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Performance of pacu juveniles fed diets supplemented with L-carnitine

Abstract – The objective of this work was to determine the effect of L-carnitine supplementation on the productive performance and physiology of juvenile pacu (*Piaractus mesopotamicus*). A total of 288 pacu, with an initial average weight of 9.62 ± 0.74 g, were fed experimental diets supplemented with 400, 800, 1,200, 1,600, and 2,000 mg kg⁻¹ L-carnitine and a control diet (without supplementation), for 128 days. The following were evaluated: growth performance; carcass centesimal composition; intestinal, muscle, and hepatic histomorphologies; and oxidative stress. The fish hepatosomatic and viscerosomatic fat indexes increased with the inclusion of L-carnitine in the diets. The evaluation of carcass centesimal composition showed that the diets supplemented with 2,000 mg kg⁻¹ L-carnitine caused a reduction in protein content and an increase in that of ethereal extract. Intestinal histomorphology indicated changes in the villi with L-carnitine supplementation. Moreover, hepatic lipid peroxidation occurred with the inclusion of 2,000 mg kg⁻¹ L-carnitine. The supplementation with L-carnitine in the diets of pacu juveniles does not influence the development of the fish until the rate of 1,600 mg kg⁻¹. However, high carcass lipid levels, as well as an increase in the hepatosomatic and viscerosomatic fat indexes, are observed in fish fed diets containing 2,000 mg kg⁻¹.

Index terms: *Piaractus mesopotamicus*, animal nutrition, carcass composition, histomorphology, oxidative stress.

Desempenho de juvenis de pacu alimentados com rações suplementadas com L-carnitina

Resumo – O objetivo deste trabalho foi determinar o efeito da suplementação de L-carnitina no desempenho produtivo e na fisiologia de juvenis de pacu (*Piaractus mesopotamicus*). Um total de 288 pacus, com peso inicial médio de $9,62 \pm 0,74$ g, foram alimentados com dietas experimentais suplementadas com 400, 800, 1.200, 1.600 e 2.000 mg kg⁻¹ de L-carnitina e com uma dieta controle (sem suplementação), por 128 dias. Foram avaliados: desempenho produtivo; composição centesimal da carcaça; histomorfologias intestinal, muscular e hepática; e estresse oxidativo. Os índices hepatossomático e de gordura viscerossomática dos peixes aumentaram com a inclusão de L-carnitina nas rações. A avaliação da composição centesimal da carcaça mostrou que as dietas suplementadas com 2.000 mg kg⁻¹ de L-carnitina causaram redução no conteúdo de proteína e aumento no de extrato etéreo. A histomorfologia intestinal indicou alterações nas vilosidades com a suplementação de L-carnitina. Além disso, a peroxidação lipídica hepática ocorreu com a inclusão de 2.000 mg kg⁻¹ de L-carnitina. A suplementação de L-carnitina em dietas para juvenis de pacu não influencia o desenvolvimento dos peixes até a dose de 1.600 mg kg⁻¹. Entretanto, observam-se elevados níveis de lipídeos na carcaça, assim como aumento nos índices hepatossomáticos e de gordura viscerossomática, em peixes alimentados com rações contendo 2.000 mg kg⁻¹.

Termos para indexação: *Piaractus mesopotamicus*, nutrição animal, composição da carcaça, histomorfologia, estresse oxidativo.

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Introduction

An important challenge in aquaculture practices is improving fish sustainable production, to reduce costs and minimize negative effects on the environment. In fish production, feeding represents the highest cost, reaching up to 70% of the total (Nass et al., 2020). This shows the need of assessing the effects of additives and/or supplements, such as L-carnitine, on fish growth and physiology (Rodrigues et al., 2015).

L-carnitine is synthesized from lysine and methionine with the help of vitamin C (Harpaz, 2005). This molecule has attracted interest because it is a multi-physiological additive, with a considerable positive effect on the growth and lipid metabolism of some fish species (Mohseni & Ozorio, 2014). It acts in the transport of long-chain fatty acids, through the mitochondrial membrane, for the oxidation and production of adenosine triphosphate in peripheral tissues (Longo et al., 2016). Its highest concentration is in the skeletal and cardiac musculatures (Harpaz, 2005). When there is a deficiency in L-carnitine, hepatic lipid oxidation is reduced, diverting fatty acids for the synthesis of triacylglycerol in the liver (Wray-Cahen et al., 2004).

L-carnitine can improve the productive performance of fish when included in their diet. As an amine, it has a protein-sparing effect, directing dietary and body lipids to maintaining body energy (Tonini et al., 2011). It can also prevent the formation of free radicals, minimizing the peroxidation of membrane lipids and the aggression to tissue and membrane proteins (Safari et al., 2015). In Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) fingerlings, the inclusion of 450 mg kg⁻¹ L-carnitine in the diets was able to reduce protein requirement from 30 to 20%, without affecting productive performance (El-Sayed et al., 2010).

Several authors have reported the positive effects of L-carnitine on some fish species, including: improved growth, reduction of muscle fat, better response against stress due to confinement, and adaptation to high levels of ammonia and high temperature variations (Gonçalves et al., 2010; Yang et al., 2012). However, the literature also shows contradictory results, which may be related to differences among the studied species, such as experimental conditions, management, and leaching and levels of the supplemented L-carnitine, among others (Gonçalves et al., 2010).

Despite these reports, there are still no known studies on the inclusion of L-carnitine in the diet of pacu (*Piaractus mesopotamicus* Holmberg, 1887), which is native to South America and has an opportunistic omnivorous food habit, being well adapted to different production systems (Vaz et al., 2000). The species is also one of the most studied in Brazilian aquaculture and the sixth most cultivated in the country (Produção..., 2018). Its main characteristics include: rusticity, adaptability to artificial feeding, and easy management, which provide positive production results (Nunes et al., 2013). However, since the species is considered as a fat fish (Ramos Filho et al., 2008), researches with the aim of reducing its celomatic fat content are vital to improve its performance.

The objective of this work was to determine the effect of L-carnitine supplementation on the productive performance and physiology of juvenile pacu.

Materials and Methods

The research project was approved by the animal ethics committee of Universidade Estadual do Oeste do Paraná (protocol number 42/17), located in the municipality of Toledo, in the state of Paraná, Brazil. A total of 288 pacu juveniles, with an average initial weight of 9.62±0.74 g, were distributed in 24 cylindrical-conical tanks with the capacity of 0.5 m³, equipped with a water recirculation system containing a mechanical filter and a central air blower for constant aeration. The experimental design was completely randomized, with six treatments and four replicates (the tanks were the experimental unit), with 12 fish each. Weekly, the following water physical and chemical parameters were measured using the YSI Professional Plus multiparameter meter (YSI Incorporated, Yellow Springs, OH, USA): pH, 7.03±0.11; electrical conductivity, 80.7±0.68 µS cm⁻¹; and dissolved oxygen, 8.54±0.50 mg L⁻¹. Daily, temperature (22.5±1.6°C) was measured using a digital thermometer. The values obtained for the water quality parameters are within the production range for the species, but the temperature is at the minimum recommended (Tavares & Santeiro, 2013).

The experimental diets were formulated as isoenergetic (3,200 kcal kg⁻¹ digestible energy) and isoproteic (23% digestible protein) (Table 1). Based on National Research Council (NRC, 2011), the treatments were supplemented with 0, 400, 800, 1,200, 1,600, and

2,000 mg kg⁻¹ L-carnitine. The used feed ingredients were ground individually in a hammer-type grinder, manually blended according to each formulation, extruded in the Ex Micro extruder (Extec Máquinas, Ribeirão Preto, SP, Brazil), and oven dried at 55°C for 24 hours. After cooling, the feed was packed in bags

Table 1. Chemical composition of the experimental diets containing different levels of L-carnitine fed to juvenile pacu (*Piaractus mesopotamicus*).

Ingredient (%)	L-carnitine (mg kg ⁻¹)					
	0	400	800	1,200	1,600	2,000
45% soybean meal	32.90	32.90	32.90	32.90	32.90	32.90
Corn	31.20	31.20	31.20	31.20	31.20	31.20
Wheat bran	19.55	19.55	19.55	19.55	19.55	19.55
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00
Poultry viscera meal	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	4.03	4.03	4.03	4.03	4.03	4.03
Limestone	0.72	0.72	0.72	0.72	0.72	0.72
Mineral and vitamin supplement ⁽¹⁾	0.50	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	0.42	0.42	0.42	0.42	0.42	0.42
Common salt	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine hydrochloride	0.26	0.26	0.26	0.26	0.26	0.26
Antifungal calcium propionate	0.10	0.10	0.10	0.10	0.10	0.10
BHT antioxidant	0.02	0.02	0.02	0.02	0.02	0.02
L-carnitine	0.00	0.04	0.08	0.12	0.16	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrients						
Starch (%)	30.00	30.00	30.00	30.00	30.00	30.00
Total arginine (%)	1.81	1.81	1.81	1.81	1.81	1.81
Calcium (%)	1.00	1.00	1.00	1.00	1.00	1.00
Digestible energy (kcal kg ⁻¹)	3,200	3,200	3,200	3,200	3,200	3,200
Total phenylalanine (%)	1.23	1.23	1.23	1.23	1.23	1.23
Total phosphorus (%)	0.80	0.80	0.80	0.80	0.80	0.80
Total histidine (%)	0.66	0.66	0.66	0.66	0.66	0.66
Total isoleucine (%)	1.10	1.10	1.10	1.10	1.10	1.10
Total leucine (%)	2.08	2.08	2.08	2.08	2.08	2.08
Total lysine (%)	1.64	1.64	1.64	1.64	1.64	1.64
Total methionine (%)	0.41	0.41	0.41	0.41	0.41	0.41
Digestible protein (%)	23.00	23.00	23.00	23.00	23.00	23.00
Total threonine (%)	1.01	1.01	1.01	1.01	1.01	1.01
Total tryptophan (%)	0.32	0.32	0.32	0.32	0.32	0.32
Total valine (%)	1.25	1.25	1.25	1.25	1.25	1.25
Analyzed chemical composition (natural matter)						
Dry matter (%)	93.04	91.71	89.66	91.72	91.69	91.69
Crude protein (%)	27.11	27.12	27.93	27.76	27.06	26.92
Ethereal extract (%)	7.98	8.12	8.37	8.58	8.07	7.29
Mineral matter (%)	7.45	7.86	7.69	7.67	7.40	7.73
Gross energy (kcal kg ⁻¹)	4,461	4,459	4,352	4,446	4,412	4,356
Crude fiber (%)	5.43	5.99	5.01	5.10	5.42	4.97

⁽¹⁾Guarantee levels per kilogram of product: 500.000 IU vitamin A, 250.000 IU vitamin D3, 5.000 mg vitamin E, 500 mg vitamin K3, 1.500 mg vitamin B1, 1.500 mg vitamin B2, 1.500 mg vitamin B6, 4.000 mg vitamin B12, 500 mg folic acid, 4.000 mg calcium pantothenate, 10.000 mg vitamin C, 10 mg biotin; 1.000 inositol, 7.000 nicotinamide, 10.000 mg choline, 10 mg cobalt, 1.000 mg copper, 5.000 mg iron, 200 mg iodine, 1.500 mg manganese, 30 mg selenium, and 9.000 mg zinc. The values of gross and digestible energy, as well as of crude protein (%), were estimated for pacu according to Abimorad & Carneiro (2004).

and stored under refrigerated conditions, at -20°C , until feeding. L-carnitine was included at the same time as the other micro-ingredients of the formulation.

The fish were acclimated for 8 days and received the experimental diet for 120 days. During the total experimental period, the fish were fed four times a day at 8:00 a.m., 11:00 a.m., 2:00 p.m., and 5:00 p.m., ad libitum. At the end of the experimental period, the fish were fasted for 24 hours to empty their gastrointestinal tract and, subsequently, desensitized in a benzocaine solution at 100 mg L^{-1} water, in order to measure their individual weight (g) and total length (cm). Then, three fish from each tank were euthanized in a benzocaine solution at 250 mg L^{-1} water and packed in ice for the removal of their liver, intestine, white muscle, and viscerosomatic fat. After evisceration, the fish carcasses were analyzed to determine carcass composition.

The obtained biometric values were tabulated to determine the following production performance data: average final weight (AFW, g); average final length (AFL, cm); survival (%) = $100 \times (\text{final fish number} / \text{initial fish number})$; weight gain (g) = final body weight - initial body weight; feed conversion rate (FCR) = feed supplied / weight gain; specific growth rate (SGR, %) = $100 \times [(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{experimental period}]$; protein efficiency ratio (PER, %) = $100 \times (\text{weight gain} / \text{consumed crude protein})$; hepatosomatic index (%) = $100 \times (\text{liver weight} / \text{final body weight})$; and viscerosomatic fat index (%) = $100 \times (\text{viscerosomatic fat weight} / \text{final body weight})$.

For determination of carcass centesimal composition, the fish samples were pre-dried in a forced-air ventilation oven, at 55°C , for 72 hours. Subsequently, the moisture analysis was carried out by oven drying, at 105°C , for 8 hours. Ash content was obtained in a muffle furnace, at 550°C , for 6 hours. Crude protein was determined by the Kjeldahl method, and the ethereal extract content was obtained using a Soxhlet extractor with ether as the solvent, all according to the methodology described by Association of Official Analytical Chemists (AOAC) (Horwitz, 2005).

For the histological analysis, with the aid of a blade, a 15-mm white muscle sample was removed from the left side of the fish, above the lateral line. Later, the fish were eviscerated and 15-mm samples of the median intestine and liver were collected. A total of eight fish per treatment were used. The samples were conditioned in formalin-acetic acid-alcohol solution

for 24 hours and then preserved in 70% alcohol, at 20°C , until the analyses were performed. For processing, the samples were dehydrated in increasing concentrations of alcohol, diaphanized in xylol, and embedded in histological paraffin. For the cuts, the HM 340E Electronic Microtome (Thermo Scientific Inc., Waltham, MA, USA) was used. The taken cross sections were $5\text{ }\mu\text{m}$ from the liver and $7\text{ }\mu\text{m}$ from the muscle and intestine. The samples were stained using the hematoxylin-eosin method (Behmer et al., 1976).

The smallest diameter of at least 200 muscle fibers of each fish was classified according to morphometry, as $<20\text{ }\mu\text{m}$, $20\text{--}50\text{ }\mu\text{m}$, and $>50\text{ }\mu\text{m}$, in order to evaluate the contribution of hyperplasia and hypertrophy to muscle growth (Almeida et al., 2008). In the intestine, the number of hepatocytes per area ($2,000\text{ }\mu\text{m}^2$) was quantified, and two cuts of each liver were examined. The morphometric analysis of the intestinal mucosa was performed in ten villi per animal, to measure villi height, total villi height, villi width, villi thickness, and tunica thickness.

Photo documentation was done using light microscopy, under the Olympus BX50 optical P1 microscope (Olympus Co., Ltd., Manila, Philippines), coupled to the Olympus PMC 35 B camera (Olympus Europa SE & Co. KG, Hamburg, Germany), with 40X objective lens for the analysis of the muscle and liver and 20X objective lens for that of the intestine. For measurements, the cellSens, version 1.15, standard software (Olympus Corporation, Tokyo, Japan) was used.

For the analysis of thiobarbituric acid reactive substances (TBARS), glutathione-S-transferase (GST), and catalase (CAT), two fish from each tank were packed in ice for the removal of a liver sample, which was stored in liquid nitrogen for further analysis. Upon thawing, the liver was homogenized in $90\text{ }\mu\text{L}$ of 0.3 mol L^{-1} sodium phosphate buffer (140 mmol L^{-1} KCl, pH 7.4) per gram of tissue. The mixture was composed of a protease inhibitor (not degrading the enzymes) and phenylmethylsulfonyl fluoride (PMSF) in a 100-nmol L^{-1} concentration diluted in isopropanol (0.03484 g PMSF in 2.0 mL isopropyl alcohol). A total of $10\text{ }\mu\text{L}$ PMSF were added to each milliliter of the buffer.

TBARS was determined according to Buege & Aust (1978), and the results were expressed in nmol mg^{-1} malondialdehyde protein. CAT was evaluated as in

Nelson & Kiesow (1972) at a wavelength of 240 nm, and the results were expressed as $\mu\text{mol mg}^{-1}$ protein per minute. GST activity was obtained following the method of Ellman (1959), using 20% trichloroacetic acid in the proportion 0.2 g tissue:1.0 mL trichloroacetic acid. Readings were performed in a spectrophotometer, at a wavelength of 412 nm, and activity was expressed in $\mu\text{mol L}^{-1} \text{mg}^{-1}$ protein.

The data were subjected to Levene's test to check for normality and homoscedasticity, and, given these assumptions, were subjected to the analysis of variance, at 5% probability. When there was a significant difference, means were compared by Tukey's test, at 5% probability, using the Statistica, version 7.1, software (TIBCO Software, Inc., Palo Alto, CA, USA).

Results and Discussion

The inclusion of L-carnitine in the diets of pacu juveniles did not affect the AFW, AFL, weight gain, survival, FCR, PER, and SGR variables (Table 2). However, it may promote the energy efficiency of fatty acids, due to the increased mitochondrial lipid oxidation. It should be noted that the obtained results may differ according to experimental conditions, studied species, fish development phase, stress conditions, among other factors (Gonçalves et al., 2010). Li et al. (2020) found that the action of L-carnitine supplementation in fish diets is directly linked to the abundance of individual macronutrients, including fatty acids, glucose, and amino acids. Therefore, the "protein-sparing effect"

will be effective when the diets contain an adequate amount of lipids.

During the experimental period, the temperature remained within the recommended minimum limit for pacu cultivation, which may have hindered fish development, as supported by the indexes of weight gain and specific growth rate (Sanchez et al., 2016). Regarding the influence of temperature, Hosam et al. (2016) observed that Nile tilapia fed diets supplemented with L-carnitine showed increased survival at low temperatures, besides improved performance up to the rate of 1,200 mg kg^{-1} .

The supplementation with L-carnitine also promoted an increase in the hepatosomatic index (Table 2), which is influenced by the diet and represents higher energy reserve rates and, supposedly, the deposition of fat and glycogen in the liver, indicating an increase in the weight of this organ (Barbosa et al., 2011). When studying zebrafish (*Danio rerio* Hamilton, 1822), Li et al. (2017) reported an improvement in lipid catabolism with the inclusion of L-carnitine in the diets, but also an increase in glycogen deposition in the entire body. These results were confirmed by the expression of a gene related to glycolysis (PFK and PK) in the muscle and to gluconeogenesis (PECK1 and G6Pa) in the liver, showing that the increase of lipid catabolism in fish decreases the energy from the degradation of glucose, causing an increased gluconeogenesis and glycogen synthesis.

When included in the diet, L-carnitine has the tendency to increase lipid oxidation in the metabolism,

Table 2. Growth performance of juvenile pacu (*Piaractus mesopotamicus*) after 128 days fed diets containing different levels of L-carnitine⁽¹⁾.

Variable ⁽²⁾	L-carnitine (mg kg^{-1}) ⁽³⁾						P-value
	0	400	800	1,200	1,600	2,000	
AFW (cm)	9.90±0.74	9.52±0.80	9.87±0.95	9.27±0.35	9.68±0.54	9.48±0.62	-
AFL (cm)	13.19±0.63	13.38±0.55	13.25±0.94	12.97±1.16	12.92±0.71	12.87±0.37	0.92
WG (g)	31.45±4.71	32.71±3.64	33.13±6.99	31.17±5.83	30.21±5.13	30.08±2.91	0.96
Survival (%)	87.50±12.50	83.33±11.78	85.42±10.82	91.67±8.33	97.92±3.61	97.92±3.61	0.27
FCR	2.15±0.27	2.20±0.58	2.03±0.40	2.08±0.18	1.79±0.28	2.09±0.15	0.75
SGR (%)	1.19±0.09	1.24±0.05	1.22±0.08	1.22±0.10	1.17±0.08	1.19±0.09	0.92
PER (%)	0.18±0.03	0.19±0.02	0.20±0.05	0.18±0.04	0.18±0.03	0.18±0.02	0.96
HSI (%)	1.74±0.14a	2.02±0.05ab	2.24±0.34b	2.15±0.16b	2.03±0.05ab	2.24±0.22b	0.003
VFI (%)	2.36±1.04a	2.49±0.62a	2.83±0.45ab	3.21±0.72ab	3.20±0.64ab	3.96±0.43b	0.004

⁽¹⁾Means followed by equal letters do not differ by Tukey's test, at 5% probability. ⁽²⁾AFW, average final weight; AFL, average final length; WG, weight gain; FCR, feed conversion rate; SGR, specific growth rate; PER, protein efficiency rate; HSI, hepatosomatic index; and VFI, viscerosomatic fat index. ⁽³⁾Mean ± standard deviation.

promoting a lower accumulation of fat and, consequently, a high protein content in the body of fish (Yang et al., 2012). In the present study, there was a reduction in fish body fat with the supplementation of 1,600 mg kg⁻¹ L-carnitine, but an increase in lipid content with 2,000 mg kg⁻¹ (Table 3). Similar results were observed for Caspian Sea Kutum (*Rutilus frisii kutum* Kamensky, 1901), when fed diet supplemented with different L-carnitine levels (Nekoubin et al., 2012), and for common carp (*Cyprinus carpio* Linnaeus, 1758) fed 1,000 mg kg⁻¹ L-carnitine (Sabzi et al., 2017). Although the mechanisms that lead L-carnitine to promote this effect are still not known, they could be related to the limit requirement of each species. After being synthesized, L-carnitine is transported to the tissues, being higher in the organisms that use fatty acids as a primary energy source (Longo et al., 2016). Therefore, after synthesis in the liver and

kidneys, it is transported to the muscle where it is used in β -oxidation, being converted from free-form L-carnitine to L-carnitine ethers (Ozório et al., 2002).

The analysis of hepatocyte quantification and muscle histomorphometry showed no significant difference between the fish fed diets supplemented with L-carnitine and those fed the control (Table 4 and Figures 1 and 2). During the early stages of life, fish tissues exhibit rapid growth rates (Almeida et al., 2008). Therefore, hyperplasia (diameter <20 μ m) is very frequent during muscle development in the juvenile phase, whereas hypertrophy (diameters >50 μ m) is more frequent in adulthood (Almeida et al., 2008). Diameters smaller than 20 μ m represented between 30 and 38% of the diameter of the evaluated fibers (Table 4). In this case, the larger fibers started as normal and became hyperplastic, showing activity in the process of muscle growth during the developmental

Table 3. Carcass centesimal composition of juvenile pacu (*Piaractus mesopotamicus*) after 128 days fed diets containing different levels of L-carnitine⁽¹⁾.

Variable	L-carnitine (mg kg ⁻¹) ⁽²⁾						P-value
	0	400	800	1,200	1,600	2,000	
Moisture (%)	70.13±1.09	69.75±0.75	69.52±0.67	69.47±1.67	69.87±0.88	68.56±0.42	0.59
Crude protein (%)	18.73±0.75a	16.37±0.75b	17.65±0.24ab	16.91±0.43b	17.28±0.46b	17.75±0.29ab	0.00
Lipids (%)	10.16±0.50ab	10.61±0.50ab	10.23±0.04ab	9.90±0.29ab	9.62±0.60a	10.76±0.30b	0.04
Ash (%)	3.01±0.10	2.98±0.04	3.10±0.17	3.16±0.05	3.14±0.07	3.15±0.13	0.21

⁽¹⁾Means followed by equal letters do not differ by Tukey's test, at 5% probability. ⁽²⁾Mean±standard deviation.

Table 4. Intestinal histomorphometry, quantification of hepatocytes, and frequency of the smallest diameter of the muscle fibers of juvenile pacu (*Piaractus mesopotamicus*) after 128 days fed diets containing different levels of L-carnitine⁽¹⁾.

Variable	L-carnitine (mg kg ⁻¹) ⁽²⁾						P-value
	0	400	800	1,200	1,600	2,000	
Intestine							
Height (μ m)	399.31±92.01ab	312.65±106.70a	466.66±90.83ab	477.78±50.42ab	478.62±38.48ab	539.67±48.35b	0.01
Total height (μ m)	450.49±106.81	416.15±142.99	507.69±91.26	528.85±41.38	537.55±23.94	592.86±49.47	0.10
Width (μ m)	121.97±12.20	110.49±3.26	122.76±18.83	108.98±3.19	120.42±14.11	111.25±14.29	0.29
Thickness (μ m)	52.27±0.81ab	56.61±6.22ab	57.60±9.45ab	60.93±0.98ab	63.72±5.14b	50.05±4.96a	0.02
Tunica (cm)	40.34±2.66a	53.90±3.36c	44.17±5.79ab	44.42±2.24ab	57.34±2.71c	51.28±2.62bc	0.00
Liver							
Hepatocytes ⁽³⁾	142.00±7.57	157.58±34.43	136.33±19.28	125.67±9.18	136.50±11.79	136.42±9.14	0.28
Muscle (%)							
<20 μ m	30.00±4.43	34.75±9.00	33.50±6.49	35.63±3.94	38.00±11.11	30.50±4.71	0.60
20–50 μ m	66.75±4.70	65.00±8.40	66.00±6.42	61.75±2.53	61.12±11.02	65.50±4.43	0.79
>50 μ m	0.83±1.44	0.50±0.86	0.66±1.15	1.67±1.75	1.17±1.15	3.00±1.32	0.12

⁽¹⁾Means followed by equal letters do not differ by Tukey's test, at 5% probability. ⁽²⁾Mean±standard deviation. ⁽³⁾Average number of hepatocytes in 2,000 μ m².

stage, confirming the lower percentage (less than 3%) of hypertrophic fibers.



Figure 1. Histological image of the muscle tissue of pacu (*Piaractus mesopotamicus*) after 128 days fed diets containing different L-carnitine levels. The smallest diameter of each muscle fiber was measured using the cellSens standard software (Olympus Corporation, Tokyo, Japan), through a light microscope with 40x objective lens. The fibers were divided into three measurement classes, which showed no significant difference between the levels of dietary inclusion by Tukey's test, at 5% probability.

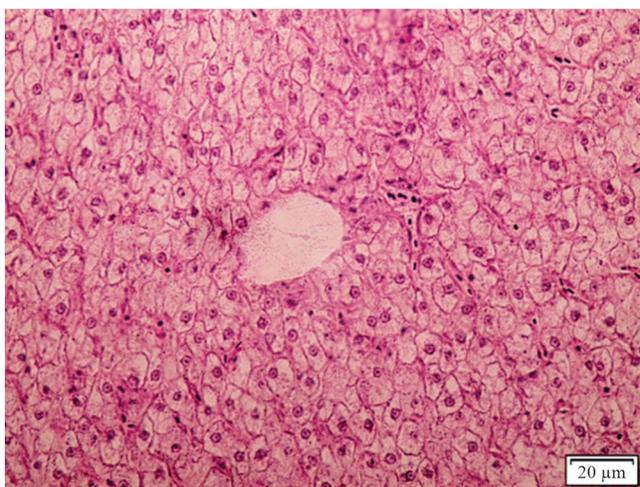


Figure 2. Histological image of the liver tissue of pacu (*Piaractus mesopotamicus*) after 128 days fed diets containing different L-carnitine levels. Hepatocytes were quantified using a 40x light microscope. No significant difference was observed between the levels of dietary inclusion by Tukey's test, at 5% probability.

Fish fed 2,000 mg kg⁻¹ L-carnitine had taller and less-thick villi (Table 4 and Figure 3), which represents an adaptation of the tissue to the food, suggesting an improvement in the surface area for the absorption of nutrients (Hisano et al., 2018). In pacu, the intestine – the main organ for nutrient digestion and absorption in fish – is medium sized and lined by folds, which are distributed longitudinally, being wider and more complex in the median portion, where there is a greater nutrient absorption (Corrêa, 2016). According to Yuan et al. (2011), L-carnitine can protect the intestinal wall from lesions and bacterial infections because it improves the absorption of nutrients.

L-carnitine acts on β -oxidation as a carrier of long-chain fatty acids, has a regulatory function in the energy metabolism of animals, and may also act on resistance to stress due to the β -oxidation of mitochondrial fatty acids (Li et al., 2017). According to these authors, although the limited carnitine synthesis in Nile tilapia did not induce oxidative stress, it significantly inhibited the β -oxidation of mitochondrial fatty acids and decreased resistance to pathogens and nitrogenous ammonia, indicating that carnitine is necessary for the β -oxidation of mitochondrial fatty acids.



Figure 3. Image of the histological section of the intestine of pacu (*Piaractus mesopotamicus*) after 128 days fed diets containing different L-carnitine levels, showing: villi height (A), total villi height (B), villi width (C), villi thickness (D), and tunica thickness (E). The height and thickness of the villi and thickness of the tunica differed significantly between treatments by Tukey's test, at 5% probability.

Table 5. Concentration of substances reactive to catalase (CAT), glutathione S-transferase (GST), and thiobarbituric acid (TBARS) in the liver of juvenile pacu (*Piaractus mesopotamicus*) after 128 days fed diets containing different levels of L-carnitine⁽¹⁾.

Variable ⁽²⁾	L-carnitine levels (mg kg ⁻¹) ⁽³⁾						P-value
	0	400	800	1,200	1,600	2,000	
CAT	0.58±0.14	0.57±0.18	0.58±0.18	0.52±0.39	0.46±0.20	0.59±0.14	0.96
GST	0.87±0.17	0.90±0.06	0.82±0.18	0.90±0.11	0.97±0.17	0.88±0.14	0.85
TBARS	1.50±0.59a	1.69±1.09ab	1.83±0.38ab	1.50±1.29a	1.00±0.37a	3.57±1.26b	0.02

⁽¹⁾Means followed by equal letters do not differ by Tukey's test, at 5% probability. ⁽²⁾CAT, $\mu\text{mol mg}^{-1}$ protein per minute; GST, $\mu\text{mol GS-DNB min}^{-1}$ mg per protein; and TBARS, $\eta\text{mol mg}^{-1}$ protein. ⁽³⁾Mean±standard deviation.

There was no influence of L-carnitine on the CAT and glutathione peroxidase enzymes (Table 5). Low-molecular weight enzymes, such as CAT, glutathione peroxidase, glutathione reductase, and GST, are known to have a high antioxidant activity, which forms complex defense networks against oxidative stress (Silva et al., 2017). Moreover, even though the CAT enzyme does not act directly on lipid peroxidation products, it may affect the initial peroxidation phase, decreasing the concentrations of hydrogen peroxide (Lushchak & Bagnyukova, 2006).

The inclusion of 2,000 mg kg⁻¹ L-carnitine in the diet of pacu significantly increased TBARS (Table 5), and the viscerosomatic fat index and carcass lipid content were also higher at this rate. These substances allow measuring the lipid peroxidation in a tissue, whose high fat levels may include saturated fatty acids, which are substrates for lipid peroxidation (Yu et al., 2017). TBARS, when at a high level in the liver, indicates a reduction in the antioxidant status of fish tissues and, as representative compounds of the final products of the lipid peroxidation process, they increase and consequently cause damages to the cytoplasm (Kaur & Jindal, 2017). Therefore, the obtained results are indicative that, up to the level of 1,600 mg kg⁻¹ L-carnitine in the diet, there is no alteration in the lipid peroxidation in the hepatic tissue.

Conclusion

The inclusion of L-carnitine in the diets of pacu (*Piaractus mesopotamicus*) juveniles does not influence the development of the fish up to 1,600 mg kg⁻¹, but increases carcass lipid levels, as well the hepatosomatic and viscerosomatic fat indexes, at 2,000 mg kg⁻¹.

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