels of lipid and plant protein sources, it can be necessary to supplement LC in fish diets. L-carnitine supplementation can be especially necessary for young fish due to their limited endogenous production (Harpaz, 2005).

In recent years, several studies aimed to determine the level of LC supplementation to promote growth, reproduction features, and other physiological responses in different fish species (Lu *et al.*, 2019; Rodrigues *et al.*, 2019; Wang *et al.*, 2019). However, results obtained until now remain controversial, even if related to the same species, such as in studies with African catfish (*Clarias gariepinus*) (Torreele *et al.*, 1993; Ozório *et al.*, 2001a,b; 2002; Naz *et al.*, 2005), rainbow trout (*Oncorhynchus mykiss*) (Schuhmacher & Gropp, 1998; Dikel *et al.*, 2010; Jalali-Haji-Abadi *et al.*, 2010; Selcuk *et al.*, 2010; Ozório *et al.*, 2012), and

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European seabass (*Dicentrarchus labrax*) (Santulli & D'Amelio, 1986; Chatzifotis *et al.*, 1995; Dias *et al.*, 2001). This variability of effects from dietary LC supplementation in fish is attributed to different factors, among which the nutritional profile of diets is considered significant (Harpaz, 2005; Mohseni & Ozório, 2014).

Thus, this study aimed to evaluate the effects of digestible dietary protein (DP), crude fat (CF) and DL-carnitine levels, and their interactions on growth, whole-body composition, and nutrient retention efficiency in Nile tilapia fingerlings fed high-fat plant-based diets.

MATERIALS AND METHODS

All experimental procedures were previously approved by the Ethics Committee on the Use of Animals of the Federal Rural University of Pernambuco (License N°009/2013).

Experimental diets

Eight experimental diets were formulated to contains two levels of dietary DP (250 and 290 g kg^{-1}), CF (100 and 150 g kg^{-1}) and DL-carnitine - DLC (0 and 1 g kg^{-1}) levels (Table 1), in a completely randomized experimental design and 2×2×2 factorial scheme (n = 3). Carnitine was added to diets as DLC hydrochloride (Sigma Aldrich®) that contains 50% of the active biological form (LC). Digestible protein levels were established to supply the minimum nutritional requirement (290 g kg^{-1}) for Nile tilapia (*Oreochromis niloticus*) according to NRC (2011) and below 250 g kg^{-1} to verify a possible protein-sparing effect by dietary supplementation of CF and DLC. Diets with low CF level (100 g kg^{-1}) were formulated to supply the minimum digestible energy (DE) requirement (14.2 MJ kg^{-1}) established by NRC (2011) for Nile tilapia. DE and DP of diets were calculated from data of apparent digestibility coefficients for Nile tilapia (Furuya *et al.*, 2010). The chemical composition of all ingredients was determined before diet formulation. Ingredient's digestible energy (DE) and DP were estimated from apparent digestibility coefficients for Nile tilapia calculated from Furuya *et al.* (2010). Diet ingredients were grounded through a 1.0 mm sieve, mixed, moistened with distilled water (30% v/w), and pelleted (2.0 mm) in a mincer and then dried in a forced ventilation oven (50ºC; 24 h). Dried pellets were hermetically packed in plastic bags and stored at -4ºC until use.

Experimental system and animals

Nile tilapia juveniles were provided by Companhia de Desenvolvimento do Vale do São Francisco e Parnaíba-CODEVASF (Porto Real do Colégio, Alagoas, Brazil). Fish were acclimatized to experimental condition for seven days, feeding on a 40% CP commercial diet.

Nile tilapia juveniles (1.55 ± 0.03 g) were randomly stocked into 24 glass aquaria (75-L capacity, filled to 60-L, 18 fish per aquarium). Each aquarium was supplied with a flow rate of 0.7 L min^{-1}, connected to a recirculation system, under continuous aeration, biological and mechanical filtration, temperature control, and 12:12 h light:dark photoperiod. Water oxygen (4.6 ± 0.2 mg L^{-1}) and temperature (27.0 ± 0.2ºC) were monitored daily with the aid of an oximeter (YSI model 550A). Alkalinity (22.5 ± 3.3 mg $CaCO_3$ g L^{-1}), pH (6.9 ± 0.3), hardness (91.3 ± 19.4 mg $CaCO_3$ L^{-1}), nitrite (0.99 ± 0.72 mg L^{-1}) and ammonia (0.01 ± 0.01 mg L^{-1}) were checked weekly using a commercial kit of the colorimetric methods (Alfakit, Florianopolis, SC, Brazil).

Fish were carefully hand-fed to apparent satiation daily (09:00, 13:00 and 17:00 h) for 74 days with the experimental diets. Feed intake was recorded for each experimental unit during the feeding trial. Fish were fasted for 24 h, sedated in alcoholic benzocaine solution (50 mg L^{-1}), and weighted every 14 days to evaluate growth performance.

Fish whole-body and diet chemical analyses

Initially, a pooled sample of fish from the original population was previously fasted (24 h) and euthanized by anesthetic overdoses (0.5 g L^{-1} benzocaine) to determine whole-body composition. After 74 days, fish from each aquarium were fasted as described above, sedated (0.05 g L^{-1} benzocaine solution), weighed, and counted. Ten fish were randomly sampled from each aquarium, euthanized by anesthetic overdose as described before, grounded, and freeze (-20ºC) until chemical analysis.

The chemical composition of the ingredients, experimental diets, and fish samples was analyzed according to the procedures recommended by the Association of Official Analytical Chemists (AOAC, 1990). Moisture was determined by the gravimetric method in an oven at 105°C until constant weight. Ash content was determined using the gravimetric method in a muffle furnace at 550°C for 24 h. Crude protein (N × 6.25) was determined by the micro-Kjeldahl method, and crude fat was determined by the Soxhlet method. Crude fiber content was determined by acid/base digestion.

Growth parameters

The following growth parameters were calculated: weight gain (WG, g $fish^{-1}$) = final weight - initial weight;

Table 1. Composition (g kg^{-1}) and chemical composition (g kg^{-1} dry matter) of experimental diets. [1]Guaranteed level (kg^{-1} product): vit. A, 1,000,000 IU; vit. D3, 312,500 IU; vit. E, 18,750 IU; vit. K3, 1,250 mg; vit. B1 (thiamine), 2,500 mg; vit. B2 (riboflavin), 2,500 mg; vit. B6 (pyridoxine), 1,875 mg; vit. B12, 4 mg; vit. C, 31,250 mg; nicotinic 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 sulfate, 6,250 mg; copper sulfate, 625 mg; zinc sulfate, 6,250 mg; manganese sulfate, 1,875 mg; sodium selenite, 13 mg; calcium iodate, 63 mg; and cobalt sulfate, 13 mg. [2]DL-carnitine hydrochloride (50% L-carnitine) - Sigma Aldrich® (C9500), [3]Cellulose MC101 - Rhoster®. Araçoiaba da Serra-SP, [4]Calculate from Furuya *et al.* (2010) and Rostagno *et al.* (2011).

Ingredient	Crude fat 100 g kg^{-1}				Crude fat 150 g kg^{-1}			
	Digestible protein 250 g kg^{-1}		Digestible protein 290 g kg^{-1}		Digestible protein 250 g kg^{-1}		Digestible protein 290 g kg^{-1}	
	DL-carnitine 0 g kg^{-1}	1.0 g kg^{-1}	0 g kg^{-1}	1.0 g kg^{-1}	DL-carnitine 0 g kg^{-1}	1.0 g kg^{-1}	0 g kg^{-1}	1.0 g kg^{-1}
Soybean meal	400	400	470	470	400	400	470	470
Corn grain	170	170	170	170	170	170	170	170
Corn gluten meal	81	81	100	100	81	81	100	100
Starch	187	187	100	100	147	147	60	60
Soy oil	65	65	63	63	105	105	103	103
Yeast	50	50	50	50	50	50	50	50
Dicalcium phosphate	14	14	14	14	14	14	14	14
Vitamin and mineral supplement[1]	10	10	10	10	10	10	10	10
L-histidine	8	8	8	8	8	8	8	8
DL-methionine	6	6	6	6	6	6	6	6
DL-carnitine[2]	-	1	-	1	-	1	-	1
Inert[3]	6.2	5.2	6.2	5.2	6.2	5.2	6.2	5.2
BHT	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Chemical composition								
Dry matter (g kg^{-1})	953.1	929.1	952.4	945.8	952.6	932.4	951.5	942.9
Crude protein (g kg^{-1})	312.7	300.4	347.4	350.5	304.2	303.8	346.8	349.1
Crude fiber (g kg^{-1})	26.4	27.4	29.4	34.6	27.7	26.3	27.5	38.3
Crude fat (g kg^{-1})	106.6	102.5	119.2	105.2	151.7	149.6	157.4	160.5
Ash (g kg^{-1})	48.2	46.1	53.2	53.1	48.0	46.7	53.7	54.0
Digestible energy (MJ kg^{-1})[4]	14.0	14.0	14.2	14.2	15.0	15.0	15.2	15.2

specific growth rate (SGR, % biomass d^{-1}) = 100 × [(ln final weight - ln initial weight) / days of the trial]; survival rate (SUR, %) = 100 × (final number of fish each aquarium / initial number of fish); daily feed intake (DFI, % biomass d^{-1}) = {feed intake / [(final weight + initial weight) / 2] / days of trial×100}; feed efficiency ratio (FER) = weight gain / total feed intake; protein productive value (PPV, %) = [(final body weight × final whole-body protein $_{(\%\ wet\ basis)}$) - (initial body weight × initial whole-body protein $_{(\%\ wet\ basis)}$)] / total protein intake; and energy productive value (EPV, %) = [(final body weight × final whole-body energy $_{(\%\ wet\ basis)}$) - (initial body weight × initial whole-body energy $_{(\%\ wet\ basis)}$)] / total energy intake.

Statistical analysis

Results were previously tested for normality (Shapiro-Wilk test) and homoscedasticity of variances (Bartlett's test) and submitted to a three-way analysis of variance (ANOVA). Significant effects of the treatments were compared by Tukey's test ($P < 0.05$). All analyses were carried out in the software SAS v.9.1.

RESULTS

There was no significant effect of treatments (CF, DP, and DLC levels) and interaction among them on fish growth and survival rate (Table 2). DFI of fish decreased ($P < 0.05$) and FER increased ($P < 0.05$) with increasing dietary DP levels (Table 3). FER of fish was significantly affected by CF×DP interaction (Fig. 1). Additionally, there was a significant interaction between CF and DLC on DFI and FER (Fig. 2). Fish fed 100 CF/1 g kg^{-1} DLC diets increased ($P < 0.05$) feed intake. FER also was significantly affected by CF×DLC interaction, in which increasing levels of CF without DLC supplementation resulted in a decrease ($P < 0.05$) of feed efficiency of fish. FER of fish also was reduced ($P < 0.05$) by DLC supplementation when fish fed 100 g kg^{-1} CF.

Fish fed diets containing 290 g kg^{-1} of DP showed lower PPV ($P < 0.05$) than fish fed with the 250 g kg^{-1} DP diets. A significant triple interaction (CF×DP×DLC) was registered on PPV (Table 3, Fig. 3). The DLC supplementation did not influence ($P > 0.05$) the PPV in any combination of CF×DP. However, when DLC was supplemented and DP level increased in 100 g kg^{-1} CF diets, there was a reduction of PPV ($P < 0.05$). The same trend was registered when DP increased in unsupplemented diets and 150 g kg^{-1} of CF. On the other hand, EPV increased with carnitine supplementation ($P < 0.05$) and significantly decreased when dietary CF and DP levels increased, but no significant interaction of CF, DP, or DLC levels was registered on energy retention of fish.

The whole-body composition of Nile tilapia *Oreochromis niloticus* is shown (Table 4). Increasing dietary DP resulted in increased body protein contents ($P < 0.05$) and decreased moisture and lipid contents ($P < 0.05$). On the other hand, increase CF from 100 to 150 g kg^{-1} resulted in increased body lipid contents ($P < 0.05$). There was a significant effect of DLC supplementation interacting with CF on whole-body protein and ash (Table 4, Fig. 4). Body protein decreased significantly due to DLC supplementation in fish fed with 150 g kg^{-1} CF diets. However, no significant effect on body protein was registered in fish fed diets with the lowest CF level, regardless of dietary DLC supplementation. Whole-body ash as significantly reduced in fish fed 150 g kg^{-1} CF and DLC supplemented.

DISCUSSION

As previously reported for other fish species, LC supplementation for tilapia also presented controversial results. Similar to this study, Nile tilapia *Oreochromis niloticus* fed diets supplemented with LC from 0.25 g kg^{-1} (Yang *et al.*, 2009) to 1.0 g kg^{-1} (Erdogan *et al.*, 2015) also presented no effects on growth parameters. On the other hand, supplementation of 0.15, 0.50, and 0.90 g kg^{-1} LC increased weight gain, respectively, for hybrid tilapia (*O. niloticus* × *O. aureus*) (Becker *et al.*, 1999), Nile tilapia (Dikel *et al.*, 2003) and Mozambique tilapia *O. mossambicus* (Jayaprakas *et al.*, 1996).

Although the role of LC in fish growth is still unclear (Ma *et al.*, 2008), its participation on mitochondrial fatty acids β-oxidation could improve the efficiency of diet energy utilization and protein-sparing effect resulting in better growth (Harpaz, 2005; Arslan, 2006; Mohseni & Ozório, 2014). Contradictory results of carnitine supplementation on fish growth can be attributed to several factors, such as development stage of animals, diet composition, the relationship between LC and dietary lysine/methionine, experimental time and environmental conditions, supplementation level, possible leaching of carnitine in feed pellets, the chemoattractant action of carnitine, the stability of pellets and metabolic requirements of fish (Dias *et al.*, 2001; Ozório *et al.*, 2001b; Harpaz, 2005).

For instance, Becker *et al.* (1999), Dikel *et al.* (2003), and Jayaprakas *et al.* (1996) performed the experimental trial in intensive outdoor systems. In outdoor systems, fish are subjected to different sources of stress (*e.g.*, xenobiotics, temperature variation, cyanotoxins) in which carnitine supplementation provided some protection (Harpaz *et al.*, 1999; Schlechtriem

Table 2. Growth performance of Nile tilapia fingerlings feeding dietary levels of crude fat (CF), digestible protein (DP), and DL-carnitine (DLC) for 74 days. FW: final weight, WG: weight gain, SGR: specific growth rate, SR: survival rate.

CF ($g\ kg^{-1}$)	DP ($g\ kg^{-1}$)	DLC ($g\ kg^{-1}$)	FW ($g\ fish^{-1}$)	WG ($g\ fish^{-1}$)	SGR $\%\ d^{-1}$	SR (%)
100			23.1 ± 2.3	21.6 ± 2.4	3.7 ± 0.2	91.2 ± 7.7
150			21.3 ± 2.4	21.3 ± 2.4	3.5 ± 0.2	89.8 ± 8.2
	250		22.3 ± 2.9	20.8 ± 2.8	3.6 ± 0.2	93.1 ± 5.9
	290		22.1 ± 2.3	20.6 ± 2.3	3.3 ± 0.1	87.9 ± 8.5
		0	21.8 ± 3.0	20.2 ± 3.0	3.6 ± 0.2	92.6 ± 5.5
		1	22.6 ± 2.0	21.1 ± 2.0	3.6 ± 0.1	88.4 ± 9.0
Three-way ANOVA (*P*- values)						
CF			0.0906	0.0931	0.0676	0.6639
DP			0.8368	0.8097	0.9786	0.1242
DLC			0.4084	0.3880	0.4729	0.2033
CF×DP			0.3618	0.3792	0.3287	0.8845
CF×DLC			0.2013	0.1785	0.1537	0.4719
DP×DLC			0.2618	0.2362	0.2097	0.6643
CF×DP×DLC			0.8051	0.8354	0.9146	0.3174

Table 3. Feed and nutrient utilization of Nile tilapia fingerlings feeding dietary levels of crude fat (CF), digestible protein (DP), DL-carnitine (DLC) for 74 days. DFI: daily feed intake (% biomass d^{-1}), FER: feed efficiency ratio, PPV: productive protein value (%), EPV: productive energy value (%). Values in the same column with different superscript denote significant differences according to Tukey's test ($P < 0.05$).

CF ($g\ kg^{-1}$)	DP ($g\ kg^{-1}$)	DLC ($g\ kg^{-1}$)	DFI	FER	PPV	EPV
100			3.33 ± 0.17	0.72 ± 0.04	28.6 ± 1.6	39.1 ± 6.5^{a}
150			3.33 ± 0.09	0.71 ± 0.02	29.0 ± 1.9	31.5 ± 5.2^{b}
	250		3.38 ± 0.11^{a}	0.70 ± 0.02^{a}	30.0 ± 1.1^{a}	39.7 ± 5.9^{a}
	290		3.27 ± 0.13^{b}	0.73 ± 0.03^{b}	27.6 ± 1.2^{b}	30.8 ± 4.7^{b}
		0	3.29 ± 0.13	0.72 ± 0.04	28.9 ± 1.5	33.8 ± 6.2^{a}
		1	3.37 ± 0.13	0.71 ± 0.02	28.7 ± 1.9	36.8 ± 7.5^{b}
Three-way ANOVA (*P*- values)						
CF			0.9558	0.1594	0.3605	<.0001
DP			0.0228	0.0060	<.0001	<.0001
DLC			0.1080	0.0760	0.1463	0.0502
CF×DP			0.1312	0.0223	0.1183	0.4505
CF×DLC			0.0285	0.0094	0.7498	0.9230
DP×DLC			0.5209	0.0511	0.0594	0.4336
CF×DP×DLC			0.3013	0.5360	0.0155	0.9552

et al., 2004; Guzmán-Guillén *et al.*, 2013). On the other hand, this trial was performed in controlled and ideal conditions, without stressful factors and in this case, no effect of dietary DLC supplementation on fish growth was observed, in accordance to Akbari *et al.* (2014).

Fish consume feed to meet their energy requirements, and consequently, the concentration of this nutrient in formulated diet limits feed intake. In this trial, fish fed diets containing 100 g kg^{-1} CF meet the minimum DE requirement, according to NRC (2011). However, Nile tilapia can make adjustments in their consumption, even at energy concentrations slightly above or below the minimum nutritional requirement (Fortes-Silva & Sánchez-Vázquez, 2012). In this case, DLC supplementation in 100 g kg^{-1} CF diets stimulated fish feed intake to metabolize the substrate to perform the β-oxidation due to the increased levels of circulating LC. It should be noted that feed intake of fish fed 150 g kg^{-1} CF diets was not different regardless of carnitine supplementation (Fig. 2).

There is a close relationship between dietary LC, lipids levels, and body LC concentration in fish (Gaylord & Gatlin, 2000; Ozório *et al.*, 2001a). For example, Ozório *et al.* (2010) recorded an increase of

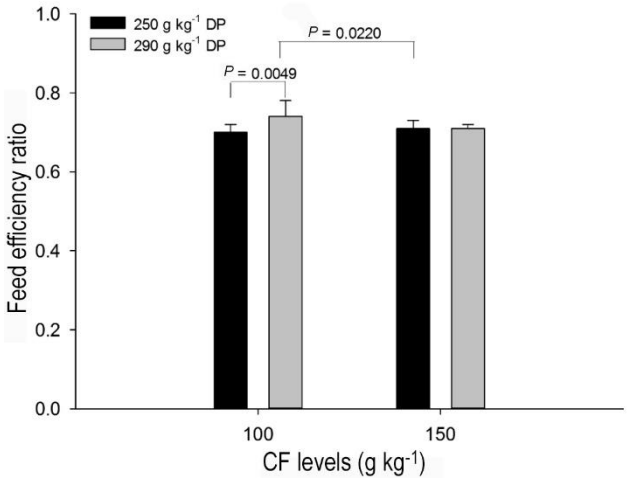

Figure 1. Interaction between dietary crude fat (CF) and digestible protein (DP) levels on feed efficiency ratio of Nile tilapia juveniles. Bars show the significant differences with respective *P* values between these factors.

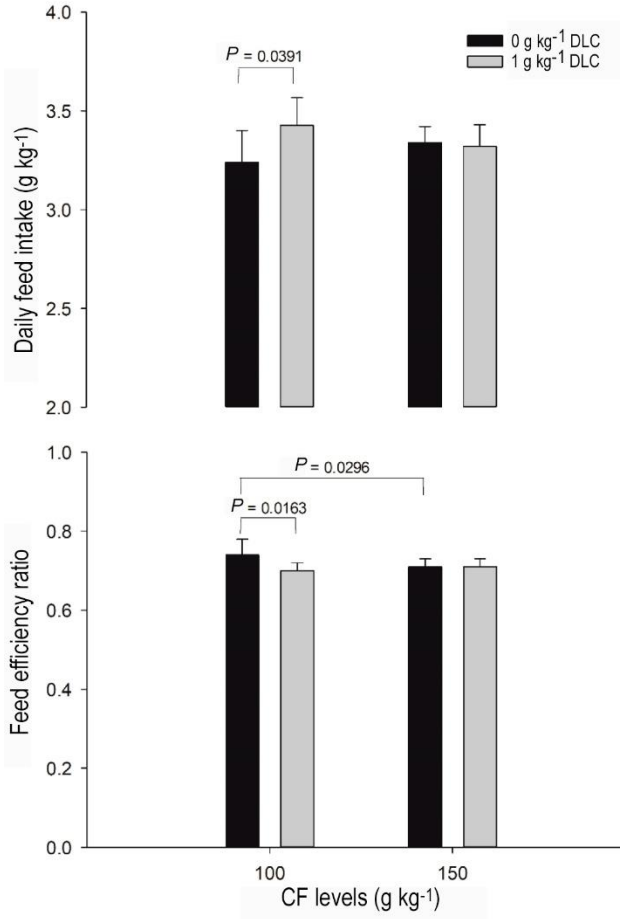

Figure 2. Interaction between dietary crude fat (CF) and DL-carnitine (DLC) levels on intake and use of feed by Nile tilapia juveniles. Bars show the significant differences with respective *P* values between these factors.

30% in muscle and plasma carnitine levels in African catfish *Clarias gariepinus* when dietary lipid increased from 100 to 180 g kg^{-1}. The LC circulating in the body is a combination of supplemental sources, through biosynthesis and reabsorption rates (Rebouche & Seim, 1998). In the present study, when lipid content increased in DLC-unsupplemented diets, it was observed a reduction in feed efficiency (Fig. 2). Additionally, it was registered a decrease in FER when 100 g kg^{-1} CF diets were supplemented with DLC (Fig. 2). These results suggest that endogenous LC concentration in Nile tilapia juveniles was not enough to oxidize dietary lipid content. Gaylord & Gatlin (2000) reported variations in muscle concentrations of LC of striped bass × white bass hybrids (*Morone saxatilis* × *M. chrysops*) supplemented with 3.0 g kg^{-1} LC diet as a function of the dietary lipid content (5 and 20%).

The mechanism by which this occurs is still unclear. However, Ozório *et al.* (2010) concluded that the diet's lipid level could influence the transport and use of LC. Therefore, LC concentrations required to improve feed efficiency may be directly linked to the CF concentration of the diet, corroborating the observations of Torreele *et al.* (1993), Ozório *et al.* (2001a) and Jalali-Haji-Abadi *et al.* (2010). Moreover, the decrease feed efficiency herein observed in fish fed DLC at 100 g kg^{-1} CF diets can be explained by additional energy expenditure due to excessive circulating LC that was not used in the β-oxidation of the fatty acids.

The whole-body protein retention rate decreased when dietary DP was increased from 250 to 290 g kg^{-1}. Therefore, it was observed that dietary DP reduced whole-body protein retention, indicating that 250 g kg^{-1} was sufficient to meet fish protein requirements and, that 290 g kg^{-1} of dietary DP provided protein excess. These results suggest that excess protein intake from the diet can reduce the efficiency of protein utilization by fish and decreased growth. When dietary amino acids are not utilized to protein synthesis, it deviates as an energy source, lipogenesis or gluconeogenesis.

The present study used DLC as a source of a biologically active form of carnitine (LC). An important aspect of supplementation of LC in aquafeeds is the high price of purified levocarnitine form, once effective levels to increase fish growth are 10-15 times higher than preconized for poultry (Golzar-Adabi *et al.*, 2011). Becker *et al.* (1999) suggested that dietary supplementation over 0.15 g kg^{-1} of LC for tilapia is economically infeasible. Therefore, Harpaz (2005) recommended a cost/benefit analysis when LC is included in fish diets. On the other hand, DLC is near ten times low cost than pure LC form and, probably due

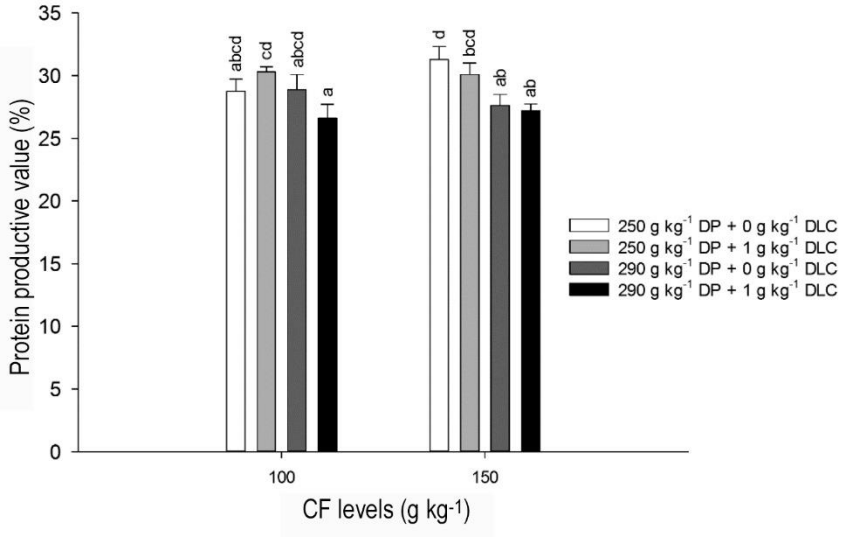

Figure 3. Interaction between dietary crude fat (CF), digestible protein (DP) DL-carnitine (DLC), and levels on protein retention by Nile tilapia juveniles. Different lowercases show significant ($P < 0.05$) differences by Tukey's test.

Table 4. Whole-body composition (wet basis) of Nile tilapia fingerlings feeding dietary levels of crude fat (CF), digestible protein (DP), and DL-carnitine (DLC) for 74 days. Values in the same column with different superscript denote significant differences according to Tukey's test ($P < 0.05$).

CF (g kg^{-1})	DP (g kg^{-1})	DLC (g kg^{-1})	Moisture	Crude fat	Ash	Crude protein
100			24.51 ± 0.79^{a}	5.78 ± 0.78^{a}	2.94 ± 0.18	13.09 ± 0.33
150			25.10 ± 0.63^{b}	6.50 ± 0.76^{b}	2.87 ± 0.22	13.16 ± 0.53
	250		25.20 ± 0.70^{a}	6.66 ± 0.69^{a}	2.94 ± 0.25	12.95 ± 0.46^{a}
	290		24.43 ± 0.65^{b}	5.63 ± 0.64^{b}	2.88 ± 0.14	13.27 ± 0.34^{b}
		0	24.55 ± 0.49	5.95 ± 0.66	2.95 ± 0.23	13.17 ± 0.43
		1	24.97 ± 0.92	6.33 ± 0.98	2.88 ± 0.17	13.07 ± 0.43
Three-way ANOVA (*P* values)						
CF			0.0503	0.0036	0.5863	0.8828
DP			0.0136	0.0002	0.2586	0.0326
DLC			0.1325	0.0873	0.1756	0.2387
CF×DP			0.1050	1.0000	0.6714	0.3112
CF×DLC			0.5726	0.1158	0.0179	0.0021
DP×DLC			0.0571	0.1733	0.0292	0.6178
CF×DP×DLC			0.6654	0.3562	0.0471	0.1981

to this feature, has been used in several pharmaceutical formulations and food supplements commercialized in different countries (Lopes *et al.*, 2004; Sánchez-Hernández *et al.*, 2010a,b).

According to Rebouche & Seim (1998), D-isomer competitively inhibits the carnitine acetyltransferase activity and, consequently, decreases the oxidation of fatty acids and the mitochondrial transport of long fatty acids. On the other hand, LC is assimilated in the intestine 1.6 to 2.0 times faster than the D-form (Gross & Henderson, 1984). Thus, the increase in EPV in fish fed DLC-diets shown a slight but effective biological effect of DLC on lipid metabolism, as reported by Silva *et al.* (2018).

Fish body and muscle lipid content can be reduced by LC supplementation due to its role in the oxidation of fatty acids (Harpaz, 2005). Additionally, LC supplementation provides an increase in body protein and muscle content (Keshavanath & Renuka, 1998). This typical response from the body and muscle composition by dietary LC supplementation has been observed in some fat fish, such as rohu (*Labeo rohita*) (Keshavanath & Renuka, 1998) and mrigal carp (*Cirrhinus cirrhosus*) (Singh *et al.*, 2008).

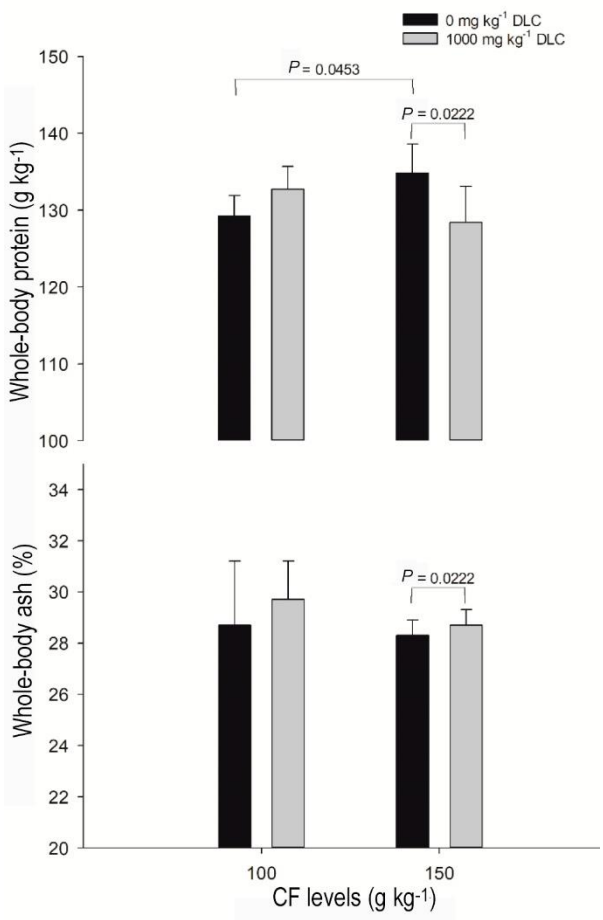

Figure 4. Interaction between dietary crude fat (CF) and DL-carnitine (DLC) levels on whole-body protein and ash contents of Nile tilapia juveniles. Bars show the significant differences with respective *P*-values between these factors.

In this trial, the whole-body lipid of the Nile tilapia juveniles was only influenced by the dietary CF and DP concentrations. Considering that the muscles are the main storage site of LC (Harpaz, 2005), Jalali-Haji-Abadi *et al.* (2010) hypothesized that the determination of muscle lipids in fat considered fish would be a better indicator of the lipid oxidation than the body fat. However, the Nile tilapia is considered a lean-meat species from the human consumption viewpoint (Jobling, 2001), and at first, there are no reasons for differences in the effect of DLC on the composition of the muscle and whole-body lipid. Besides, no effect of LC supplementation has been recorded on the chemical composition of other species (Berger & Sachan, 1991; Gaylord & Gatlin, 2000; Twibell & Brown, 2000; Dias *et al.*, 2001). Thus, as previously described for growth performance, the effects of LC supplementation on the body chemical composition of fish are still unclear, at least, contradictory.

In conclusion, dietary LC supplementation using DLC as a source did not improve the growth of Nile tilapia juveniles. Additionally, despite the LC source used in the present study, it was demonstrated a strong interaction between DLC and CF levels on several parameters related to feed and nutrient use. Thus, further studies are recommended to investigate the interaction between DLC and CF, aiming to determine an ideal dietary CF:LC ratio.

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