

SUPPLEMENTATION OF L-CARNITINE IN DIETS FOR SILVER CATFISH AND ITS EFFECTS ON REPRODUCTIVE ASPECTS

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ABSTRACT

The objective of the present study was to assess the reproductive performance of silver catfish *Rhamdia quelen* fed with diets supplemented with growing levels of L-carnitine. 288 juveniles with average initial weight of 94.36 ± 3.76 g, fed during 126 days with diets containing 400, 800, 1200, 1600 and 2000 mg. kg⁻¹ of L-carnitine and one control diet. Females fed with diets supplemented with 400 mg. kg⁻¹ of L-carnitine presented increase in fertilization and fertility, reduction in levels of triglycerides and plasma calcium when fed with diets supplemented with 1600 mg. kg⁻¹ of L-carnitine. Males fed with diets supplemented with 1600 mg. kg⁻¹ of L-carnitine presented increase in spermatozoa and improvement in semen quality. Structural changes were observed in the development of germ cells in females and males, even though all fish were in final stages of maturation in all treatments. The offspring from breeding animals fed with 400 mg. kg⁻¹ of L-carnitine presented better potentials of development during the embryonic process. Therefore, the L-carnitine supplementation in diets stimulates the gonad development of breeding animals and also speeds up the initial development of silver catfish offspring.

Keywords: animal nutrition; native species; biotechnology; energy direction.

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SUPLEMENTAÇÃO DE L-CARNITINA EM DIETAS PARA JUNDIÁ E SEUS EFEITOS SOBRE OS ASPECTOS REPRODUTIVOS

RESUMO

O objetivo deste estudo foi avaliar o desempenho reprodutivo de jundiá *Rhamdia quelen* alimentados com dietas suplementadas com níveis crescentes de L-carnitina. Utilizou-se 288 juvenis com $94,36 \pm 3,76$ g de peso inicial médio, alimentados durante 126 dias com dietas contendo 400, 800, 1200, 1600 e 2000 mg. kg⁻¹ de L-carnitina e uma dieta controle. As fêmeas alimentadas com dietas suplementadas com 400 mg. kg⁻¹ de L-carnitina apresentaram aumento na taxa de fertilização e fecundidade. E aquelas que consumiram dietas contendo 1600 mg. kg⁻¹ de L-carnitina demonstraram redução nos níveis de triglicerídeos e cálcio plasmático. Os machos alimentados com dietas suplementadas com 1600 mg. kg⁻¹ L-carnitina apresentaram aumento na produção de espermatozoides e melhora na qualidade seminal. No desenvolvimento das células germinativas em fêmeas e machos apresentaram modificações estruturais, embora todos os peixes encontravam-se em estágios finais da maturação em todos os tratamentos. A prole proveniente de reprodutores alimentados com 400 mg. kg⁻¹ L-carnitina apresentaram melhores potenciais de desenvolvimento no decorrer do processo embrionário. Portanto, a suplementação dietética de L-carnitina estimula o processo de desenvolvimento gonadal dos reprodutores e ainda acelera o desenvolvimento inicial das proles de jundiá.

Palavras-chave: nutrição animal; espécie nativa; biotecnologia; direcionamento energético.

INTRODUCTION

Intensive breeding of fish requires wide knowledge on reproductive biology of target species, which makes possible the exploration of their productive capacity due to the growing demand of the aquaculture sector. The improvement of production systems and assistance of artificial induction with egg laying in controlled cycles, associated to the use of nutritionally balanced diets for breeding animals, generate increase in viable offspring's (Zaniboni Filho and Weingartner, 2007; Murgas et al., 2011; Andrade et al., 2015).

Moreover, nutritional aspect exercise significant influence in fish reproductive quality, and such characteristics orient the preparation of diets to promote higher availability of nutrients that constitute feeds. According to Bilinski and Jonas (1970), benefits attributed to the use of L-carnitine in diets provide increase in intracellular lipid oxidation, maximizing energy efficiency. It also acts in the balance of hypothalamic-pituitary-gonad axis' physiological events, directly influencing gametes' quality (Costa et al., 2012), in addition to directing protein energy gain for incorporation in muscular tissue and, consequently, fish growth (Tonini et al., 2011), promotes higher resistance of animals to stressing factors for abiotic changes and handling during management, since it increases the efficiency of enzymes that act in oxidative stress (Harpaz, 2005).

Carnitine is a quaternary amine (Carr and Chaney, 1976) and lysine and methionine serve as substrates for its formation (Borum, 1983) in the presence of ascorbic acid, vitamin B6 and vitamin B3, in addition to iron ion, resulting from metabolic processes in the organism. This molecule promotes the provision of energy by the cell, acting in reactions to transfer cytosol's free fat acids to mitochondrion' matrix (Hathcock and Shao, 2006), facilitating oxidation and energy generation (ATP), therefore improving animals' growth and body composition (El-Hattab and Scaglia, 2015; Robinson et al., 2016; Shi et al., 2017).

L-carnitine biosynthesis and stimulation of increase in protein synthesis also have the effect of influencing the direction of remaining energy to the development of germ cells and regulatory hormone system, acting in gonad maturation process (burkert, 2007; Costa et al., 2012). The primary energy acting in sperm motility is influenced by the levels of carnitine stored in spermatocytes (Costa et al., 2012) improving fish fertility. The offspring can develop endogenous reduction in the production and assimilation of L-carnitine, influencing its synthesis capacity, being necessary food supplementation in the initial phases of life (Ozório, 2009).

Under appropriate concentrations, L-carnitine, in addition to inhibiting lipid peroxidation, promotes cells' growth, avoiding pathological changes in different tissues, because it activates regulatory enzymes of blood defense cells and acts in cellular antioxidant activity (Guzmán-Guillén et al. 2015; Wang et al., 2016), it also promotes increase in digestive enzymes activity (amylase, protease and lipase) throughout the entire intestinal tract, stimulating better absorption of diets' nutrients (Jayaprakas et al., 1996).

Some of the effects produced by L-carnitine in fish reproductive development were observed by Jayaprakas et al. (1996), improvement in semen quality in males of *Oreochromis mossambicus*, and increase in offspring of *Poecilia reticulata* (Schreiber et al., 1997). The multifunctional attributions of L-carnitine in the development of several species were characterized by Harpaz (2005), highlighting its efficiency in fertility and offspring development. Wang et al. (2016) highlighted its protective activity in ovarian cells of *Ctenopharyngodon idellus* when induced to oxidative stress. However, information on L-carnitine influence in fish reproductive variables should be better elucidated.

Silver catfish, *Rhamdia quelen*, is particularly outstanding for its reproductive characteristics viable for manipulation, maturation

and egg laying in captivity, acceptance of artificial diets due to omnivorous habit, quick growth and resistance to variations in water quality and also growing acceptance by consumers (Carneiro et al., 2003; Fracalossi et al., 2004).

The importance of breeding animals' nutrition leads to constant search for information that can make feasible offspring obtention, providing success for captive breeding. Thus, the objective of this study was to assess the effect of diets supplemented with L-carnitine in reproductive and embryonic responses of *R. quelen*.

MATERIAL AND METHODS

All experimental procedures adopted were approved by the Committee for Ethics in Use of Animals of Universidade Estadual do Oeste do Paraná – Unioeste (CEUA/Protocol N° 3/16). 288 juveniles of *R. quelen* were used, with average initial weight of 94.36 ± 3.76 g, randomly distributed in 24 fish tanks with usable volume of 500 L, adapted in water recirculation system, controlled temperature and constant aeration. During the experimental period, water quality variables were kept in average of oxygen dissolved 6.38 ± 0.35 mg. L⁻¹, pH 7.05 ± 0.04 , electric conductivity 398.2 ± 1.8 μ S.cm⁻¹ and temperature 26.8 ± 0.2 °C.

The experimental design was fully random with six treatments and four repetitions. For the design adopted the supplementation of diets with five growing levels of L-carnitine (400, 800, 1200, 1600 and 2000 mg.kg⁻¹) and one control diet (without supplementation) isoenergetic (3,000 kcal of digestible energy.kg⁻¹ of feed) was considered (Table 1). Fish were fed four times a day until apparent satiation during 126 days (when they reach the first sexual maturation).

Breeding animals were selected according to external morphological characteristics, a total of six females (147.10 ± 31.80 g and 24.72 ± 3.25 cm) and six males (132.23 ± 45.83 g and 22.30 ± 2.48 cm) per treatment and hormone induced with carp pituitary extract – CPE (Reidel et al., 2010; Diemer, et al., 2014). The females selected received 0.5 mg of CPE.kg⁻¹ of live weight related to preparatory dose and, after 12 hours, 5.0 mg of CPE.kg⁻¹ of live weight. Males received one single dose of 2.5 mg of CPE.kg⁻¹ of live weight in the moment of the second application of females. After 240 hours-degree of the second induction extrusion was performed through abdominal massage in cephalocaudal direction for collection of gametes.

Fertilization was performed by the dry method (Zaniboni Filho and Weingartner, 2007) with homogenization of oocytes with semen, and then 100 ml of eggs were transferred to 20-liter experimental incubators and kept until the moment of hatching.

For determination of oocytes' diameter (Chatakondi and Kelly, 2013), before the first hormone induction, a sample of each repetition was collected using human urethral probe n°8 (Romagosa et al., 2001) and fixed in Gilson solution (Simpson, 1951). After induction, oocytes were collected on Petri dishes for total gauging of oocytes released per kg of fish. One aliquot of these oocytes was fixed in Gilson solution to compare oocytes' diameter before and after induction. The oocytes' diameter was gauged using BEL View magnifier, version 7.0 (BEL Engineering s.r.l., Monza, Italy) and diameter was calculated via arithmetic mean of the largest horizontal and vertical axis.

Table 1. Percent and nutritional composition of diets supplemented with L-carnitine for breeding animals of *R. quelen* raised in net-tanks.

Ingredients (%)	Levels of L-carnitine supplementation (mg Kg ⁻¹)					
	0	400	800	1200	1600	2000
Soy bran 45%	46.39	46.39	46.39	46.39	46.39	46.39
Corn	28.40	28.40	28.40	28.40	28.40	28.40
Rice grits	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal 55%	5.00	5.00	5.00	5.00	5.00	5.00
Chicken meat flour	5.00	5.00	5.00	5.00	5.00	5.00
Premix ¹	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine HCL	0.01	0.01	0.01	0.01	0.01	0.01
DL-methionine	0.17	0.34	0.35	0.34	0.34	0.35
Dicalcium phosphate	0.97	0.97	0.97	0.97	0.97	0.97
Common salt	0.30	0.30	0.30	0.30	0.30	0.30
Antifungal ²	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant ³	0.02	0.02	0.02	0.02	0.02	0.02
Soy oil	2.77	2.77	2.77	2.77	2.77	2.77
Limestone	0.33	0.33	0.33	0.33	0.33	0.33
Vehicle ⁴	0.04	0.04	0.04	0.04	0.04	0.04
Nutrients (%) ⁵						
Calcium	1.00	1.00	1.00	1.00	1.00	1.00
E. D. (kcal/kg) ⁶	3000.00	3000.00	3000.00	3000.00	3000.00	3000.00
Phosphorus total	0.80	0.80	0.80	0.80	0.80	0.80
Lysine total	1.74	1.74	1.74	1.74	1.74	1.74
Methionine total	0.64	0.64	0.64	0.64	0.64	0.64
Threonine total	1.17	1.17	1.17	1.17	1.17	1.17
Tryptophan total	0.37	0.37	0.37	0.37	0.37	0.37
Chemical composition (% Natural matter)						
Dry matter	93.042	91.715	89.658	91.717	91.692	91.686
P. B. ⁶	27.11	27.12	27.93	27.76	25.06	26.92
Ether 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
E. B. (Kcal.Kg ⁻¹) ⁶	4461	4459	4352	4446	4412	4356
Crude fiber	5.43	5.99	5.01	5.10	5.42	4.97

¹Premix composition: Levels of guarantee per kilo of product: Vit. A, 1,750,000UI; Vit. D3, 375,000UI; Vit. E, 20,000UI; Vit. K3, 500mg; Vit. B1, 2,000mg; Vit. B2, 2,500mg; Vit. B6, 2,500mg; Vit. B12, 5,000mg; Folic ac. 625mg; Pantothenate Ca, 7,500mg; Vit. C, 37,500mg; Biotin, 50mg; Inositol, 12,500mg; Niacin, 8,750mg; Co, 50mg; Cu, 1,250mg; Fe, 15,000mg; I, 100mg; Mn, 3,750mg; Se, 75mg; Zn, 17,500mg. ²Calcium propionate; ³BHT (Butylated hydroxytoluene) ⁴L-Carnitine mixed with bran corn. ⁵Calculated values. ⁶Values of gross and digestible energy, and crude protein (%) estimated for *Rhamdia quelen* proposed by ReideL et al. (2010); Freitas et al. (2011).

Approximately 12 hours after fertilization, three samples of eggs from each experimental unit were collected to estimate Fertilization Rate ($FR = \text{number of viable eggs} \times 100 / \text{total number of eggs}$), being considered only oocytes presenting translucent aspect (Okawara et al., 2015).

Hatching rate was calculated approximately 30 hours after fertilization; for such, larvae from each incubator were homogenized and three 50 ml samples were collected for determination of number of larvae existing in each sample, estimating the total number of larvae in each incubator.

The semen of each fish was collected in 10 ml Falcon tube (± 0.5 mL for gauging of volume) and kept in styrofoam box with ice at $\pm 12^\circ\text{C}$ until the end of analyses. pH was measured with colorimetric method using litmus paper Merck® (Asturiano et al., 2001) and sperm concentration was measured by diluting the semen in saline-buffered formaldehyde (1:1000) and counting was made with Neubauer hematometric chamber (Sanchez et al., 2011).

Normality was assessed by fixation in saline-buffered formaldehyde (1:1000 semen:fixer) of 500 μL of semen, later colored with rose bengal, then the extension plates were prepared and analyzed in light microscope (obj. 40x) (Caneppele et al., 2015), counting

300 spermatozoa of each fish, which were then classified as normal and abnormal according to CBRA (1998).

For follow-up of embryonic development, samples of eggs were collected in moments after fertilization until hatching (0, 6, 12 and 24 hours) and fixed in neutral formaldehyde at 4% (Eiras et al., 2000) to obtain measurements in total diameter, yolk sac diameter and perivitelline space in optical microscope Nikon Eclipse-50 photomicroscope (Nikon Instruments Inc., Melville, NY, USA) coupled with digital camera. For morphometric analysis of images cellSens Standard 1.15[®] software was used. The definitions of embryonic phases used followed terminologies according to Pereira et al. (2006).

After gametes collection, three females of each experimental unit were anesthetized with benzocaine 100 mg L⁻¹ (Gomes et al., 2001) and submitted to blood collection by puncture of caudal vessel, using disposable syringes containing 10% of EDTA (ethylenediamine tetracetic acid). Assessment of levels of plasma calcium and triglycerides was made using samples of the plasma obtained by centrifugation at 2.500 rpm, for five minutes, for quantitative determination using commercial kits (Gold analisa Diagnostica[®]) and later the reading in spectrophotometer.

After blood collection fish were euthanized with benzocaine solution (250 mg L⁻¹) to remove gonads, liver and fat to obtain gonadosomatic index (GSI), hepatosomatic index (HSI) and viscerosomatic fat index (VSFG) (*Indices: organ weight/fish total weight X 100*) respectively.

Then ovaries and testicles' samples were fixed in ALFAC for histological analysis. The histological cuts were colored with Hematoxylin and eosin (HE), plates were analyzed in light microscope (NIKON Eclipse-50) and classified according to gonad maturation stage (Vazzoler, 1996).

In the histomorphometric analysis of gonads, images of ovaries were captured. For females' measurements of oocyte diameter and average height of follicular cells' layer, previtellogenic and vitellogenic oocytes were obtained. For males, measurements of seminiferous tubules' diameter and height of germinal epithelium were made.

Data obtained, meeting normality and homoscedasticity assumptions, were submitted to ANOVA and when significant, means were compared in Duncan test at 5% assisted by Statistica 7.0[®] software.

RESULTS

For females of *R. quelen* fed with feed containing L-carnitine the fertilization rate did not differ among levels of supplementation and relative fecundity was reduced when 2000 g of L-carnitine. Kg⁻¹ was used with average of 92.40 oocytes.gr⁻¹ (p<0.05) (Table 2).

Hepatosomatic and visceral fat indices did not present significant difference among treatments (p>0.05) (Table 2). Oocytes' diameter was superior (833.48 µm) (p<0.05) in females fed with 1200 mg of L-carnitine.Kg⁻¹, while those fed with 400 mg of L-carnitine. Kg⁻¹ produced oocytes with smaller diameter (736.38 µm) (p<0.05).

Plasma calcium and triglycerides presented reduction (p<0.05) when 1600 mg of L-carnitine.Kg⁻¹ was used against the other levels of supplementation (Table 2).

Males of *R. quelen* fed with feed containing L-carnitine presented superior sperm normality (p<0.05) against those fed without supplementation, with average of 93.65% (Table 3). Sperm concentration (1.16 x 10⁹.mL⁻¹) was superior (p<0.05) in males fed with diets containing 1600 mg of L-carnitine.Kg⁻¹, however, it did not differ from other treatments, except when supplementation exceeded this level (2000 mg.Kg⁻¹) the average concentration was 0.67 x 10⁹.mL⁻¹ (Table 3).

In histomorphometry of testicles of *R. quelen* in final maturation stage, the seminiferous tubule diameter (87.90 µm) was superior (p<0.05) in fish fed with diets containing 1200 mg of L-carnitine. Kg⁻¹, while the shortest diameter (45.73 µm) was observed in fish fed with 1600 mg.Kg⁻¹ (p<0.05) (Table 4). The average height of germinal epithelium was shorter (p<0.05) in fish fed with diets containing more supplementation of L-carnitine (1600 and 2000 mg.Kg⁻¹) (Table 4). A higher accumulation of spermatozoa was also microscopically observed in fish fed with diets containing 400 and 800 mg.Kg⁻¹ of L-carnitine, which were not fully released. Fish fed with 0, 1200, 1600 and 2000 mg.Kg⁻¹ levels, on the other hand, presented a higher presence of sperm cells in formation, due to a higher semen release, once again preparing for maturation.

Based on histological observations of *R. quelen* ovaries in final maturation stage, for presenting asynchronous oocyte development characteristic of the species, larger previtellogenic oocytes were observed in supplementation levels superior to 800 mg.Kg⁻¹ of

Table 2. Reproductive parameters of females of *R. quelen* fed with feed containing different levels of L-carnitine.

Parameters	Levels of L-carnitine (mg Kg ⁻¹)					
	0	400	800	1200	1600	2000
FR (%)	52.98±14.43ab	87.9±6.36a	54.46±4.08ab	44.60±35.01ab	17.48±8.36b	28.36±24.68b
TFC (oocyte.gr⁻¹)	226.78±100.98ab	323.97±81.51a	154.53±83.07ab	275.27±115.66ab	153.47±107.35ab	92.40±58.62b
GSI (%)	21.61±6.91	16.94±8.06	17.35±9.32	11.14±0.64	12.24±6.81	9.86±6.64
HSI (%)	1.88±0.70	1.79±0.13	1.77±0.27	2.71±1.36	1.51±0.32	1.75±1.74
VSFG (%)	1.28±0.52	1.85±0.96	0.96±0.81	1.36±0.32	1.48±0.81	1.89±1.74
OD (mm)	794.16±43.80bc	736.38±64.12e	816.76±49.86ab	833.48±66.01a	768.76±52.53cd	758.65±58.14de
PC (mg/dL⁻¹)	22.33±0.04b	23.08±1.20b	23.04±0.94b	23.60±0.89b	19.87±0.66a	22.05±0.64b
T (mg/dL⁻¹)	183.78±47.91ab	150.80±61.70ab	202.43±73.88b	192.70±55.14ab	99.17±74.24a	150.60±50.84ab

Fertilization rate (FR), Fecundity rate (TFC), gonadosomatic index (GSI), Hepatosomatic index (HSI), viscerosomatic fat index (VSFG), Oocytes' diameter (OD), Plasma calcium (PC), Triglycerides (T). Means followed by lower case indicate significant effect of L-carnitine supplementation for each diet by Duncan test with 5% probability.

Table 3. Reproductive parameters of males of *R. quelen* fed with diets containing different levels of L-carnitine.

Parameters	Levels of L-carnitine (mg Kg ⁻¹)					
	0	400	800	1200	1600	2000
Normality (%)	86.23±6.63b	93.35±3.95a	93.34 3.57a	92.7±3.02a	95.77±3.95a	93.11±3.48a
Concentration (SPZx 10 ⁹ .mL ⁻¹)	1.07±0.27ab	0.87±0.11ab	0.98±0.39ab	0.99±0.18ab	1.16±0.16a	0.67±0.24b
pH	7.00±00	7.00±00	7.00±00	7.00±00	7.00±00	7.00±00
Volume (mL)	5.22±0.82	4.38±1.02	4.55±0.59	4.70±1.76	4.12±0.96	4.18±1.76
Survival (%)	91.79±6.97	86.70±6.91	90.97±2.67	84.96±4.15	94.88±0.49	95.74±3.77

Means followed by lower case letters indicate significant effect of L-carnitine supplementation for each diet by Duncan test with 5% probability.

Table 4. Gonad histomorphometry of males and females of *R. quelen* fed with diets containing different levels of L-carnitine.

Males (µm)	Levels of L-carnitine (mg Kg ⁻¹)					
	0	400	800	1200	1600	2000
DST	70.02±11.36ab	74.85±9.02b	62.30±9.35c	87.90±8.79a	45.73±8.55d	62.52±8.96c
AHGE	22.19±6.69abc	25.63±10.70a	23.67±4.82ab	17.62±4.28abc	14.28±2.69bc	13.56±2.40c
Females (µm)	0	400	800	1200	1600	2000
DPO	89.89±19.78c	103.86±36.92bc	128.80±24.97a	140.34±25.50a	122.88±28.28ab	120.18±38.56ab
DVO	203.49±53.48bc	191.00±22.95c	227.39±19.73ab	248.23±31.24a	235.79±24.62a	225.60±14.60ab
AHFC	18.60±5.21b	21.65±5.82ac	17.55±4.85b	22.82±4.01a	18.88±2.90bc	23.32±3.77a

Diameter of seminiferous tubule (DST); Average height of germinal epithelium (AHGE); Diameter of previtellogenic oocytes (DPO); Diameter of vitellogenic oocyte (DVO); Average height of follicular cells (AHFC). Means followed by lower case letters indicate significant effect of L-carnitine supplementation for each diet by Duncan test with 5% significance.

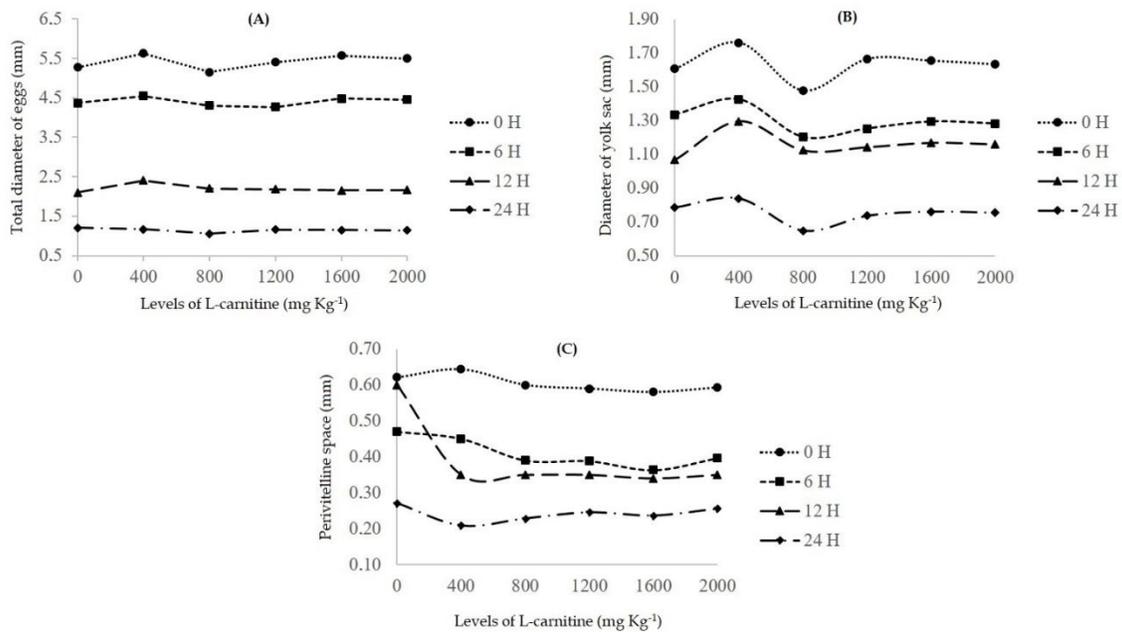


Figure 1. Embryonic development of *R. quelen* from breeding animals fed with diets containing different levels of L-carnitine in times 0h, 6h, 12h and 24h. (A) Total diameter of eggs; (B) Diameter of yolk sac; (C) Perivitelline space. Means followed by lower case letters indicate significant effect of L-carnitine supplementation for each diet by Duncan test with 5% probability.

L-carnitine, while animals fed without L-carnitine or with 400 mg.Kg⁻¹ presented the smallest diameters (p<0.05) (Table 4). Vitellogenic oocytes with larger diameters were observed when levels above 800 mg.Kg⁻¹ of L-carnitine (p<0.05) were used. The average height of follicular cells varied among treatments, being higher when 2000 mg.Kg⁻¹ of L-carnitine (p<0.05) was used (Table 4).

In *R. quelen* embryos it was observed that the total diameter of eggs was larger when coming from females fed with diets supplemented with 400 mg.Kg⁻¹ of L-carnitine from 0h to 24h after fertilization, as well as increase in yolk sac diameter (p<0.05) (Figure 1). However, for eggs under level of 800 mg.Kg⁻¹ of L-carnitine, there was reduction in yolk sac diameter in times

0h and 24h ($p < 0.05$). Perivitelline spaces were smaller in eggs from females that received diets containing all levels of supplementation with L-carnitine in their composition starting from 12h of development ($p < 0.05$), when compared to eggs from females that received control diet. In other times there was no significant difference among treatments.

DISCUSSION

L-carnitine supplementation in diets positively influenced the semen quality of *R. quelen* with higher percent in spermatozoa normality when compared to control treatment. According to Jayaprakas et al. (1996), the energetic metabolism promoted by L-carnitine acted as of 900 mg.Kg^{-1} concentration in males of tilapias, improving sperm feasibility due to the efficient use of fat acids in spermatogenesis process (Al-Daraji and Tahir, 2014).

Increase in sperm concentration occurred when 1600 mg of L-carnitine. Kg^{-1} was used in males' diet, and the improvement in semen quality promoted by the use of L-carnitine possibly occurs due to its efficiency as chemoprotective/antioxidant, hindering the formation of free radicals in the semen (Agarwal and Said, 2004). Moreover, lipids are important constituents of sperm metabolism and fertilization (Navarro et al., 2010).

According to Agarwal and Said (2004), L-carnitine stimulates the sperm metabolic process through its derivation into acetyl-L-carnitine, which acts as primary energy source for spermatozoa due to its storage in germinal cells while keeping cellular structure and so providing better efficiency to the spermatogenic process, cells' maturation and sperm motility.

The *R. quelen* testicles presented larger diameter of seminiferous tubule in fish fed with diets containing 1200 mg of L-carnitine. Kg^{-1} . L-carnitine induces the energetic absorption through the action of glucose-lactate-glucose pathway by Sertoli cells, influencing their stimulation and directing the control of the spermatogenic process, with maturation and maintenance of germinal cells, in addition to removing the excess of Acetyl-CoA (Palmero et al., 2000).

The germinal epithelium average height was shorter than in fish fed with diets containing higher levels of L-carnitine. According to Agarwal and Said (2004), the concentration of L-carnitine accumulated in gonads along the growth process keeps cells acting, directing their energy load to gametogenesis by energetic balance, demonstrating stabilizing effect without limiting the metabolism through phospholipids acetylation.

The increase in concentration of L-carnitine directed to the cell development in males of *R. quelen* caused reduction of the fish spermatogenic structures. According to Jeulin et al. (1987), such fact may influence the spermatogenic concentration in lumen and spermatozoa motility.

According to the results observed in this study, fertilization and fecundity presented higher rates when supplementation levels of 400 mg of L-carnitine. Kg^{-1} were used when compared to control treatment and to higher levels of supplementation, meeting the needs of the fish to potentiate outcomes.

L-carnitine influence in gonads probably occurred in the mobilization of lipids to oocytes due to their capacity for reabsorption and biosynthesis, and though the levels of intracellular lipid of oocytes are highly variable among species, providing rich energetic support to maturation and development of oocytes, thus decisively contributing to improve fertilization and fecundity rates (Genicot et al., 2005; Turini, 2008; Sturmey et al., 2009).

The active triggering of beta oxidase through L-carnitine promotes more feasibility of oocytes because it induces the development of gametes (Dunning and Robker, 2012), acting in the protection and growth of ovarian cells, improving its viability and protecting from oxidative process through intracellular balance via regulation of antioxidant enzymes, which reduces its effect when levels of L-carnitine are raised (Wang et al., 2016).

In this study, females fed with 400 mg of L-carnitine. Kg^{-1} presented smaller oocyte diameter, however, the best responses in fertilization and fecundity rates. According to Romagosa et al. (2001), females from rhiphelic species present success of fertilization in oocytes with diameters smaller than 1 mm , when compared to females presenting larger oocytes.

L-carnitine increases phospholipids' synthesis necessary for stabilization of the oocyte membrane and intrinsic processes in energy generation favoring the quality of oocytes and their viability (Felizardo et al., 2012; Patel et al., 2012). Thus, supplementation in ideal doses of L-carnitine becomes a potential energetic precursor for females of *R. quelen* in reproductive process.

The level of plasmatic calcium was reduced in females fed with diet containing 1600 mg.Kg^{-1} of L-carnitine and the most expressive concentration occurred when 1200 mg.Kg^{-1} L-carnitine was used, though not differing from the other levels of supplementation, corroborates the increase in diameter of oocytes from females in the same treatment, probably presented more deposition of vitellogenin, protein that rises Ca molecules circulating in the blood (Gillespie and Peyster, 2004).

The association of the vitellogenic oocyte size to the diameter of oocytes from females fed with 800 and 1200 mg.Kg^{-1} levels of L-carnitine, shows increase in cellular development, though the hypercalcemic effect during the maturation process of females can be kept equalized in females in development stage fit to egg laying (Sanchez, 2006), as could be observed in females fed with control diet and supplementation levels of 400 , 800 and 2000 mg.Kg^{-1} of L-carnitine.

The administration of L-carnitine in diets for fish promotes reduction in plasmatic levels of triglycerides, due to the action of carnitine-acyltransferase I, which regulates the speed of entry of fat acid in mitochondria controlling lipoprotein activity and reducing levels of plasmatic triglycerides (Kurban and Mehmetoglu, 2006; Li et al., 2007; Yavuz and Kurtoğlu, 2014). However, females fed with 800 and 1200 mg of L-carnitine. Kg^{-1} presented higher levels of plasmatic triglycerides and obtained larger diameter of oocytes, but did not positively reflect in gametes fertilization and fecundity processes.

The oocyte development in females of *R. quelen* was influenced by L-carnitine supplementation in diets, with observation of larger previtellogenic and vitellogenic oocytes when L-carnitine above

800 mg.Kg⁻¹ was used, for conferring a protective effect on the viability of germinal cells and promoting its growth (Wang et al., 2016), there is stimulation of the cellular energy metabolism by the control of carbohydrates and lipid catabolism involved in β -oxidation, therefore improving the stability and efficiency of hormone stimulations for the development of cells in final phases of maturation (Canbaz et al., 2007; Calabrese et al., 2012).

The average height of follicular cells varied among treatments, and was higher when 2000 mg of L-carnitine.Kg⁻¹ was used. In the course of oocyte development in advanced maturation, phase that secondary growth oocytes and vitellogenic oocytes have accumulated through intracellular transport molecules of lipids and proteins, due to the active cellular synthesis during vitellogenesis (Vazzoler, 1996), L-carnitine provides the cells increase in inter-cell space, due to actions of cells that produce glycoprotein.

While assessing the embryonic development of *R. quelen*, it was observed that the total diameter of eggs was larger when coming from females fed with diets containing 400 mg of L-carnitine. Kg⁻¹, thus reflecting the larger diameter of yolk sac, resulting in larger and more resistant larvae.

The metabolic energy acquired during the process of gamete formation, possibly, continues to be transformed during the development of the offspring of *R. quelen* from matrices fed with L-carnitine, occurring increase of additional energy (Dias et al., 2001) to the egg, because its growth and migration in initial phases into fish need fat acids (Sargent et al., 1999; Tocher, 2003) and therefore demands carnitine to interact in their metabolic processes.

The use of cell energy source to maintain the egg's growing process can be minimized due to the metabolic change promoted by L-carnitine via direction of enzymes responsible for oxidation of fat acids (Ji et al., 1996). However, when level of 800 mg of L-carnitine.kg⁻¹ was used, reduction in diameter of yolk sac occurred, probably due to inefficiency in the process of oxidation of unsaturated fat acids, which caused increase in cell energy use and promoted quick absorption of yolk sac directed to embryo growth.

The Perivitelline space in eggs from females that received diets supplemented with L-carnitine, regardless of the level used, were reduced at 12 h of development (p<0.05), and when compared to eggs from females that received control diets there was no reduction in the efficiency of their final development, thus acting in the egg protection and therefore higher survival of the embryo (Sanches et al., 2001).

CONCLUSIONS

The supplementation of 400 mg of L-carnitine.Kg⁻¹ in diets for females of *Rhamdia quelen* provided better reproductive performance of females. For males, there was improvement in the production of spermatozoa and semen quality when fed with diets containing 1200 mg of L-carnitine.Kg⁻¹. L-carnitine speeds up the development of the offspring in the first hours of embryonic phase, when generated from breeding animals fed with supplementation of 400 mg of L-carnitine.Kg⁻¹ in the diet.

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