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Vitamin C supplementation in aquaculture activities

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ABSTRACT

This study investigated the effects of different levels of vitamin C in diet of fish with oxidative stress biomarkers and hematological parameters of silver catfish. Juvenile fish were divided into three groups, and fed with different amounts of vitamin C: 1) 0, 2) 500, and 3) 1,000 mg kg^{-1}. Ten fish per group were collected at 0, 14, and 28 days after the beginning of the experiment. Liver catalase activity increased in group 1 after 28 days. The GPx activity in gills decreased in all groups, and the hepatic GPx activity increased in groups fed with vitamin C. SOD hepatic activity and NPSH levels in gills and liver were not affected. In all groups, the GST activity in gills decreased, and the GST activity in liver increased. The levels of lipid peroxidation decreased in liver and gills for groups 2 and 3. This study's results indicate that both diets with 500 or 1,000 mg/kg of vitamin C contributed to the decrease in the oxidative damage of *Rhamdia quelen*, suggesting that vitamin C supplementation in the present experimental model could be useful in aquaculture to overcome possible oxidative changes induced by adverse environmental conditions.

Index terms: antioxidant, ascorbic acid, catfish, food.

Ideias centrais

- Vitamina C, importante antioxidante não proteico, melhora o status da saúde dos peixes na atividade de aquicultura
- Efeitos dos níveis de vitamina C na dieta de peixes sobre biomarcadores do estresse oxidativo e parâmetros hematológicos em *Rhamdia quelen*
- Redução dos níveis de peroxidação lipídica nos grupos alimentados com 500 a 1.000 mg kg^{-1} de vitamina C, em brânquias e fígado
- Dietas com 500 ou 1.000 mg kg^{-1} de vitamina C contribuem para diminuir o dano oxidativo de *Rhamdia quelen*
- Na aquicultura, a suplementação de vitamina C pode ser útil para superar possíveis mudanças oxidativas induzidas por condições ambientais adversas

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Suplementação de vitamina C em atividades de aquicultura

RESUMO

Neste estudo, investigaram-se os efeitos de diferentes níveis de vitamina C na dieta de peixes com biomarcadores de estresse oxidativo e parâmetros hematológicos em jundiás. Os juvenis foram divididos em três grupos e alimentados com diferentes quantidades de vitamina C: 1) 0, 2) 500 e 3) 1.000 mg kg^{-1}. Dez peixes por grupo foram coletados em 0, 14 e 28 dias após o início do experimento. A atividade da catalase hepática aumentou no grupo 1 após 28 dias. A atividade da GPx nas brânquias diminuiu em todos os grupos, e a GPx hepática aumentou nos grupos alimentados com vitamina C. A atividade hepática da SOD e os níveis de NPSH nas brânquias e no fígado não foram afetados. Em todos os grupos, a atividade GST diminuiu nas brânquias e aumentou no fígado. Os níveis de peroxidação lipídica diminuíram no fígado e nas brânquias para os grupos 2 e 3. Os resultados deste estudo indicam que ambas as dietas, com 500 ou 1.000 mg/kg de vitamina C, contribuíram para diminuir o dano oxidativo de *Rhamdia quelen*, sugerindo que a suplementação de vitamina C no presente modelo experimental pode ser útil na aquicultura para superar possíveis mudanças oxidativas induzidas por condições ambientais adversas.

Termos para indexação: antioxidante, ácido ascórbico, peixe-gato, alimento.

INTRODUCTION

The nutrients required by fish for any normal physiological functions are almost the same as those of land animals. On the other hand, feeding fish in their aquatic environment is more complex than feeding land animals, because in aquatic environment the following features must be taken into account: the nutrient contribution of natural aquatic organisms, the effect of feeding on water quality, and the loss of nutrients that were not consumed immediately (Lovell, 2013). Therefore, in fish farming, as the fish are kept confined, natural food becomes scarce, thus, animals need a complete and nutritionally balanced diet in order to avoid reduction of fish resistance to the spread of disease or reduction in growth (Campeche et al., 2009; Garcia et al., 2009; Lovell, 2013).

Moreover, fish from fish farming have problems with gene pool alterations, competition, and disease transmission due to interbreeding and cohabiting within the same environment. This fact, associated with changes in foraging behavior, aggression, individual swimming and group behavior, sometimes results in a stress state (Paehlke, 1995; Martins et al., 2012). In this context, since vitamin C is an important non-protein antioxidant, it could improve the status of fish health in such conditions.

Vitamin requirements depend on the environmental conditions, the interrelationship with other nutrients present in the diet, and fish health (Navarro et al., 2009). Teleost fish are unable to synthesize vitamin C due to the absence of enzyme L-gulonolactone oxidase (GLO), which catalyzes the conversion of L-gulonic acid to ascorbic acid (Wang et al., 2003; Andrade et al., 2007; Vélez-Alavez et al., 2014). However, vitamin C is an indispensable micronutrient, required in diet to maintain the physiological processes of certain animals, including most fish. This vitamin is a water-soluble nutrient, essential for the synthesis of red blood cells and blood vessels, and for tissue repair (Wang et al., 2003; Vélez-Alavez et al., 2014). Many vitamin C deficiency symptoms in fish have been described, including internal bleeding, immunosuppression, increased susceptibility to bacterial infections, reduced growth, skeletal muscle injury, structural deformities, and changes in natural pigmentation, among others (Lee & Dabrowski, 2003; Navarro et al., 2009; Vélez-Alavez et al., 2014).

Moreover, diets lacking vitamin C can lead to increased cellular oxidation caused by the imbalance between the production of free radicals and the antioxidant response. The production of reactive oxygen species (ROS) occurs in a normal situation due to cellular metabolism, and organisms are able to control this situation. However, when fish are submitted to environmental stress, like pollutants, oxidative damage is frequently recorded (Bloomer et al., 2005; Salaro et al., 2013). In order to inhibit or reduce oxidative damage caused by increased ROS and free radicals, the organism has an efficient antioxidant defense system. Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) are part of the endogenous enzymatic antioxidant defense system, while reduced glutathione (GSH) and vitamins A, C and E belong to the non-enzymatic system (Paniz et al., 2007).

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The silver catfish (*Rhamdia quelen*) is a teleost fish that belongs to the order Siluriformes, family Heptapteridae and subfamily Heptarinae (Borges et al., 2007). It is a promising species for fish cultivation in southern South America and other subtropical climate regions of the world, due to its zootecnic and organoleptic characteristics, which are propitious to its development, and due to tolerance to low temperatures that provide continuous growth in winter (Ferreira et al., 2010; Fukushima et al., 2012).

The present study was delineated to investigate the effects of different levels of dietary vitamin C on oxidative stress markers and hematological values of silver catfish in order to verify the antioxidant potential of vitamin C. The results obtained with vitamin addition in food could be used in future studies to improve the antioxidant capacity of *Rhamdia quelen* against toxicity caused by exogenous stressors like pesticides applied in nearby aquaculture.

MATERIALS AND METHODS

Fish

Ninety silver catfish juveniles of both sexes (length 10.61 ± 1 cm; weight 9.87 ± 1 g) were obtained from fish farming close to Santa Maria County (state of Rio Grande do Sul, Brazil). The fish were acclimated to the laboratory conditions for 15 days and fed with commercial feed. They were kept in plastic boxes with 170 liters capacity, continuously aerated with a biofilter system and a natural photoperiod (12h light/12h dark). Feces and pellet residues were removed by suction. The water parameters averages were: dissolved oxygen 7.22 ± 1.0 mg L^{-1}, temperature 23.0 ± 1.0 °C, pH 6.9 ± 0.2, ammonia 0.04 ± 0.01 µg L^{-1}, and nitrite 0.05 ± 0.01mg L^{-1}. After the acclimation period, the fish were divided into experimental groups.

Diet preparation and experimental design

The experimental diets were prepared at the aquaculture laboratory of the Federal University of Santa Maria (UFSM). Diets were elaborated containing different concentrations of vitamin C: group 1 – 0 mg kg^{-1}; group 2 – 500 mg kg^{-1}; and group 3 – 1,000 mg kg^{-1} (Table 1). All ingredients were mixed, and pellets of approximately 5 mm diameter were formed by grinding the mixture through a meat grinder. After processing, all the diets were packed into small bags and kept at 4 ºC. The fish were divided randomly into three groups, thirty silver catfish to each diet. During the experiment, the fish were fed with 3% of their body weight per day (Menezes et al., 2014a), and daily diet was divided into two equal meals at 9 a.m. and 4 p.m. The biofilter system was used to maintain water quality, and

Table 1. Control diet composition[(1)].

Ingredients	Amount (g)
Fish flour	60.890
Corn starch	17.000
Cellulose[(1)]	12.580
Vitamin premix	3.000
Dicalcium phosphate	2.000
Calcareous	1.500
Soy oil	1.170
Cod-liver oil	1.000
Salt	0.500
Di-methionine	0.360
Vitamin C coated	0.000
Total	1,000.000

[(1)]The feeds containing 500 to 1,000 mg kg^{-1} vitamin C were also prepared according to Table 1; however, cellulose was removed according to the addition of vitamin C (500 or 1,000 mg kg^{-1}).

the water parameters averages were maintained equal to those of the acclimation period. Feces and pellet residues were removed by suction.

Sample preparation

During the supplementation of vitamin C, ten fish per diet were randomly collected at days 0, 14 and 28. In each collection, the fish were anaesthetized with eugenol (50 μg L^{-1}), according to Cunha et al. (2010), and then weight and length were measured. After that, the blood from caudal vein was collected using syringes previously heparinized. Then, the fish were euthanized by medullary section. Quantitative determinations of blood cells were performed using a Pentra 80 ABX diagnostics (France). The hematological parameters evaluated were: red blood count (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). After the blood collection, samples of tissues (liver and gills) were quickly removed and stored at -80 °C for further evaluation of ascorbic acid levels, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) activities, non-protein thiols (NPSH) levels, glutathione S-transferase (GST) activity, lipid peroxidation (TBARS assay) and protein carbonyl levels. The animal experimentation was performed according to the National Institute of Health Guide for Animal. All experimental protocols were approved by the Ethics Commission on Animal Use of the University Federal of Santa Maria (protocol number 4302190116).

Oxidative stress assays

Liver and gills were homogenized with Tris-HCl buffer [50 mM] pH 7.4 and centrifuged at 3,000 rpm for 10 minutes. This homogenate was used for all of the following analyzes. Protein was determined by the Coomassie Blue Method using bovine serum albumin as standard. Absorbance of samples was measured at 595 nm, according to Bradford (1976). Ascorbic acid was determined in liver and gills by Roe method (Roe, 1954). 300 μL homogenate plus 100 μL distilled water, 100 μL TCA [13.3%] and 75 μL 2,4 dinitrophenylhydrazine (DNPH) were incubated at 37 °C in a water bath for 3 hours. Following the incubation, 500 μL sulfuric acid (H_2SO_4) 65% was added to stop the reaction. The absorbance was read at 520 nm. The ascorbic acid content was expressed as μmol g^{-1} of tissue. In the liver, the CAT (EC 1.11.1.6) activity was assayed by ultraviolet spectrophotometry according to Nelson & Kiesow (1972). The assay mixture consisted of 2.0 mL potassium phosphate buffer (TFK) [50 mM] pH 7.0, 10 μL homogenate, and 50 μL hydrogen peroxide (H_2O_2) [0.3 mM]. Changes of H_2O_2 were measured in 1 minute; the reading was performed in kinetic mode at 240 nm. CAT activity was expressed in μmol min^{-1} mg $protein^{-1}$. SOD (EC 1.15.1.1) activity was performed in liver by modification of Misra & Fridovich's (1972) assay. This method was based on the inhibition of the radical superoxide reaction with adrenaline. The SOD present in the sample competes with the detection system for radical superoxide. The oxidation of adrenaline leads to the formation of the colored product, adrenochrome, which is detected using a spectrophotometer. Distinct volumes of homogenate (5, 10, 15 and 20 μL) were pipetted into microplate, and 190, 185, 180 and 175 μL of glycine-NaOH [50 mM] pH 10.6 (maintained at 37°C in a water bath), respectively, and 5 μL of the adrenalin [60 mM]. The absorbance was read in kinetic mode at 480 nm for 10 minutes. The SOD activity was expressed in UI SOD mg $protein^{-1}$. A unit of SOD was defined as the amount of enzyme that inhibits the rate of oxidation of adrenaline by 50%. GPx (EC 1.11.1.9) activity was determined in liver and gills by modifying the Paglia & Valentine's (1967) method. The assay mixture consisted of 10 μL homogenate and 260 μL of the system containing: NADPH, GSH, GR (glutathione reductase), azide and potassium phosphate buffer (TFK) [100 Mm] pH 7.0. The absorbance was read in kinetic mode at 340 nm, for 2 minutes. The specific activity was determined using the extinction coefficient of 6.22 mM cm^{-1}, and the GPx activity was expressed in μmol min^{-1} mg $protein^{-1}$. NPSH levels were determined in liver and gills by modifying Ellman's method (Ellman, 1959). 100 μL of the homogenate was mixed with 100 μL 10% trichloroacetic acid (TCA), followed by centrifugation at 3,000 rpm, for 10 minutes. 30 μL of the supernatant was pipetted into microplate, 70 μL distilled water, 100 μL potassium phosphate buffer (TFK) [50 mM] pH 7.0, and 10 μL 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) [10 mM]. The microplate was incubated in the dark for 20 minutes. The absorbance was recorded in endpoint mode at 412 nm. NPSH levels were expressed as μmol SH g^{-1} of tissue. GST (EC

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2.5.1.18) activity was determined in liver and gills by modification of procedure described by Habig et al. (1974). The assay mixture consisted of 10 µL homogenate, 150 µL potassium phosphate buffer (TFK) [20 mM] pH 6.5 (maintained at 37 °C, in a water bath), 50 µL reduced glutathione (GSH) and 50 µL 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. The absorbance was read in kinetic mode at 340 nm, for 2 minutes. The specific activity of GST was determined using the extinction coefficient of 9.6 mM cm^{-1} and expressed in GS-DNB min^{-1} mg $protein^{-1}$.

Oxidative damage assays

Lipid peroxidation was measured in liver and gills by thiobarbituric acid reactive species (TBARS) production, according to Buege and Aust method (Buege & Aust, 1978), with some modifications. 100 µL of the homogenate was mixed with 100 µL 10% TCA, followed by centrifugation at 3,000 rpm, for 10 minutes. 100 µL of the supernatant was mixed with 100 µL 2-thiobarbituric acid (TBA) [0.67%]. The mixture was incubated at 100 °C in a water bath for 30 minutes. 150 µL of the supernatant was pipetted into microplate. The absorbance was read in endpoint mode at 532 nm. The lipid peroxidation was expressed as nmol MDA mg $protein^{-1}$. Protein carbonyl content was assayed in liver and gills by modification of the method described by Yan et al. (1995). 200 µL of the homogenate plus 800 µL distilled water were reacted with 150 µL 2,4 dinitrophenylhydrazine (DNPH) [10 nm] in 2N hydrochloric acid. After incubation at room temperature in the dark for 60 minutes, 125 µL sodium dodecyl sulfate (SDS) 3%, 500 µL ethanol and 500 µL of heptane were subsequentially added. This mixture was vortexed for 30 seconds and centrifuged at 3,000 rpm for 15 minutes. Then, the protein isolated from the interface was washed by resuspension in ethanol/ethyl acetate (1:1). After that, 250 µL sodium dodecyl sulfate (SDS) 3% and vortexed again were added. The mixture was pipetted into a microplate. Assay was performed in duplicate and one blank tube incubated with 2 N HCl without DNPH was included for each sample. The absorbance was recorded in endpoint mode at 370 nm. The total carbonylation was calculated using a molar extinction coefficient of 22.000 M cm^{-1}. The protein carbonyl content was expressed as nmol carbonyl mg $protein^{-1}$.

Statistical analysis

All data were analyzed by two-way ANOVA to test the effects of the dietary treatments at the different times, followed by Tukey's multiple comparisons test. Homogeneity of variances between groups was tested with Levene test. The values are presented as mean ± S.E.M. Differences were considered to be significant at a probability level of $p < 0.05$ between groups.

RESULTS

No statistical difference was observed between the groups in relation to body weight and length (data not shown). In relation to blood parameters, the data show increase in the Hb, in all groups, after 14 days of feeding, which did not change until the day 28 (Table 2). There were no significant changes in RBC, MCV, HCT and MCH in relation to the different vitamin C diets (data not shown).

Table 2. Blood parameters: effect of different amounts of vitamin C in relation to blood parameters and the feeding time.

Diets/Days (mg kg^{-1})	Hb (g dL^{-1})		
	0	14	28
0	5.38 ± 0.31^{a}	7.15 ± 0.67^{b}	6.42 ± 1.39^{b}
500	5.40 ± 0.30^{a}	6.97 ± 0.39^{b}	7.00 ± 1.00^{b}
1,000	5.42 ± 0.31^{a}	6.90 ± 0.69^{b}	7.15 ± 0.90^{b}
1,000	22.62 ± 2.00^{a}	24.00 ± 2.13^{a}	26.53 ± 5.96^{a}

Hemoglobin (Hb) and hematocrit (HCT). Different letters indicate differences between groups ($p < 0.05$). Data are reported as mean ± S.E.M. (n = 10).

The level of ascorbic acid in the gills increased after 28 days of feeding in groups 2 and 3 when compared to day 0. However, in the liver, there were no significant changes in the level of ascorbic in all groups (Table 3).

Table 3. Analyzed dietary concentration of vitamin C (µmol g^{-1} tissue) during different days of feeding.

Diets/Days (mg kg^{-1})	0	14	28
		Gill	
0	7.78 ± 1.74[a]	8.65 ± 2.41[a]	8.06 ± 1.92[a]
500	8.01 ± 1.58[a]	7.87 ± 2.53[a]	12.39 ± 2.06[b]
1,000	9.11 ± 1.88[a]	8.53 ± 1.83[a]	12.61 ± 1.67[b]
		Liver	
0	10.51 ± 3.27[a]	11.35 ± 1.94[a]	8.85 ± 1.87[a]
500	11.41 ± 2.79[a]	11.46 ± 2.13[a]	11.52 ± 2.52[a]
1,000	11.57 ± 2.62[a]	13.60 ± 3.20[a]	11.42 ± 1.62[a]

Different letters indicate differences between groups ($p < 0.05$). Data are reported as mean ± S.E.M. ($n = 10$).

Considering the oxidative parameters, a significant decrease in the lipid peroxidation of liver was observed in groups 2 and 3 after 28 days of feeding (Figure 1 A). The levels of hepatic lipid peroxidation did not change for group 1. In gills, TBARS were significantly reduced in groups 2 and 3 after 14 days, and such reduction was not evident for group 1 (Figure 1 B). The activity of GPx in liver increased in fish fed for 28 days with the diets 2 and 3 when compared to day 0 and compared to group 1 (Figure 1C). GPx activity in gills (Figure 1D) decreased after 28 days of feeding in all groups.

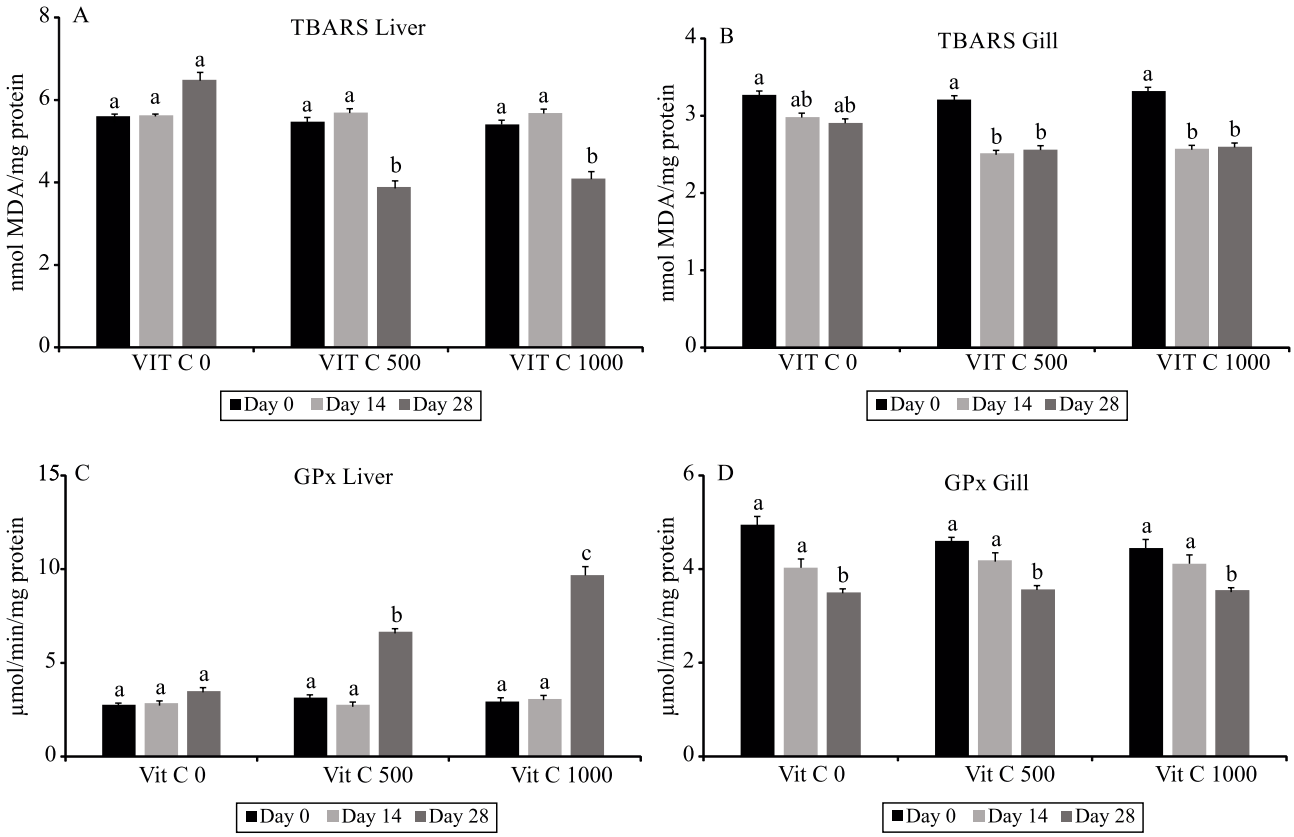

Figure 1. Lipid peroxidation level (TBARS) in liver (A) and gill (B), and GPx activity in liver (C) and gill (D) in silver catfish fed with different vitamin C levels (0, 500 and 1,000 mg kg^{-1}). Different letters indicate differences between groups ($p < 0.05$). Data are reported as mean ± S.E.M. ($n = 8$).

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In liver, the carbonyl content (Figure 2 A) increased in group 1 after 14 days and returned to day 0 level at 28 days. Levels of hepatic carbonyl did not vary in groups 2 and 3. The carbonyl protein content increased significantly in gills (Figure 2 B) after 28 days in group 1 when compared to day 0 and compared to the other groups. In CAT, activity was observed just in group 1 after 28 days of feeding compared to day 0 and compared to the other groups (2 and 3) (Figure 2 C).

In relation to hepatic GST (Figure 2 D), in group 1 an increase occurred after 14 days, which did not change until the day 28; in groups 2 and 3, an increase occurred after 14 days, and after 28, the activity started to return to day 0 level. The GST activity in gills (Figure 2 E) decreased after 14 days of feeding in all groups. There were no significant changes in SOD activity in relation to the different vitamin C diets (data not shown). There were no statistical differences in NPSH levels between the groups both in gill and in liver (data not shown).

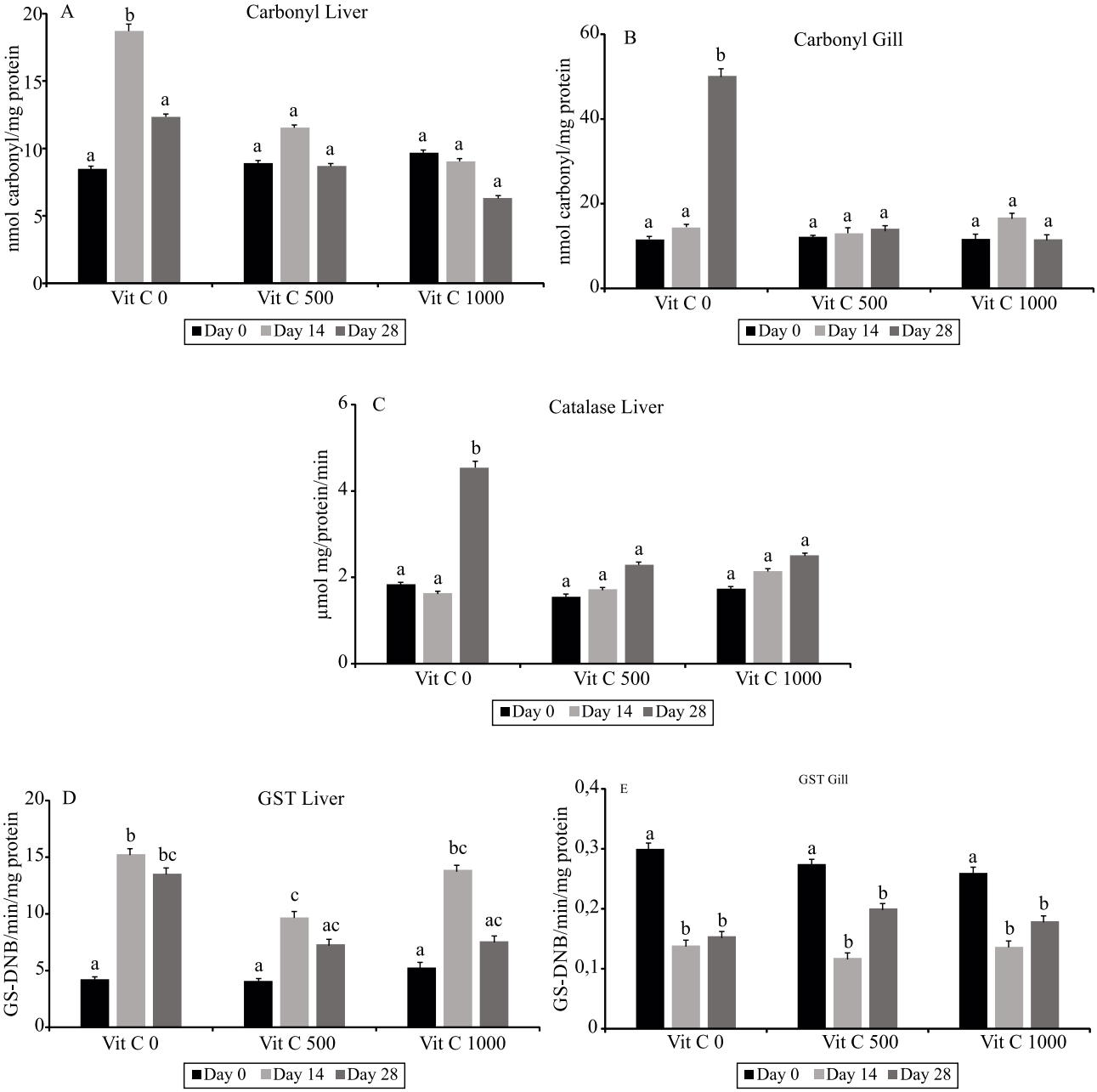

Figure 2. Carbonyl protein content in liver (A) and gill (B), catalase activity in liver (C), and GST activity in liver (D) and gill (E) of silver catfish fed with different vitamin C levels (0, 500 and 1,000 mg kg^{-1}). Different letters indicate differences between groups ($p < 0.05$). Data are reported as mean ± S.E.M. (n = 8).

DISCUSSION

The beneficial effects of diphenyl diselenide on growth, oxidative damage and antioxidant response in silver catfish (Menezes et al., 2016), and its protection towards pesticides are already known (Menezes et al., 2014b). Therefore, the study intends to investigate the antioxidant effects of vitamin C in order to conduct future studies using its protective action against toxicity caused by pesticides. Vitamin C or 2-oxo-L-threo-hexagon-1,4-lactone-2,3-enediol is an important dietary antioxidant, capable of neutralizing reactive oxygen species, peroxyl, superoxide anion, hydroperoxyl radicals and reactive nitrogen radicals.

Blood reflects the organism's whole body pathophysiologic condition and changes in its components; blood cell formation and function are good indicators of nutrition, stress and the overall health of fish (Adhikari et al., 2004; Buentello et al., 2007). In relation to the hematological parameters, the study results showed an increase in number of Hb observed in all groups. Therefore, it is difficult to associate the increase of these parameters to vitamin C since this increase occurred in all groups, even the group not supplemented. There were no significant changes in RBC, MCV, HCT and MCH in relation to the different vitamin C diets. Therefore, it can be stated that these different concentrations of vitamin C or feeding time were not enough to interfere with the values of RBC, MCV, HCT and MCH.

According to literature data, vitamin C acts in association with vitamin E, reducing lipid peroxidation and oxidative damage. The results of lipid peroxidation of the present study show a reduction of their levels in groups fed with 500 to 1,000 mg kg^{-1} of vitamin C, in gill and liver, when compared to day zero and the group without vitamin C. Considering the absence of such reduction in groups without vitamin C, it can be assumed that this reduction in lipid peroxidation levels is associated with vitamin C present in the feed. On the other hand, it is known that one of the functions of GPx is the reduction of lipid peroxidation products (Menezes et al., 2016). From this point of view, it can be deduced that the increase of GPx activity in liver in groups 2 and 3 (day 28) may be associated with decreased lipid peroxidation in these same groups. However, in the gills, unlike the liver, there were no significant changes in GPx activity in relation to the different vitamin C diets. This fact pointed out the fish tissue specific response to vitamin C in diet. Liver is the central organ of metabolism and detoxification processes, thus, in relation to vitamin C, the liver could be important to metabolize vitamin C and reduce peroxidation levels, but gills play another role in fish physiology as osmoregulation control (Menezes et al., 2014a).

Protein carbonyl levels are the most commonly used marker for the presence of protein oxidation. The use of protein carbonyl groups is advantageous because of their rapid formation; besides that, they present some stability after formation (Dalle-Donne et al., 2003; Dayanand et al., 2012). The results show that there was an increase in protein carbonyl levels in liver in group 1. This carbonylation seems to be associated with the same stress, which has also caused the increase of CAT. Although the activity of CAT has not varied in groups supplemented with vitamin C, CAT is one of the most important antioxidant enzymes involved in the elimination of H_2O_2. This enzyme catalyzes the conversion of H_2O_2 into molecular oxygen and water. The ascorbate (AsA), an ionized form of vitamin C, is one of the most important non-enzymatic antioxidants, which, along with GSH, participates in the cycle of ascorbate-glutathione to eliminate H_2O_2 by peroxidation of AsA. Therefore, CAT and ascorbate-glutathione cycle are important in the removal of H_2O_2 and, although their properties and requirements are different, they can effectively work in parallel (Barbosa et al., 2014). Therefore, since catalase and AsA work in parallel in the elimination of H_2O_2, it can be deduced that the increased activity of CAT (days 21 and 28) observed in group 1 may be due to the absence of AsA in fish reserves, and consequently greater demand of CAT for elimination of H_2O_2 present in the fish body.

GST is a well known detoxification enzyme that makes the compounds more hydrophilic when conjugating them with GSH (Lushchak, 2011). The observed variations in GST activity both in liver

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and gills may be related to its other functions in the organism as transport of hormones, and even the elimination of hydroperoxides and aldehydes produced during lipid peroxidation. Although GSH is the most representative NPSH and is part of the cycle of vitamin C and GST, there was no difference in their levels between the groups fed with different concentrations of this vitamin, in gill and liver. Also, there were no significant changes in SOD activity in relation to the different vitamin C diets. SOD is a metalloenzyme belonging to the first line of antioxidant defense, whose function is to dismutate superoxide anion into H_2O_2. Thus, it cannot be stated that its decrease or its increase is related to different concentrations of vitamin C in the diet.

Through the above results, it can be stated that fish fed with diet containing 500 or 1,000 mg kg^{-1} vitamin C had higher antioxidant capacity compared to the group fed with a diet containing 0 mg kg^{-1} of vitamin C. These results were more pronounced in the liver than in the gills, which can be explained by the fact that liver is the target organ of metabolism and detoxification. Maybe some results could be better understood in a longer supplementation experiment, like the improvement of antioxidant enzymes (hepatic GPx) and the reduction of oxidative damage (hepatic protein carbonylation and lipid peroxidation).

In conclusion, the study results indicate that diets containing 500 or 1,000 mg kg^{-1} of vitamin C contributed to improve the antioxidant capacity of *Rhamdia quelen*, suggesting that vitamin C supplementation in the amounts of 500 or 1,000 mg kg^{-1} may be useful in aquaculture, preventing possible damage caused by oxidative stress.

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