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Note

Effect of dietary supplementation of L-carnitine on growth, feed utilisation and liver histology in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) fry

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ABSTRACT

This study was undertaken to investigate the effect of dietary supplementation of L-carnitine on the growth performance and liver histology in fry of Nile tilapia, *Oreochromis niloticus*. Five isonitrogenous and isolipidic experimental diets were formulated to contain 0 (control), 250, 500, 750 and 1000 mg L-carnitine kg^{-1}, respectively. Fish were randomly distributed in groups of 20 fish per 120 l glass aquaria and fed the diets three times daily to apparent satiation in triplicate for 120 days. Final body weight, specific growth rate, feed conversion ratio, protein efficiency ratio, feed intake and whole body composition for fish fed with diets containing different levels of L-carnitine were not significantly different compared to fish fed the control diet. Fish exhibited normal liver histological structure. However, there was a comparatively low hepatic fat acumulation with increase in dietary L-carnitine supplementation. Results indicated that supplemental dietary L-carnitine had no beneficial effect on improving growth performance, feed utilisation and body composition of Nile tilapia fry.

Keywords: Feed utilisation, Growth, L-carnitine, Liver histology, *Oreochromis niloticus*

L-carnitine is a non-essential amino acid compound that is naturally biosynthesised from methionine and lysine in animal liver and kidney (Harpaz, 2005). It is required for transport of long chain fatty acids into the mitochondria for oxidation (Ozorio *et al*., 2002). Thus, supplementation of L-carnitine in animal feeds has a potential role to support growth, protein sparing effect of fat and consequently contributes to reduction of body fat deposition (Harpaz, 2005; Yang *et al*., 2012). Growth promoting effects of dietary L-carnitine supplementation were reported in tilapia (Jayaprakas *et al*., 1996; El-Sayed *et al*., 2010), common carp (Focken *et al*., 1997), black seabream (Ma *et al*., 2008), beluga sturgeon (Mohseni *et al*., 2008), Indian major carp (Singh *et al*., 2008), Asian catfish (Desai *et al*., 2010), rainbow trout (Dikel *et al*., 2010; Haji-Abadi *et al*., 2010) and silver perch (Yang *et al*., 2012). In contrast, some researchers failed to observe any beneficial effect of L-carnitine on growth performance in various species (Gaylord and Gatlin, 2000; Dias *et al*., 2001; Ozorio *et al*., 2002; Seyfabadi *et al*., 2003; Schlechtriem *et al*., 2004; Yilmaz *et al*., 2004; Yang *et al*., 2009; Kordi *et al*., 2012; Ozorio *et al*., 2012). Researchers indicated variability in response to L-carnitine on growth performance and tissue lipid composition in fish. The present study evaluated the effects of dietary L-carnitine supplementation on the growth and body composition of Nile tilapia fry with special emphasis on the effect of dietary L-carnitine on liver histology.

Five isonitrogenous (40% crude protein), isolipidic (6% crude lipid) and isoenergetic (3550 kcal g^{-1} DE) experimental diets were formulated to contain different levels of L-carnitine (0, 250, 500, 750 and 1000 mg kg^{-1}). Diets were prepared as per Aydın and Gumus (2013). Details of formulation of diets and their proximate composition are shown in Table 1.

Experiment was carried out at the fish research unit, Ortaca Vocational School, Mugla Sıtkı Kocman University, Turkey, from 21 July, 2009 to 20 October, 2009. Nile Tilapia fry were obtained from the same fish research unit. Fish were fed with control diet three times daily during the acclimation period for about 2 weeks prior to initiation of the experiment. At the beginning of the experiment, 20 fry (average weight 2 g) per aquarium (120 l) were randomly distributed into 15 glass aquaria. Dietary treatments were randomly assigned to triplicate aquaria and fish were hand fed to apparent satiation three times daily (09:00, 12:00 and 16:00 hrs) for 120 days. Fish weights and amount of feed consumed were measured weekly. Any uneaten feed was collected 1 h after each feeding, dried to constant weight at 70°C and reweighed. Dechlorinated tap water was supplied to the experimental

Table 1. Formulation and proximate composition of the experimental diets.

Ingredients (%)	Experimental treatments (L-carnitine content mg kg^{-1})				
	0	250	500	750	1000
Fish meal	46.00	46.00	46.00	46.00	46.00
Soybean meal	26.50	26.00	25.50	25.00	24.50
Corn meal	9.50	8.75	8.50	8.25	9.00
Maize starch	2.00	3.00	3.50	4.00	3.50
L-carnitine	0.00	0.25	0.50	0.75	1.00
Fish oil	3.00	3.00	3.00	3.00	3.00
Mineral premix[1]	3.00	3.00	3.00	3.00	3.00
Vitamin premix[2]	2.00	2.00	2.00	2.00	2.00
$CaHPO_4 2H_2O$[3]	4.00	4.00	4.00	4.00	4.00
Sodium chloride (NaCl)	1.00	1.00	1.00	1.00	1.00
Cellulose	1.00	1.00	1.00	1.00	1.00
Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00
Methionine	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100
Proximate composition (%, wet wt.)[4]					
Moisture	9.34±0.16	9.53±0.20	9.62±0.26	9.64±0.16	9.68±0.24
Crude protein	40.74±0.77	40.16±0.04	40.54±0.57	40.53±0.79	40.89±0.34
Crude lipid	6.40±0.19	5.94±0.21	5.96±0.37	5.93±0.11	5.96±0.07
Crude ash	15.88±0.31	16.01±0.26	16.10±0.42	16.38±0.60	16.31±0.21
Crude fiber	3.96±1.53	4.36±1.45	5.11±1.87	6.27±0.97	6.79±1.05
Dietary energy (kcal kg^{-1})	3561	3548	3538	3527	3523

[1] per kg mix: 60000 mg manganese, 10000 mg iron, 75000 mg zinc, 5000 mg copper, 1000 mg cobalt, 2500 mg iodine, 100 mg selenium, 65000 mg magnesium
[2] per kg mix: 4 000000 IU vitamin A, 600000 IU vitamin D3, 40000 mg vitamin E, 2400 mg vitamin K3, 5000 mg vitamin B1, 8000 mg vitamin B2, 4000 mg vitamin B6, 12 mg vitamin B12, 40000 mg vitamin C, 50000 mg niacin, 1400 mg folic acid, 8000 mg calcium D-pantothenate, 50 mg D-biotin, 40000 mg inositol
[3]Calcium hydrogen phosphate
[4]Means (mean±SD) of three replicate analysis

tanks. Continuous aeration was supplied to each tank with compressed air from a central compressor. Water temperature was also maintained constant with a 100 W automatic heater set. Water temperature and dissolved oxygen were recorded daily using a Model WTW Oxi 330i multi-oxygen meter (WTW Wissenschaftlich-Weilheim, Germany). Photoperiod (14 h L/10 h D) and light intensity (100 lux) were kept constant.

On termination of the experiment, all fish in each aquarium were collected, anesthetised with 200 mg l^{-1} clove oil and weighed individually. Five fish were sampled per treatment for carcass analyses. The carcasses were stored at -20°C for the whole body composition analysis. Chemical compositions of fish and experimental diets were performed according to AOAC (1995). Moisture content was determined by drying to constant weight at 105°C, crude protein (N×6.25) was determined by the Kjeldahl method after an acid digestion (Gerhardt Analytical Systems; Konigswinter, Germany). Crude lipid was determined by the ether extraction method using the Soxtec System HT (Behr Soxtec System KV5M; Dusseldorf, Germany). Ash content was determined in a muffle furnace at 550°C for 8 h, fibre by acid and alkaline extraction using a Whelp model extractor.

Liver samples for histological examinations were collected from 3 fish per tank at the end of the experiment. Samples were fixed in 10% phosphate buffered formalin with a pH of 7.2 and stored at room temperature. After dehydration following standard procedures, the samples were embedded in paraffin. Serial sections (5 μm thick) were cut and stained with hematoxylin and eosin (H&E) (Martoja and Martoja-Pierson, 1970). Histological sections were examined by light microscopy (Olympus CX41, Japan).

Following growth parameters were calculated at the end of the experiment: Specific growth rate (SGR, % day^{-1}) = 100 x [Ln final wt (g) - Ln initial wt (g)] / days; Feed conversion ratio (FCR) = feed intake (g) / [final wt (g) - initial wt (g)]; Protein efficiency ratio (PER) = [final wt (g) - initial wt (g)] / protein intake (g); Hepatosomatic index (HSI, %) = 100 x [liver wt (g) / final wt (g)]; Viscerosomatic index (VSI, %) = 100 x [visceral wt (g) / final wt (g)]; Survival rate (SR, %) = 100 x [final fish number / initial fish number].

All data were subjected to one-way ANOVA in SPSS version 15.0 (SPSS INC. Chicago, IL, USA). Differences among the means were compared by Duncan's multiple range test at a 5% probability level. Results are presented as mean±SD.

Water quality parameters, such as temperature (26 ± 1°C), pH (8.12 ± 0.10), dissolved oxygen (5.34 ± 0.05 mg L^{-1}), nitrite (0.11 mg L^{-1}) and nitrate (8.98 mg L^{-1}) were measured weekly and were within acceptable limits during the experimental period. The growth performance and feed utilisation values of experimental fish fed different diets are given in Table 2. Survival rate of experimental fish ranged between 81.67% and 96.67%, and significantly affected by dietary treatments ($p<0.05$). Survival rate decreased as level of L-carnitine increased in the diet. The growth performance and feed utilisation were significantly affected by dietary L-carnitine supplementation ($p<0.05$). The final weight, SGR, PER, feed intake (FI) and FCR values for fish fed diets with inclusion up to 750 mg kg^{-1} L-carnitine were similar to fish fed the control diet. Lower growth performance and feed utilisation were recorded above 750 mg kg^{-1} dietary L-carnitine supplementation. Increased growth performance and feed utilisation by L-carnitine supplementation in the diets have been reported for Atlantic salmon at an L-carnitine level of 740 mg kg^{-1} (Ji *et al*., 1996), rohu at 500 mg kg^{-1} (Keshavanat and Renuka, 1998), beluga sturgeon at 300 mg kg^{-1} (Mohseni *et al*., 2008), black seabream at 240 mg kg^{-1} (Ma *et al*., 2008), Indian major carp at 250 mg kg^{-1} (Singh *et al*., 2008), Nile tilapia at 450 mg kg^{-1} (El-Sayed *et al*., 2010), and rainbow trout at 1 mg kg^{-1} (Haji-abadi *et al*., 2010). However, insignificant growth effects of dietary L-carnitine were observed in European seabass (Dias *et al*., 2001), catfish (Ozorio *et al*., 2002; Yilmaz *et al*., 2004), tilapia (Schlechtriem *et al*., 2004; Yang *et al*., 2009), Caspian roach (Kordi *et al*., 2012) and rainbow trout (Ozorio *et al*., 2012).

In the present study, no statistically significant difference was recorded in VSI ($p>0.05$), but dietary L-carnitine significantly affected the HSI of fish ($p<0.05$) (Table 2). HSI values decreased significantly ($p<0.05$) with dietary L-carnitine supplementation and reached the lowest in fish fed diet containing up to 500 mg of L-carnitine kg^{-1} diet. However, the value of HSI increased at supplementation levels above 750 mg kg^{-1} dietary L-carnitine. Similar results have been previously reported in Indian major carps (Singh *et al*., 2008). Histological examination of liver tissue in this study indicated no abnormalities in various treatments (Fig. 1). Liver of fish fed the experimental diets demonstrated normal hepatocytes. However, comparatively low hepatic lipid acumulation was observed in all treatment groups compared to the control group.

There were no significant differences in final whole body crude protein, lipid, or ash content of fish fed the experimental diets except in the moisture content ($p>0.05$) (Table 3), similar to the findings in common carp (Focken *et al*., 1997), Caspian white fish (Seyfabadi *et al*., 2003) and hybrid tilapia (Yang *et al*., 2009). In contrast, whole

Table 2. Growth parameters in Nile tilapia fry (*Oreochromis niloticus*) fed with different levels of L-carnitine[1].

Parameters[2]	Experimental treatments (L-carnitine mg kg^{-1})				
	0	250	500	750	1000
IBW (g $fish^{-1}$)	2.05	2.05	2.06	2.04	2.07
FBW (g $fish^{-1}$)	13.02±1.43^{a}	11.55±0.70^{ab}	13.00±0.62^{a}	12.28±0.58^{ab}	9.91±0.51^{b}
BWG (g $fish^{-1}$)	10.97±1.42^{a}	9.50±0.70^{ab}	10.94±0.63^{a}	10.24±0.56^{ab}	7.84±0.52^{b}
SGR (% day^{-1})	1.53±0.08^{a}	1.44±0.05^{ab}	1.53±0.04^{a}	1.50±0.03^{a}	1.31±0.05^{b}
FI (g $fish^{-1}$)	321.43±3.64^{a}	317.66±1.58^{ab}	315.97±0.85^{ab}	319.15±4.06^{a}	309.12±2.72^{b}
FCR	1.53±0.17^{a}	1.76±0.11^{ab}	1.52±0.08^{a}	1.70±0.13^{a}	2.15±0.18^{b}
PER	1.59±0.18^{a}	1.40±0.10^{ab}	1.62±0.10^{a}	1.50±0.10^{ab}	1.19±0.08^{b}
HSI (%)	2.03±0.35^{b}	1.70±0.36^{b}	1.75±0.24^{b}	2.31±0.12^{ab}	3.16±0.44^{a}
VSI (%)	9.08±0.81	9.29±0.42	9.87±0.59	10.07±0.15	10.30±0.75
SR (%)	96.67±1.67^{a}	91.67±1.67^{a}	93.33±4.41^{a}	88.33±1.67^{ab}	81.67±3.33^{b}

[1]Means (mean±SD) of three replicate analysis; $^{a-b}$Values in the same row with different superscripts are significantly different from each other ($p<0.05$).
[2]IBW: Initial body weight; FBW: Final body weight; BWG: Body weight gain; SGR: Specific growth rate; FI: Feed intake; FCR: Feed conversion ratio; PER: Protein efficiency ratio; HSI: Hepatosomatic index; VSI: Viscerosomatic index; SR: Survival rate.

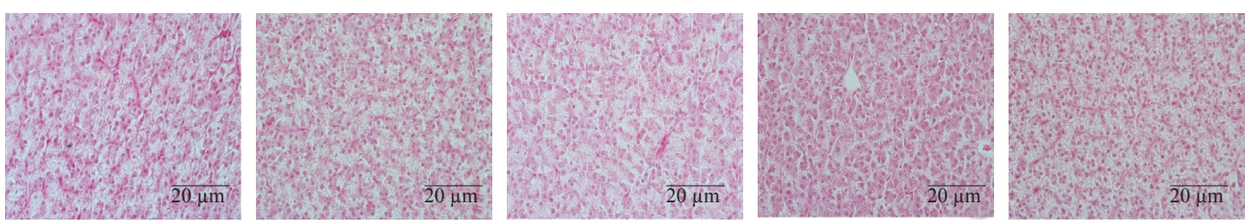

Fig. 1. Histological sections of liver from fish fed with different levels of L-carnitine for 120 days: (a) 0 mg kg $diet^{-1}$, (b) 250 mg kg $diet^{-1}$, (c) 500 mg kg $diet^{-1}$, (d) 750 mg kg $diet^{-1}$, (e) 1000 mg kg $diet^{-1}$ (H & E; X 400)

Table 3. Effect of dietary L-carnitine on proximate (% wet wt) composition of Nile tilapia fry fed the experimental diets.

Parameters	Experimental treatments (L-carnitine content mg kg^{-1})				
	0	250	500	750	1000
Crude protein	15.35±0.18	15.46±0.09	15.20±0.04	15.27±0.24	15.29±0.11
Crude lipid	3.21±0.22	3.36±0.92	3.39±0.29	3.52±0.47	3.25±0.19
Crude ash	3.25±0.05	3.25±0.04	3.34±0.01	3.27±0.09	3.33±0.02
Moisture	76.99±0.15^{a}	76.23±0.10^{c}	76.57±0.06^{b}	76.74±0.12^{ab}	76.83±0.26^{ab}

Values in the same row with different superscripts are significantly different ($p<0.05$). Proximate composition values are mean (±SD) of triplicate analysis.

body lipid concentrations reduced in Mozambique tilapia (Jayaprakas *et al*., 1996), Indian major carps (Singh *et al*., 2008), Asian catfish (Desai *et al*., 2010) and silver perch (Yang *et al*., 2012) fed diets containing different levels of L-carnitine. Results of the present study demonstrated that supplemental dietary L-carnitine had no beneficial effects on improving growth performance, feed utilisation and body composition in Nile tilapia (*O. niloticus*) fry.

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