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Vitamin C supplementation on growth performance and gonadal development in Nile tilapia

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ABSTRACT. The study aimed to verify the vitamin C supplementation on performance and gonadal development of Nile tilapia. 160 fingerlings distributed in 16 tanks with 65 L capacity and constant water renewal in a closed recirculation system were used. The design was completely randomized with four treatments (0, 150, 300, and 600 mg of monophosphate L-ascorbic acid kg^{-1} diet) and four replications. We evaluated the weight gain parameters, apparent feed conversion, apparent feed intake and carcass yield to evaluate of production performance. We analyzed the sexual maturation stage and gonadosomatic index (GSI) to evaluate reproductive performance. There was no statistical difference in the performance parameters. It was observed significant difference to the GSI. No significance was found regarding other reproduction parameters. We recommend the use of 150 mg kg^{-1} of vitamin C to the diet of Nile tilapia from to the reproductive index.

Keywords: ascorbic acid, *Oreochromis niloticus*, reproduction.

Suplementação de vitamina C sobre o desempenho e desenvolvimento gonadal de tilápia-do-Nilo

RESUMO. O estudo objetivou verificar a suplementação de vitamina C sobre o desempenho produtivo e desenvolvimento gonadal da tilápia-do-Nilo. Foram utilizados 160 alevinos, distribuídos em 16 aquários com capacidade de 65 L e renovação de água constante em sistema fechado de recirculação. O delineamento foi inteiramente casualizado com quatro tratamentos (0, 150, 300, e 600 mg de monofosfato de ácido L-ascórbico kg^{-1} de ração) e quatro repetições. Foram avaliados os parâmetros de ganho em peso, conversão alimentar aparente, consumo aparente de ração e rendimento de carcaça para avaliação de desempenho produtivo. Foram analisados o estádio de maturação sexual e índice gonadossomático (IGS) para avaliação de desempenho reprodutivo. Não houve diferença estatística para os parâmetros de desempenho. Foi observado diferença significativa para o índice gonadossomático. Não foi observada significância em relação a outros parâmetros reprodutivos. Recomenda se a utilização de 150 mg kg^{-1} de Vitamina C à ração de tilápia-do-Nilo para o índice reprodutivo.

Palavras-chave: ácido ascórbico, *Oreochromis niloticus*, reprodução.

Introduction

In intensive and super-intensive production systems, natural food is restricted and nutritional requirements are meeting by the food supplied, which should contain highly digestible ingredients at appropriate proportions for maximum utilization by the animal. In the context nutrition, animal growth and development can be influenced by the bioavailability of nutrients and by the concentration of vitamins and minerals (Navarro, Lanna, Donzele, Matta, & Souza, 2007; Fernandes Junior et al., 2010; Salaro et al., 2013).

The requirements for vitamins may be affected by intrinsic factors to the animal (growth rate, body size, species, reproductive status), diet composition and interaction with other nutrients and are also influenced by the production system (Sanches, Bombardelli, & Marcos, 2010; Combs, 2012).

Most teleost does not synthesize vitamin C by the absence of the enzyme L-gulonactone oxidase, which catalyzes the last reaction in the synthesis of ascorbic acid from glucose, therefore, it must be added to the diet (Borba, Fracalossi, & Freitas, 2007; National Research Council [NRC], 2011).

A diet free from or deficient in ascorbic acid reduces the availability of vitamin C in the ovary, reducing the number of eggs and their hatchability and increases both the number of larvae with

deformities and the mortality rate (Navarro, Filho, Ferreira, & Pereira, 2009). Some authors have reported the influence of vitamin C in the reproduction and performance of fish (Zhou, Wang, Wang, Xie, & Wang, 2012, Nguyen et al., 2012, Gao, Shunsuke, Ishikawa, Yokoyama, & Mamauag, 2014).

In this context, this study aimed to analyze the growth performance and gonadal development in Nile tilapia supplemented with different levels of vitamin C.

Material and methods

The experiment was conducted in the Laboratory of Aquaculture, University of Brasilia – UnB.

One hundred and sixty male of Nile tilapia (*Oreochromis niloticus*) with an average initial weight of 8,00±1 g, from the Department of Fish Farming of the Department of Agriculture of the Federal District were distributed in 16 tanks of 65 L with constant water renewal (750 mL min^{-1}) in a closed water recirculation system. The whole procedure was approved by the Ethics Committee on the Use of Animals (CEUA) with protocol 43079/2012.

The experiment was set up according to a completely randomized design with four treatments of vitamin C supplementation (0, 150, 300, and 600 mg kg^{-1} L-ascorbic acid monophosphate) to an iso-protein feed of 32% Crude protein and iso-caloric of 3600 kcal DE kg^{-1} with four replications. The acclimation period lasted five days. The feed was divided into two daily meals, with the feed given at 8:00 and 17:00 hours. Biometric measurements were taken every seven days, in order to adjust the feed supplied at 5% of body weight.

Water temperature was monitored daily at 7:00 and 17:00 hours, while the pH, dissolved oxygen and ammonia were monitored every seven days.

We analyzed the nutritional parameters of weight gain (WG), apparent feed intake (AFI), apparent feed conversion (AFC), carcass quality and yield (CY), according Equation 1:

$$\begin{aligned} &WG\ (g) = \text{final weight} - \text{initial weight} \\ &AFC = AFI / WG \\ &CY\ (\%) = [(DCW \times 100) / \text{final weight})] \end{aligned} \quad (1)$$

where:
DCW = dressed carcass weight.

For histological analysis, animals were killed by benzocaine solution with 200 mg L^{-1}. Thereafter, gonads were fixed in Bouin solution, preserved in 70% ethanol and subjected to standard processing for electron microscopy analysis. Then, gonads were stained with hematoxylin - eosin (HE) and sectioned to 5 μm thickness. Histological preparation and morphological and morphometric analyses were performed at the Pathology Laboratory, UnB.

In light microscopy, we evaluated the volumetric ratio of the components of testicular parenchyma, with the aid of an integrated eyepiece with 121 points, at 400X magnification. Points corresponding to the seminiferous tubule, intertubule, blood and lymph vessels were counted in twenty random fields for each animal.

We also calculated the viscerosomatic index (VSI), gonadosomatic index (GSI) and hepatosomatic index (HSI), according Equation 2:

$$\begin{aligned} &GSI = (GW / BW) \times 100 \\ &VSI = (\text{viscera weight} / BW) \times 100 \\ &HSI = (\text{liver weight} / BW) \times 100 \end{aligned} \quad (2)$$

where:
GW = gonad weight;
BW = body weight.

Data normality was tested according to Kolmogorov-Smirnov (Massey Junior, 1951). Analyses were run by Duncan's test at 5% significance in the software SAS®.

Results and discussion

The water quality parameters showed the following values: 21.74±0.43°C for initial temperature, 29.50±0.03°C for room temperature, 5.5 for pH; 6.1±0.36 mg L^{-1} for dissolved oxygen, 0.35 for ammonia and 0.10 for NO_2. Navarro, Navarro Filho, Ferreira, & Pereira Filho (2012) reported similar results.

The levels of vitamin C did not affect the final weight, total length, standard length, weight gain and apparent feed conversion (Table 1). A similar result was reported by Reis et al. (2011) who observed that supplementation of 600, 850, 1100, 1600 and 2600 mg vitamin C monophosphate did not affect performance parameters in catfish larvae (*Rhamdia voulezi*). These results contradict Falcon, Barros, Pezzato, & Valle (2007), which stated that ascorbic acid directly influences the growth of fish.

No significant difference was found for viscerossomatic index, hepatosomatic index and gonad weight. The results of chemical analysis of the carcass (dry matter, protein, ether extract) and carcass yield showed no significant difference (Table 2). Probably, the amount and period of supply of vitamin C for the treatments were not enough to stimulate collagen synthesis.

Table 1. Final weight (FW), total length (TL), standard length (SL), apparent feed intake (AFI), weight gain (WG) and apparent feed conversion (AFC) of Nile tilapia fingerlings fed with diets containing different levels of supplemental vitamin C.

	Vitamin C mg kg^{-1}			
	0	150	300	600
FW (g)	18.57±0.49	22.95±2.47	18.29±1.01	18.86±1.01
TL (cm)	9.89±1.00	10.26±0.96	10.03±0.96	10.26±0.75
SL (cm)	7.43±0.88	7.78±0.97	7.41±0.92	7.56±0.69
AFI (g)	21.88±1.92	29.66±7.05	23.53±3.57	19.13±4.58
WG (g)	10.50±0.49	14.87 ±2.48	10.21±1.01	10.79±1.01
AFC	2.08±0.25	1.99±0.16	2.30±0.62	1.80±0.52

Mean values ± standard error.

Table 2. Viscerossomatic index (VSI), hepatosomatic index (HSI), gonadosomatic index (GSI), gonad weight (GW), carcass yield (CY) and carcass composition in dry matter (DM), crude protein (CP) and ether extract (EE) of Nile tilapia fingerlings fed different levels of supplemental vitamin C.

	Vitamin C mg kg^{-1}			
	0	150	300	600
VSI %	12.04±3.04	11.34±3.62	11.28±4.07	12.94±3.90
HSI %	3.51±1.28	3.36±1.05	3.10±1.13	3.86±1.78
GSI %	0.49±0.09^{b}	0.53±0.13^{a}	0.58±0.15^{a}	0.54±0.13^{a}
GW(g)	0.11±0.07	0.10±0.05	0.17±0.22	0.11±0.09
CY%	86.53±1.57	87.05±1.57	86.10±1.36	85.66±2.34
DM%	25.69±2.67	26.13±1.45	26.68±5.5	26.31±1.6
CP%	48.86±3.56	48.87±2.35	48.73±7.8	48.90±3.8
EE%	27.57±2.56	27.40±4.5	29.59±4.8	28.59±8.9

Different letters in the row indicate significant differences ($p < 0.05$) by Duncan's test; Mean ± standard error.

There was a significant difference in GSI values between the treatments, with higher values for the treatments with 150 and 300 mg kg^{-1}. This result demonstrates the importance of the vitamin as an antioxidant; the main role of vitamin C is to act synergistically with vitamin E, reducing lipid peroxidation and preserving the integrity of the cell (Navarro et al., 2009). In this way, other studies registered a positive effect on fish reproduction. Mataveli, Moraes, Streit Junior, Ribeiro, and Gasparino (2010) observed an increase in sperm motility in Nile tilapia and recommended supplementation at 225 mg kg^{-1}. Soliman, Jauncey, and Roberts (1986) also verified a delay of gonadal maturation in tilapia mossambica fed diet free of ascorbic acid. Furuita et al. (2009) reported a significant improvement in the quality of eggs of *Anguilla japonica*. Navarro et al. (2010) found a significant increase in GSI in tilapia fed supplemental vitamin E.

In the microscopic analysis of gonads (Figure 1), we observed a dynamic spermatogenesis process, as described by Navarro, Sousa, Bizarro, and Navarro (2015) for the same species. No significant difference between treatments was detected for seminiferous tubules and intertubular space (Table 3). Andrade, Tonini, Burkert, Godinho, and Vidal Júnior (2010) described that in the intermediate phase maturation, there is a reduction of primary spermatogonia and primary light tubules at this stage is more enlarged than the initial stages of maturation. The intertubular compartment of the testicular parenchyma consists of blood and lymph vessels, nerves, connective cells and Leydig cells. The arrangement of these components can be different according to the species (Fawcett, Neaves, & Flores, 1973).

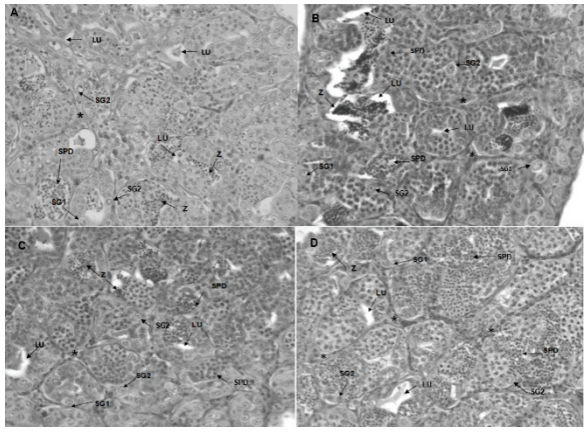

Figure 1. Light micrograph (hematoxylin-eosin staining) of testis of Nile tilapia males showing the primary spermatogonia (SG1), secondary spermatogonia (SG2), spermatids (SPD), lumen (LU), sperm (Z) and interstitial space (asterisk): A) Treatment without dietary vitamin C supplementation; B) Treatment supplemented with 150 mg vitamin C in the diet; C) Treatment supplemented with 300 mg vitamin C in the diet; and D) Treatment supplemented with 600 mg vitamin C in the diet.

Table 3. Germinal epithelium, spermatozoa, tubule lumen, connective tissue, blood vessel and Leydig cells of Nile tilapia fingerlings fed different levels of supplemental vitamin C.

	Vitamin C mg kg^{-1}			
Treatment	0	150	300	600
Germinal epithelium%	77.68±2.41	74.38±2.36	71.07±0.25	87.67±4.56
Lumen %	2.47±2.35	9.91±2.58	1.65±2.55	4.95±1.58
Spermatozoa %	4.13±0.20	1.65±4.33	13.22±0.03	1.65±1.36
Tubular space %	84.28	85.94	85.94	94.27
Connective tissue %	7.43±5.20	12.39±2.25	5.78±2.36	1.65±1.48
Vessel %	0.82±2.33	0±0.0	1.65±2.35	2.42±1.47
Leydig cell %	7.43±2.58	1.65±0.58	6.61±3.56	1.66±1.49
Intertubular space %	15.72	14.06	14.94	5.73

Leydig cells may occupy a small percentage, about 2% in rat (França & Russel, 1998), reaching 51.7% in capybara (Paula, Costa, Fonseca, & Neves, 2007). In fish, Leydig cells were found near the spermatic duct, suggesting a functional relationship with the spermatogenic process (Vilela, Silva, Peixoto, Godinho, & França, 2003). Thus, Leydig cells are the component of the intertubular compartment with the highest variation among species already studied. Navarro et al. (2010) observed a significant difference in the percentage of Leydig cell of animals of the treatment with 50 mg vitamin E per kg of feed in relation to the other treatments.

Conclusion

The supplementation of 150 and 300 mg kg^{-1} vitamin C to the diet given to Nile tilapia results in a positive increase in GSI. It is recommended a supplementation of 150 mg kg^{-1} of vitamin C to reduce feed costs.

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