# Influence of levels and forms of selenium associated with levels of vitamins C and E on the performance, yield and composition of tilapia fillet

Influência de níveis e formas de selênio associados com níveis das vitaminas C e E sobre o desempenho, rendimento e composição de filé em tilápia

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#### **Abstract**

The objective of this study was to evaluate the influence of vitamin C (VC), vitamin E (VE), and two selenium sources on the performance, yield, and composition of Nile tilapia fillet. The experiment was conducted in a completely randomized design consisting of six treatments with the addition of 100, 200, and 400 mg kg $^{-1}$  VC and VE and 0.1, 0.2, and 0.4 of two sources of selenium. Each treatment had five replicates of 30 fish each. The diet with 200 mg kg $^{-1}$  VC and VE + 0.2 mg.k $^{-1}$  organic selenium resulted in weight gain, length gain, and feed conversion ratio similar to that of the treatment with 400 mg kg $^{-1}$  VC and VE + 0.4 mg kg $^{-1}$  organic or inorganic selenium. The addition of organic selenium to the diet improved the weight gain and feed conversion ratio in comparison with the addition of inorganic selenium. The diet with 0.2 mg kg $^{-1}$  organic selenium showed glutathione peroxidase level equal to the diet with 0.4 mg kg $^{-1}$  of inorganic selenium. Carcass and fillet yields were not affected by treatments; however, there was treatment effect on the fillet's chemical composition. *Keywords: antioxidant; organic; yield.* 

#### Resumo

Objetivou-se avaliar a influência da vitamina C (VC), vitamina E (VE) e de duas formas de selênio sobre o desempenho, rendimento e qualidade do filé de tilápia Nilótica. O experimento foi realizado num delineamento inteiramente casualizado composto por seis tratamentos, os quais foram constituídos pela adição de 100, 200 e 400 mg kg $^{-1}$  das vitaminas C e E, associados com 0,1, 0,2 e 0,4 mg kg $^{-1}$  de duas formas de selênio. Cada tratamento foi constituído por cinco repetições de 30 peixes cada. A dieta com 200 mg kg $^{-1}$  de VC e VE + 0,2 mg kg $^{-1}$  de selênio orgânico proporcionou ganho de peso, comprimento corporal e fator de conversão alimentar semelhantes aos tratamentos com 400 mg kg $^{-1}$  de VC e VE + 0,4 mg kg $^{-1}$  de selênio orgânico ou inorgânico. A adição de selênio orgânico à dieta melhorou o ganho de peso e o fator de conversão alimentar em comparação com o selênio inorgânico. A dieta com 0,2 mg kg $^{-1}$  de selênio orgânico apresentou nível de glutationa peroxidase igual ao da dieta com 0,4 mg kg $^{-1}$  de selênio inorgânico. Os rendimentos de carcaça e filé não foram influenciados pelos tratamentos, entretanto houve efeito dos tratamentos na composição química do filé.

Palavras-chave: antioxidante; orgânico; rendimento.

## 1 Introduction

With the intensification of tilapia farming in several countries, including Brazil, mainly in cages where the availability of natural food is limited, there was increased incidence of nutritional disorders due to the inadequate vitamin and mineral enrichment of feed. These more intensive systems require the use of nutritionally complete diets with vitamin and mineral enrichment greater than those required by production systems in nurseries (KUBITZA, 2000).

Vitamins and minerals are substances present in small quantities in natural foods, which are essential for metabolism and their deficiency in the diet can cause diseases. Although vitamins and minerals act in various reactions in living organisms, little is known about some of their specific functions and interactions in tilapia nutrition.

Vitamin C is considered essential for some aquatic organisms due to the absence of gulonolactone oxidase enzyme, which is responsible for converting glucose into ascorbic acid

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(ALBREKTSEN; LIE; SANDNES, 1988). Vitamin C acts as a reducing agent in enzyme hydroxylation reactions, thereby acting as antioxidant (BAI; GATLIN, 1992) by improving cell respiration, favoring integrity and membranes fluidity (VERLHAC; GABAUDAN, 1994), and regenerating oxidized vitamin E by free radicals (MUKAI; NISHIMURA; KIKUCHI, 1991). Vitamin E is the primary fat-soluble vitamin responsible for protecting cell membrane of lipid peroxidation (THAKUR; SRIVASTAVA, 1996), and it is mainly found in the mitochondria and microsomal membranes. Being a cell antioxidant, vitamin E is involved in the stabilization of polyunsaturated fatty acids of phospholipid fraction of cell membranes preventing toxic lipoperoxide formation.

Vitamin E has a strong interaction with selenium to protect cell membranes against lipid peroxidation (WATANABE; KIRON; SATOH, 1997). Selenium is a trace element responsible for glutathione peroxidase (GSH-Px) enzyme activation, which acts to reduce hydroperoxides into alcohols and hydrogen peroxide into water.

Diet formulation enriched with vitamin C and E and selenium increases weight gain in tilapia (*O. niloticus*) (KIM et al., 2003) and increases selenium concentration in the muscle tissue of some species (BUCKLEY, 2000). However, there are few studies on the effects of nutrients on the performance, carcass yield, and chemical composition of tilapia fillets. Thus, this study aimed at evaluating the increased levels of organic or inorganic selenium and vitamins C and E in the diet on the performance, carcass yield, fillet's chemical composition, and glutathione peroxidase in the tilapia blood.

## 2 Materials and methods

The experiment was conducted in a completely randomized design with six treatments and five repetitions with 30 fish each. A total of 900 reversed Nile tilapia with average initial weight of 60.0 + 5.3 g and 14.49 + 0.8 cm average initial length were housed in 30 cages (1  $\times$  1  $\times$  1 m) placed in a pond of 3.0 m average depth and 1800 m² area. Fish feeding consisted of an experimental diet containing 33% crude protein and 3195 kcal ED kg¹¹ diet (Table 1), formulated according to the nutritional recommendations of NRC (NATIONAL..., 1993) and supplemented with organic and inorganic sources of selenium and vitamins C and E (Table 2). The sources of vitamins C and E used were pure ascorbic acid and Rovimix E\* (50% dl-α-tocopherol acetate), respectively. Selplex\* (1 mg kg¹¹ Se) was used as selenium source and sodium selenite (45.6% Se) as inorganic source.

The mineral and vitamin premix used did not contain selenium and vitamins C and E, which were added during the experimental diet preparation. After mixing, the diets were pelletized (Xavante<sup>TM</sup> – Brazil) and daily distributed in 60 cm diameter trays of based on the total biomass of each cage in three daily meals at 8, 13, and 16 hours.

To evaluate fish performance and adjust the diet amount to be offered, the samples were collected fortnightly by capturing randomly five fish from each cage to estimate the total length (cm) and weight (g). The physicochemical variables water pH,

Table 1. Formulation and proximate composition of experimental dieta.

Ingredient	g kg <sup>-1</sup>	Nutrient	g kg <sup>-1</sup>
Soybean meal	330	Crude proteín	330.4
Corn	272	Digestible energy (Kcal)	3195.7
Wheat bran	175	Fat	47.1
Fishmeal	200	Crude fiber	49.1
Soybean oil	13	Calcium	14.5
Vitamin and mineral premix <sup>b</sup>	10	Available phosphorus	8.4

 $^{\rm a}$ Basic diet Composition: Selenium, 0.22 mg kg $^{\rm -1}$ ; Vitamin E, 6.88 mg kg $^{\rm -1}$ ;  $^{\rm h}$ Guaranteed levels per kg of product. Vit. A, 6000 UI; Vit. D, 6000 UI; Vit. K, 6.30 mg; Vit. B1, 11.76 mg; Vit. B2, 15.36 mg; Vit. B6, 12.74 mg; Vit. B12, 40 mcg; Folic acid, 1.92 mg; Pantothenic acid, 39.20 mg; Choline, 800 mg; Niacin, 400 mg; Biotin, 0.2 mg; Antioxidant, 300 mg; Iron, 257.15 mg; Zinc, 300 mg; Manganese, 133.45 mg; Copper, 19.60 mg; Iodine, 9.40 mg.

**Table 2.** Treatments used to evaluate the effect of organic and inorganic selenium with vitamins C and E on tilapia feeding.

Treatments -		Nutrients (mg kg <sup>-1</sup>	1)
Treatments —	Vitamin C	Vitamin E	Selenium
T1	100	100	0.1 Organic
T2	200	200	0.2 Organic
Т3	400	400	0.4 Organic
T4	100	100	0.1 Inorganic
T5	200	200	0.2 Inorganic
T6	400	400	0.4 Inorganic

dissolved oxygen, and temperature were measured twice a week in the morning and afternoon.

At the end of the experiment, after fasting for 24 hours, the fish were killed by stunning (boxes with ice and water at 1:1 ratio) and the following weights were measured: live, gutted with head and fins, viscera, liver, and fillet with skin. Filleting process was carried out by a single person. The fillet yield was calculated dividing the fillet weight by the gutted and scaled fish weight. Carcass yield was achieved by dividing gutted and scaled fish weight by its body weight. Visceral-somatic index was obtained dividing the fish visceral weight by the fish weight, and the hepatic-somatic index was calculated by dividing the liver weight by the animal weight. The following formula was used to calculate the specific growth ratio (SGR): SGR = [(In final weight – In initial weight)/time]  $\times$  100, where In is the natural logarithm. Feed Conversion Ratio (FCR) was determined by the following model: FCR = (food consumed/weight gain)  $\times$  100.

In order to determine the glutathione peroxidase enzyme and the hematocrits, three animals from each treatment were captured, which were stored in a tank with constant water renewal for further sampling. Two blood samples were collected (5 ml each) from each treatment by puncturing the tail vein using heparinized syringes. The blood samples collected were stored in vacuum tubes and coated with EDTA anticoagulant (ethylenediaminetetraacetic acid) and 4 mL working volume. The collected blood samples were stored at room temperature between 2-10 °C, according to the laboratory guidelines. No sedation or anesthesia was used during sample collection since these drugs can cause changes in the activity of antioxidant

enzymes, such as glutathione peroxidase (WDZIECZAK et al., 1982).

The analysis of glutathione peroxidase was performed using spectrophotometry according to Paglia and Valentine (1967). Vitamin C determination (method 967.21) was performed according to AOAC (ASSOCIATION..., 1990), while selenium (method 985.35), protein (method 988.10), and ether extract (method 920.39, C) were analyzed according to AOAC (ASSOCIATION..., 1995). The mineral material (method 900.02) and humidity (method 926.12) were determined according to AOAC (ASSOCIATION..., 1996), and vitamin E was determined by HPLC according to Matthey, Graf and Flachowsky (1991). The hematocrit was analyzed in a hematology analyzer (ABX Micros 60, Horiba®). Selenium concentration in the muscle followed the method described by Tinggi (1999) and was analyzed using an atomic absorption spectrophotometer. Prior to the chemical composition analysis, the samples were lyophilized for 24 hours.

The means of weight gain, length gain, feed conversion ratio, specific growth ratio, glutathione peroxidase, selenium, hematocrit, carcass yield, fillet yield, and visceral and hepatic-somatic indices were compared using the "F" test at 5% probability, followed by the Tukey test at 5%. The orthogonal contrasts test was used to evaluate the effect of organic selenium versus inorganic selenium on weight gain, length gain, feed conversion ratio, and levels of glutathione peroxidase (NOGUEIRA, 2004).

## 3 Results

The the mean values of water quality measured in the morning and afternoon were: 23.7 and 25.2 °C for temperature values of 1.9 and 4.4 mg.L<sup>-1</sup>for dissolved oxygen 6.40 and pH 7.02, the temperature and oxygen were below the recommended levels for tropical fish farming. (BOYD; TUCKER, 1998).

After 74 days of breeding, tilapia survival was 100% in all treatments (Table 3). Weight gain of tilapia fed on 400 mg kg $^{-1}$  VC and VE and 0.4 mg kg $^{-1}$  Se-O was higher (P < 0.05) than that in the treatment with 100 mg kg $^{-1}$  VC and VE with 0.1 mg kg $^{-1}$  Se-O and Se-I and the treatment with 200 mg kg $^{-1}$  VC and VE with 0.2 mg kg $^{-1}$  Se-I, but it did not differ (P > 0.05) from the other treatments. The gain in length of tilapia fed on 400 mg kg $^{-1}$  VC and VE with 0.4 Se-O was greater (P < 0.05) than that of tilapia fed on 100 mg kg $^{-1}$  VC and VE with 0,1 mg kg $^{-1}$ 

inorganic selenium, but it did not differ (P > 0.05) from the other treatments. The feed conversion ratio observed in the treatment with 400 mg kg $^{-1}$  VC and VE with 0.4 mg kg $^{-1}$  Se-O was greater (P < 0.05) than that in treatments with 100 and 200 mg kg $^{-1}$  vitamins C and E with 0.1 and 0.2 mg kg $^{-1}$  Se-I, respectively (Table 3).

Tilapia fed diets with the organic form of selenium had weight gain and feed conversion ratio higher ( $P \le 0.05$ ) than those containing inorganic selenium (Figure 1); however, there was no significant effect (P > 0.05) of the selenium type on the length gain in tilapia.

Carcass yield was not affected by the treatments (P > 0.05) although the treatment with 400 mg kg<sup>-1</sup> VC and VE with 0.4 mg kg<sup>-1</sup> Se-O resulted in 1.32% more carcass yield compared to the treatment with 200 mg kg<sup>-1</sup> VC and VE with 0.2 mg kg<sup>-1</sup> Se-I (88.01% vs 86.67%). Regarding the fillet yield, there was no significant difference (P > 0.05) among the treatments (Table 4). Visceral-somatic and hepatic-somatic indices also showed no significant difference (P > 0.05) among the treatments studied (Table 4).

There was no significant effect (P > 0.05) among the treatments in terms of fillet composition: protein, fat, selenium, ash, moisture, and hematocrits in blood (Table 5).

The levels of glutathione peroxidase in tilapia blood increased with the diet enriched with selenium ( $P \le 0.05$ ) regardless of its source (Figure 2a); however, the effect was more evident ( $P \le 0.05$ ) in the diets supplemented with organic selenium (Figure 2b).

### 4 Discussion

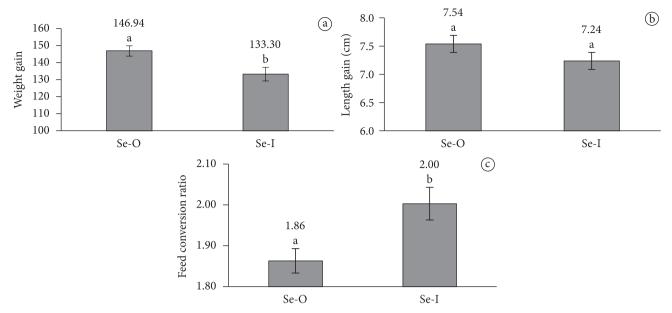
Regardless of the doses of vitamins C and E used, organic selenium promoted greater weight gain (P < 0.05), feed conversion ratio, and concentration of glutathione peroxidase in tilapia blood compared with those obtained with inorganic selenium.

The 100% fish survival in the experiment indicates that diet supplementation for juvenile tilapia bred in cages with at least 100 mg kg<sup>-1</sup> of vitamins C and E and 0.1 mg kg<sup>-1</sup> selenium contributed to the lower fish mortality. This result corroborates the findings of Kim et al. (2003), who found 100% survival of tilapia fingerlings fed on 150 mg kg<sup>-1</sup> vitamin C, 100 mg kg<sup>-1</sup> vitamin E with 0.2 mg kg<sup>-1</sup> inorganic selenium. The performance

Table 3. Performance of Nile tilapia fed diets enriched with organic and inorganic sources of selenium and vitamins C and E.

Treatments	Weight gain (g)	Specific growth ratio (%)	Length gain (cm)	Feed conversion ratio (kg/kg)	Survival (%)
T1	$138.75 \pm 9.2^{bc1}$	$1.57 \pm 0.06^{bc}$	$7.27 \pm 0.5^{ab}$	$1.96 \pm 0.2^{ab}$	$100\pm0.0^{\rm a}$
T2	$146.45 \pm 10.3^{ab}$	$1.63 \pm 0.07^{ab}$	$7.53 \pm 0.4^{ab}$	$1.88 \pm 0.3^{ab}$	$100\pm0.0^{\rm a}$
T3	$161.07 \pm 7.7^{a}$	$1.70 \pm 0.05^{a}$	$7.82 \pm 0.1^{a}$	$1.75 \pm 0.1^{a}$	$100\pm0.0^{\rm a}$
T4	$123.86 \pm 4.6^{\circ}$	$1.48 \pm 0.03^{\circ}$	$6.85 \pm 0.8^{b}$	$2.10 \pm 0.1^{b}$	$100\pm0.0^{\rm a}$
T5	$125.79 \pm 6.5^{\circ}$	$1.48 \pm 0.05^{\circ}$	$7.08 \pm 0.4^{ab}$	$2.02 \pm 0.3^{b}$	$100 \pm 0.0^a$
T6	$147.90 \pm 8.0^{ab}$	$1.65 \pm 0.05^{ab}$	$7.79 \pm 0.6^{a}$	$1.89 \pm 0.3^{ab}$	$100\pm0.0^{\rm a}$

Treatments:  $T1 = 100 \text{ mg kg}^{-1}$  of VC and VE with 0.1  $\text{mg kg}^{-1}$  of Se-O;  $T2 = 200 \text{ mg kg}^{-1}$  of VC and VE with 0.2  $\text{mg kg}^{-1}$  of Se-O;  $T3 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.4  $\text{mg kg}^{-1}$  of Se-O;  $T4 = 100 \text{ mg kg}^{-1}$  of VC and VE with 0.1  $\text{mg kg}^{-1}$  of Se-I;  $T5 = 200 \text{ mg kg}^{-1}$  of VC and VE with 0.2  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.4  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.4  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.4  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with 0.5  $\text{mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg$ 



**Figure 1.** Contrasts of weight (a) and length (b) gains and feed conversion ratio (c) of tilapia fed on organic (Se-O) and inorganic (Se-I) selenium. a, b Means followed by distinct letters differ among themselves (P < 0.05).

Table 4. Carcass and fillet yield, visceral-somatic, and hepatic-somatic indices of tilapia fed on increasing levels of selenium and vitamins C and E.

Treatments	Carcass yield (%)	Fillet yield (%)	Visceral-somatic index (%)	Hepatic-somatic index (%)
T1	$87.82 \pm 1.5^{a1}$	$37.03 \pm 2.5^{a}$	$14.19 \pm 1.5^{a}$	$1.67 \pm 0.5^{a}$
T2	$87.40 \pm 0.5^{a}$	$37.98 \pm 1.1^{a}$	$14.18 \pm 1.0^{a}$	$1.69 \pm 0.2^{a}$
Т3	$88.01 \pm 0.2^{a}$	$38.39 \pm 1.8^{a}$	$14.14 \pm 1.1^{a}$	$1.68 \pm 0.1^{a}$
T4	$87.21 \pm 0.9^{a}$	$37.26 \pm 0.9^{a}$	$14.39 \pm 1.0^{a}$	$1.33 \pm 0.2^{a}$
T5	$86.67 \pm 1.2^{a}$	$36.46 \pm 0.6^{a}$	$14.33 \pm 1.5^{a}$	$1.60 \pm 0.5^{a}$
T6	$87.95 \pm 1.3^{a}$	$37.07 \pm 0.7^{a}$	$14.28 \pm 1.6^{a}$	$1.79 \pm 0.01^{a}$

Treatments:  $T1 = 100 \text{ mg kg}^{-1}$  of VC and VE with  $0.1 \text{ mg kg}^{-1}$  of Se-O;  $T2 = 200 \text{ mg kg}^{-1}$  of VC and VE with  $0.2 \text{ mg kg}^{-1}$  of Se-O;  $T3 = 400 \text{ mg kg}^{-1}$  of VC and VE with  $0.4 \text{ mg kg}^{-1}$  of Se-I;  $T5 = 200 \text{ mg kg}^{-1}$  of VC and VE with  $0.2 \text{ mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with  $0.4 \text{ mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with  $0.4 \text{ mg kg}^{-1}$  of Se-I;  $T6 = 400 \text{ mg kg}^{-1}$  of VC and VE with T6 =

Table 5. Chemical composition and hematocrit results of tilapia fed on different sources of selenium and vitamins C and E.

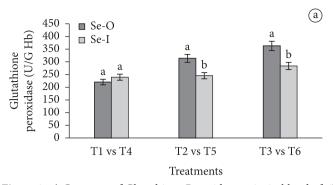
Toologic	Composition (%)			Selenium	Vitamins (mg kg <sup>-1</sup> )		Hematocrit	
Treatments -	Crude protein	Crude fat	Mineral matter	Humidity	$(mg kg^{-1})$	С	Е	(%)
T1	$18.11 \pm 1.3^{1a}$	$0.97 \pm 0.1^{a}$	$1.29 \pm 0.1^{a}$	$79.64 \pm 7.2^{a}$	$0.02 \pm 0.001^{a}$	$1240.46 \pm 30.2^{a}$	$0.36 \pm 0.03^{\circ}$	$43.95 \pm 1.8^{a}$
T2	$17.42 \pm 1.9^{a}$	$1.44\pm0.1^{a}$	$1.12\pm0.1^a$	$80.02 \pm 5.3^{a}$	$0.04 \pm 0.001^{a}$	$1263.37 \pm 40.3^{a}$	$0.87 \pm 0.02^{b}$	$38.65 \pm 1.5^{a}$
Т3	$17.47 \pm 1.5^{a}$	$1.22 \pm 0.2^{a}$	$1.26\pm0.1^a$	$80.05 \pm 4.8^{a}$	$0.05 \pm 0.003^{a}$	$1376.65 \pm 34.7^{a}$	$1.06 \pm 0.05^{a}$	$43.45 \pm 2.2^{a}$
T4	$17.98 \pm 1.8^{a}$	$1.29 \pm 0.2^{a}$	$1.26\pm0.1^a$	$79.48 \pm 5.5^{a}$	$0.04 \pm 0.002^{a}$	$1230.61 \pm 44.5^{a}$	$0.39 \pm 0.04^{\circ}$	$41.15 \pm 1.9^{a}$
T5	$17.51 \pm 1.4^{a}$	$1.06 \pm 0.1^{a}$	$1.23\pm0.1^a$	$80.19 \pm 7.1^{a}$	$0.05 \pm 0.001^{a}$	$1257.34 \pm 41.2^a$	$0.38 \pm 0.05^{\circ}$	$46.15 \pm 2.7^{a}$
Т6	$17.48 \pm 1.7^{a}$	$1.01 \pm 0.2^{a}$	$1.24\pm0.1^a$	$80.27 \pm 4.2^{a}$	$0.04 \pm 0.003^{a}$	$1362.48 \pm 53.7^{a}$	$0.54 \pm 0.09^{bc}$	$41.85 \pm 2.3^{a}$

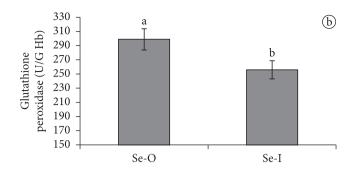
Treatments:  $T1=100 \text{ mg kg}^{-1} \text{ of VC}$  and VE with  $0.1 \text{ mg kg}^{-1} \text{ of Se-O}$ ;  $T2=200 \text{ mg kg}^{-1} \text{ of VC}$  and VE with  $0.2 \text{ mg kg}^{-1} \text{ of Se-O}$ ;  $T3=400 \text{ mg kg}^{-1} \text{ of VC}$  and VE with  $0.4 \text{ mg kg}^{-1} \text{ of Se-O}$ ;  $T4=100 \text{ mg kg}^{-1} \text{ of VC}$  and VE with  $0.1 \text{ mg kg}^{-1} \text{ of Se-I}$ ;  $T5=200 \text{ mg kg}^{-1} \text{ of VC}$  and VE with  $0.2 \text{ mg kg}^{-1} \text{ of Se-I}$ ;  $T6=400 \text{ mg kg}^{-1} \text{ of VC}$  and VE with  $0.4 \text{ mg kg}^{-1} \text{ of Se-I}$ ;  $VE=100 \text{ mg kg}^{-1} \text{ of VC}$  and  $VE=100 \text{ mg kg}^{-1$ 

of animals fed the diet with 0.2 mg kg<sup>-1</sup> Se-O, 200 mg kg<sup>-1</sup> VC and VE (Table 2) was similar to that of tilapia fed the diet containing 0.4 Se-I plus 400 mg kg<sup>-1</sup> vitamins C and E. Se-O has greater availability than the inorganic form confirming the result obtained by Paripatananont and Lovell (1997), who feeding the channel catfish (*I. punctatus*) on 0.5 mg kg<sup>-1</sup> organic and inorganic selenium observed that the selenium absorption

in the organic form was 90.8% against 62.8% for inorganic selenium (sodium selenite).

Piedras et al. (2005), evaluating the performance of silver catfish (*Rhamdia quelen*) fed diets supplemented with 0.6 mg kg<sup>-1</sup> organic selenium, confirmed that this form of selenium improved the weight gain and growth of silver catfish (*Rhamdia quelen*) compared with the same level of inorganic





**Figure 2.** a) Contrasts of Glutathione Peroxidase units in blood of tilapia fed on 0.1 mg kg<sup>-1</sup> of Se-O (T1) and Se-I (T4); 0.2 mg kg<sup>-1</sup> of Se-O (T2), and Se-I (T5); and 0.4 mg kg<sup>-1</sup> of Se-O (T3), and Se-I (T6). b) Contrasts of unit of glutathione peroxidase in blood of tilapia fed on organic (Se-O) and inorganic (Se-I) selenium.

selenium. In addition, when evaluating the inclusion of selenium and vitamin E for *Macrobrachium amazonicum*, Sampaio et al. (2004) found that combining 200 mg kg $^{-1}$  vitamin E with 0.5 mg kg $^{-1}$  selenium maximized the weight gain of this species. Toyama, Corrente and Cyrino (2000) concluded that vitamin C levels between 765 and 859.5 mg kg $^{-1}$  increase the weight gain of tilapia during sex reversal. These results demonstrate that using selenium associated with supplementation of vitamins C and E increases the weight gain of aquatic organisms such as tilapia.

The higher growth of tilapia fed on increasing levels of selenium and vitamins C and E (Table 2) can be explained by the importance of selenium and vitamin C to increase the immune resistance of animals (NATIONAL..., 1993; WANG et al., 2003) and prevent diseases such as exophthalmia, ascites, anemia, pale gills, and shapeless, as well as decreased production of erythrocytes and greater fat accumulation in the liver (MARTINS; MIYAZAKI; YAMAGUCHI, 2008). Feed conversion improved with increasing selenium and vitamins C and E contents in the diet (Table 3), suggesting the positive effects of vitamin E as probable explanation against polyunsaturated fatty acids peroxidation present in the phospholipidic membrane and glutathione peroxidase, which protects cytoplasmic organelles of oxidative stress promoted by the hydroperoxide, peroxides, and superperoxides radicals.

The increase in the glutathione peroxidase concentrations in the blood of tilapia fed on increasing levels of selenium in the diet, especially organic selenium (Table 2), corroborates the results reported by Abdel-Tawwab and Wafeek (2010), who assessing tilapia diets enriched with 5.54 mg kg<sup>-1</sup> organic selenium compared with the control without selenium addition, found higher levels of glutathione peroxidase in the fish fed on diets enriched with this mineral. Lin and Shiau (2005) assessing the addition of 0, 0.5, 1.0, 2.0, 3.0, and 5.0 mg kg<sup>-1</sup> selenomethionine in the diet of the grouper (Epinephelus malabaricus) also found increased glutathione peroxidase in response to increased dietary selenium. Wang et al. (2007), evaluating the addition of 0.5 mg kg<sup>-1</sup> organic or inorganic selenium in diets for Carassius auratus gibelio, found higher levels of glutathione peroxidase in blood plasma of fish fed on 0.5 mg kg<sup>-1</sup> organic selenium.

Results of the hematocrit analysis (Table 2) corroborate those of Martins et al. (1995), who also found no difference in blood levels of Pacu (*Piaractus mesopotamicus*) when supplementing the diet with 0, 50, 100, and 200 mg kg<sup>-1</sup> vitamin C. However, these authors reported that the lack of vitamin C affects growth and makes fish more susceptible to diseases. Evaluating the addition of 0.0, 0.25, 0.50, 1.0, and 1.5 mg kg<sup>-1</sup> organic selenium in diets for tilapia, Gomes (2008) found no significant differences in the hematocrit results , which ranged from 43.91 to 46.75%. Hilton and Hodson (1983), when testing 0.5 and 1.0 mg kg<sup>-1</sup> selenium in diets of rainbow trout (*Salmo gairdneri*), found no significant differences in the hematocrit levels either.

The carcass yield (Table 3) obtained in this study was similar to that found by Boscolo et al. (2001), who assessing carcass yield of common and Thai tilapia with 234 g obtained yields of 88.29 and 86.88% respectively, while the fillet yield (Table 3) was similar to that found by Souza and Maranhão (2001), who obtained 36.50% yield for tilapia with mean weight between 300 and 400 g. This study shows that supplementing the diet of tilapia with organic selenium stimulates weight gain, feed conversion ratio, and glutathione peroxidase when compared with inorganic selenium and, although not significant, there was an increase in the selenium concentrations in the fillets of tilapia when 0.1, 0.2 and 0.4 mg kg<sup>-1</sup> of selenium were used in the diet. This fact is associated with the reaction of the inorganic selenium positive charge to the negative charge of the intestinal mucus, hindering its absorption by the enterocytes. However, since organic selenium is in the form of a chelate bound to two molecules of amino acids of neutral charge (cysteine or methionine), it passes through the intestinal mucus without being recognized facilitating its absorption and becoming more bioavailable to the organism (RUTZ; MURPH, 2010).

# **5 Conclusion**

The addition of organic selenium to the diet improves weight gain, feed conversion ratio, vitamin E deposition in the fillet, and the level of glutathione peroxidase in the tilapia blood (O. niloticus) when compared with the addition of inorganic selenium that caused no effects on carcass and fillet yield. The use of 200 mg kg $^{-1}$  VC and VE and 0.2 mg kg $^{-1}$  organic selenium in the diet of tilapia (Oreochromis niloticus) is recommended.

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#### References

- ABDEL-TAWWAB, M.; WAFEEK, M. Response of Nile Tilapia, Oreochromis niloticus to Environmental Cadmium Toxicity During Organic Selenium Supplementation. **Journal of the World Aquaculture Society**, v. 41, p. 106-114, 2010. http://dx.doi.org/10.1111/j.1749-7345.2009.00317.x
- ALBREKTSEN, S.; LIE, O.; SANDNES, K. Ascorbyl palmitate as a dietary vitamin C source for rainbow trout (*Salmo gairdneri*). Aquaculture, v. 71, p. 359-368, 1988. http://dx.doi.org/10.1016/0044-8486(88)90205-0
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS AOAC.

  Official Methods of Analysis of the Association of Official

  Analytical Chemists. 15th ed. Arlington: AOAC, 1990. v. 2.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists. 16th ed. Arlington: AOAC, 1995. v. 2.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS AOAC.

  Official Methods of analysis of the Association of Official

  Analytical Chemists. 17th ed. Arlington: AOAC, 1996.
- BAI, S. C.; GATLIN, D. M. Dietary rutin has limited synergistic efects on vitamin C nutrition of fingerling channel catfish (*Ictalurus punctatus*). Fish Physiology and Biochemistry, v. 10, p. 183-188, 1992. http://dx.doi.org/10.1007/BF00004512
- BOSCOLO, W. R. et al. Desempenho e Características de Carcaça de Machos Revertidos de Tilápias do Nilo (*Oreochromis niloticus*), Linhagens Tailandesa e Comum, nas Fases Inicial e de Crescimento. **Revista Brasileira de Zootecnia**, v. 30, n. 5, p. 1391-1396, 2001. http://dx.doi.org/10.1590/S1516-35982001000600001
- BOYD, C. E.; TUCKER, C. S. **Pond Aquaculture Water Quality Management**. Norwell: Kluwer Academic Publishers, 1998. 720 p. http://dx.doi.org/10.1007/978-1-4615-5407-3
- BUCKLEY, W. T. Trace Element Dynamics. In: MELLO, I. D.; FELIX, J. P. (Eds.). Farm Animal Metabolism and Nutrition. Wallingfors: CAB International, 2000. p. 161-182. http://dx.doi. org/10.1079/9780851993782.0161
- GOMES, G. R. Suplementação com selênio orgânico nas dietas de tilápias do nilo (*Oreochromis niloticus*). 2008. 48 f. Dissertação (Mestrado em Aquicultura)-Universidade Estadual Paulista, São Paulo, 2008.
- HILTON, J. W.; HODSON, P. V. Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdneri*). **Journal of Nutrition**, v. 113, p. 1241-1248, 1983. PMid:6854415.
- KIM, K. W. et al. No synergistic effects by the dietary supplementation of ascorbic acid, a-tocopheryl acetate and selenium on the growth performance and challenge test of *Edwardsiella tarda* in fingerling Nile tilapia, *Oreochromis niloticus*. **Aquaculture Research**, v. 34, p. 1053-1058, 2003. http://dx.doi.org/10.1046/j.1365-2109.2003.00908.x
- KUBITZA, F. **Tilápia**: tecnologia e planejamento na produção comercial. Jundiaí: Acqua Supre, 2000. 285 p.

- LIN, Y. H.; SHIAU, S. Y. Dietary selenium requirement of grouper, Epinephelus malabaricus. **Aquaculture**, v. 250, p. 356-363, 2005. http://dx.doi.org/10.1016/j.aquaculture.2005.03.022
- MARTINS, M. L. et al. Influência de diferentes níveis de vitamina C na ração sobre parâmetros hematológicos de alevinos de *Piaractus mesopotamicus* Holmberg (Osteichthyes, Characidae). **Revista Brasileira de Zoologia**, v. 12, p. 609-618, 1995. http://dx.doi.org/10.1590/S0101-81751995000300016
- MARTINS, M. L.; MIYAZAKI, D. M.; YAMAGUCHI, M. F. Ração suplementada com vitaminas C e E influencia a resposta inflamatória aguda em tilápia do Nilo. **Ciência Rural**, v. 38, p. 213-218, 2008. http://dx.doi.org/10.1590/S0103-84782008000100034
- MATTHEY, M.; GRAF, H.; FLACHOWSKY, G. Die Bestimmung fettliis-lither Vitamine in der Milch mittels HPLC. In: SYMPOSIUM VITAMINE UND WEITERE ZUSATZ-STOFFE BEI, 3., 1991, Mensch und Tier, Stadtroda. **Proceedings**... Niederkleen: Wissenschaftlicher Fachverlag Dr. Fleck, 1991. p. 143-146.
- MUKAI, K.; NISHIMURA, M.; KIKUCHI, S. Stopped-flow investigation of the reaction of vitamin C with tocopheroxyl radical in aqueous Triton X-100 micellar solutions. The structure activity relationship of the regeneration reaction of tocopherol by vitamin C. **Journal of Biological Chemistry**, v. 266, p. 274-278, 1991. PMid:1985900.
- NATIONAL RESEARCH COUNCIL NRC. **Nutrient Requirements of Fish**. Washington: National Academy Press, 1993. 115 p.
- NOGUEIRA, M. C. S. Orthogonal contrasts: definitions and concepts. **Scientia Agricola**, v. 61, p. 118-124, 2004. http://dx.doi.org/10.1590/S0103-90162004000100020
- PAGLIA, D. E.; VALENTINE, W. N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. **Journal of Laboratory and Clinical Medicine**, v. 70, p. 158-169, 1967. PMid:6066618.
- PARIPATANANONT, T.; LOVELL, R. T. Comparative net absorption of chelated and inorganic trace minerals in channel catfish (*Ictalurus punctatus*) diets. **Journal of the World Aquaculture Society**, v. 28, p. 62-67, 1997. http://dx.doi.org/10.1111/j.1749-7345.1997. tb00962.x
- PIEDRAS, S. R. N. et al. Comparação entre o selênio orgânico e inorgânico empregados na dieta de alevinos de jundiá (*Rhamdia quelen*). **Boletim do Instituto de Pesca**, v. 31, n. 2, p.171-174, 2005.
- RUTZ, F.; MURPHY, R. Minerais orgânicos. **Aveworld**, v. 46, p. 34-44, 2010.
- SAMPAIO, F. G. et al. Níveis de vitamina E e de selênio para pós-larvas de *Macrobrachium amazonicum*. Acta Scientiarum, v. 1, n. 26, p. 129-135, 2004.
- SOUZA, M. L. R.; MARANHÃO, T. C. F. Rendimento de carcaça, filé e subprodutos da filetagem da tilápia do Nilo, *Oreochromis* niloticus (L), em função do peso corporal. Acta Scientiarum, v. 23, p. 897-901, 2001.
- THAKUR, M. L.; SRIVASTAVA, U. S. Vitamin-E metabolism and its application. **Nutrition Research**, v. 16, p. 1767-1809, 1996. http://dx.doi.org/10.1016/0271-5317(96)00196-0
- TINGGI, U. Determination of selenium in meat products by hydride generation atomic absorption spectrophotometry. **Journal of AOAC International**, v. 82, p. 364-367, 1999. PMid:10191542.
- TOYAMA, G. N.; CORRENTE, J. E.; CYRINO, J. E. P. Suplementação de vitamina C em rações para reversão sexual da tilápia do Nilo. **Scientia Agricola**, v. 57, p. 221-228, 2000. http://dx.doi.org/10.1590/S0103-90162000000200005

- VERLHAC, V.; GABAUDAN, J. F. Influence of vitamin C on the immune system of salmonids. **Aquaculture Research**, v. 25, p. 21-36. 1994. http://dx.doi.org/10.1111/j.1365-2109.1994.tb00663.x
- WANG, X. et al. Effects of the different levels of dietary vitamin C on growth and tissue ascorbic acid changes in parrot fish (*Oplegnathus fasciatus*). **Aquaculture**, v. 215, p. 203-211, 2003. http://dx.doi.org/10.1016/S0044-8486(02)00042-X
- WANG, Y. et al. Effect of different selenium source on growth performances, glutathione peroxidase activities, muscle composition and selenium concentration of allogynogenetic
- crucian carp (*Carassius auratus gibelio*). **Animal Feed Science And Technology**, v. 134, p. 243-251, 2007. http://dx.doi.org/10.1016/j. anifeedsci.2006.12.007
- WATANABE, T.; KIRON, V.; SATOH, S. Trace minerals in fish nutrition. **Aquaculture**, v. 151, p. 185-207, 1997. http://dx.doi.org/10.1016/S0044-8486(96)01503-7
- WDZIECZAK, J. et al. Comparative studies on superoxide dismutase, catalase and peroxidase levels in erythrocytes and livers of different freshwater and marine fish species. **Comparative Biochemistry and Physiology**, v. 73B, p. 361-365, 1982.