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SHORT COMMUNICATION

Effects of organic selenium on thrombocytes and parasite infestation in Nile tilapia juveniles

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Abstract The knowledge of fish mineral requirements, such as selenium, is important to promote the growth and health status for fish in captivity, but excess or deficiency must be avoided. Therefore, this study evaluated the growth performance, hematology and the parasite load of Nile tilapia (*Oreochromis niloticus*) supplemented with organic selenium (0.0; 0.25; 0.5; 1.0 and 1.5 mg/Kg). The increased levels of selenium did not affect significantly ($p<0.05$) the growth performance of tilapia. However, the increases of thrombocytes and parasite load are proportional to the increases of selenium levels. Unlike expected, supplemented diets with selenium did not benefit growth performance and supplementation of 1.5 mg/kg caused deleterious effects on the health of tilapia.

Keywords *Oreochromis niloticus* . Micronutrients . Nutrition . Selenomethionine . Health

Introduction

Selenium (Se) is an essential micronutrient that participates in various biological functions as component of selenium proteins, acting for maintenance of antioxidant defenses, cellular signaling, metabolism of thyroid hormones and immune response (Watanabe et al. 1997; Arn'er and Holmgren 2000; Pacitti et al. 2016; Takahashi et al. 2017).

Fish can absorb selenium directly from the water, but the diet is the main source of this mineral to reach the nutritional requirement. The nutritional requirement is between 0.2 and 0.5 mg/kg (Steffens 1989). Its deficiency or excess may result in reduced growth and greater mortality (Watanabe et al. 1997). In concentrations slightly above the homeostatic need, this mineral can bioaccumulate and become toxic to the fish (Hamilton 2006).

Toxic effects were reported from chronic exposure to selenium (in metallic form) provoking hematological anomalies (Seriani et al. 2012). Currently, the scientific literature has no studies about the effect on parasitic load of tilapias submitted to diet with selenium. Therefore, the health status promoted by selenium dietary supplementation must be investigated to grant the decision-making about inclusion in

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the diet for tilapia.

Thus, this study evaluated the growth performance, hematology and parasitic load in Nile tilapia submitted to dietary supplementation with organic selenium.

Materials and methods

This study used 340 Nile tilapia (*O. niloticus*) sexually reversed with initial weight 70 ± 22 g. After acclimatization period, the fish were randomly distributed in 20 tanks (300 liters), allocating 17 animals per tank equivalent to stocking density of 0.4 kg/m^3. The tanks were equipped with a water recirculation system (13.5 L/minute). The removal of waste was performed every 48 hours through siphoning at the bottom of tanks. A completely randomized design, characterized by five concentrations of organic selenium added to the diet (0; 0.25 0.5 1.0 and 1.5 mg.kg^{-1}) with four replicates were used. The fish were fed with a basic diet (Table 1) offered three times a day until apparent satiety.

The used mineral supplement in diet formulation did not contain selenium. Four levels of organic selenium (selenomethionine) were added to the diet, and its quantification was performed by digestion in water regions at 100 °C and atomic absorption with hydrides generator as recommended by Ferreira et al. (2000). These analyses were carried out at the Laboratory of Soil Biochemistry and Applied Biochemistry of the Department of Technology of FCAV / UNESP, Jaboticabal Campus and its value presented in Table 2.

The feeding period lasted 90 days and the water quality parameters as: dissolved oxygen, pH, conductivity and temperature were measured every five days and total ammonia monitored each 15 days. The temperature, dissolved oxygen, pH and conductivity were measured with electronic probes and total ammonia determined according to Apha (1998).

Growth performance was determined at the end of 90 days. All fish (340) were anaesthetized by immersion into solution of benzocaine (60 mg/L of water) then weighed and measured. Afterwards, were calculated the growth parameters as weight gain (WG = final weight – initial weight); feed conversion rate (FCR = feed intake/weight gain) and specific growth rate (SGR= 100 x (ln final weight - ln initial weight/ days of experiment).

Along the biometrics procedures, sixteen fish (16) of each treatment were collected to hematological analysis. After anesthetization, blood samples were collected by punctured of caudal vein with aid of needles and syringes moistened with anticoagulant EDTA (3%).

Blood smears were prepared and then stained according to Rosenfeld (1947) to counting thrombocytes and total leukocytes. Another small aliquot was used for the hematocrit determination according to Goldenfarb et al. (1971). The hemoglobin was measured according methodology of cianometahemoglobin in equipment CELM® 500/550. The values of total erythrocytes, hematocrit and haemoglobin were used to calculate the blood indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) (Vallada 1999).

The same selected fish for hematological analysis were also used to parasitological analysis. The fish were euthanized after deep anesthesia, and then its gills were removed and fixed as recommended by Amato et al. (1991). The monogenetic parasites *Cichlidogyrus tilapiae* were counted to calculate prevalence and mean intensity according to Bush et al. (1997).

The data were submitted to Shapiro Wilk normality test, ANOVA and Tukey test to mean comparisons ($P < 0.05$). The associations between selenium levels, blood parameters and parasite load were determined by regression analysis ($P < 0.05$).

Results

Throughout the trial period, dissolved oxygen ranged from 2.9 to 4.6 mg/L; pH 7.4 to 7.8; electrical conductivity 183.4 to 201.8 µS/cm; total ammonia 28.5 to 71.9µ g/L and temperature 28.3 to 26.5 °C. Selenium supplementation did not affect the weight gain, feed conversion rate and specific growth rate (Table 3).

As observed in the growth performance, the dietary selenium supplementation did not promote hematological changes (Table 4), except to total of thrombocytes, which increased linearly with the addition of selenium ($r^2 = 0.87$, $p = 0.019$) (Table 5).

Table 1 Composition of the basic diet selenium-free used in the experiment

Ingredients	%
Soybean meal	33
Corn bran	18
Wheat bran	15
Rice bran	9
Fish meal	16.5
Soybean oil	6
Dicalcium Phosphate	1
Mineral supplement (selenium free)	0.5
BHT*	0.02
Limestone powder	0.98
Calculated Composition **	%
Crude Protein	27.62
Lipid	10.65
Crude fiber	7.12
Dry matter (DM)	88.6
Ash	9.2
Calcium	1.88
Phosphorus	1.15

*Composition of mineral and vitamin supplement (nutrient/Kg): Iron 15000mg, copper 5000 mg, Iodine 500 mg, Manganese 17000 mg, zinc 12000 mg, Vitamin A 12000 IU, Vitamin D_3 1500 IU, Vitamin E 50 mg, Vitamin K 4 mg, Vitamin B_{12} 7 mg , Vitamin B_2 7 mg, Pantothenic acid 60 mg, Acid nicotinic 120 mg, choline chloride 600 mg, methionine 700 mg, vitamin C 300 mg, antioxidant 500 mg, vehicle 1000 g. *Butylate hydroxytoluene, DSM Nutritional Products, Switzerland. **Composition based on the analysis of the ingredients according to AOAC (1984).

Table 2 Quantification of selenium (Se) levels (mean ± SD) in experimental diets

Se. levels*	0.0	0.25	0.50	1.0	1.50
	0.11±0.003	0.23±0.001	0.59±0.008	1.07±0.02	1.59±0.03

*(mg/kg)

Table 3 Means of weight gain (WG), feed conversion rate (FCR) and specific growth rate (SGR) of *O. niloticus* supplemented with selenium (mg/kg)

	WG(g)	FCR	SGR(%/day)
Levels of Selenium (Se)	0.796	0.714	0.299
p>F	0.546 ns	0.595 ns	0.873 ns
CV (%)*	26.11	33.23	20.89
Level Se (mg/kg)			
0.00	158.99	2.06	1.28
0.25	197.10	1.54	1.46
0.50	186.13	1.57	1.42
1.00	158.18	1.89	1.36
1.50	201.74	1.54	1.47

F = Calculated F values from ANOVA; P = probability of obtaining a value of F ≥ Fc; NS = not significant. * Coefficient of variation (CV).

Selenium levels also did not affect the prevalence, but increased the mean intensity of *Cichlidogyrus tilapiae* with the increases of selenium concentration (Table 6). Also was observed to thrombocytes, however the increases of these parameters were not correlated (P = 0.0572).

Discussion

The water quality parameters were adequate to tilapia rearing (Castagnolli and Cyrino 1986) without any negative effect to the fish. According to Steffens (1986) the selenium has no any influence on growth

Table 4 Hematological parameters of Nile tilapia juveniles after feeding with dietary selenium levels (mg/kg)

Treatments Hematological parameters	0.0 mg/kg	0.25 mg/kg	0.5 mg/kg	1.0 mg/kg	1.5 mg/kg	F	P	CV (%)
Red blood Cell (x10^6µL^{-1})	2.48	2.51	2.51	2.55	2.51	0.16	0.95	9.43
Hematocrit (%)	45.3	43.9	43.9	44.6	46.7	0.31	0.86	19.01
Hemoglobin (g.dL^{-1})	13.0	13.0	12.8	13.6	13.2	0.98	0.44	9.03
MCH (picog)	524.7	522.0	511.2	535.2	536.4	3.04	0.05	4.52
MCV (fl)	183.2	176.4	176.5	178.1	187.0	0.59	0.67	13.48
MCHC (g.dL^{-1})	30.2	30.9	30.7	31.8	30.9	0.22	0.92	16.24
White cells (10^3 cel. µL^{-1})	70.5	69.5	65.8	58.3	74.0	0.85	0.51	38.15
Lymphocyte (10^3 cel. µL^{-1})	66.15	64.3	62.1	54.1	75.8	1.34	0.29	41.81
Neutrophils (10^3 cel. µL^{-1})	2.5	3.6	2.1	2.5	6.3	2.31	0.1	130.7
Monocytes (10^3 cel. µL^{-1})	1.6	1.4	1.4	1.3	1.9	1.47	0.25	52.5

F = Calculated F values from ANOVA; p= P value; NS = not significant. * Coefficient of variation (CV); fL = Fentoliters.

Table 5 Means of total thrombocytes of Nile tilapia after feeding with dietary selenium levels (mg/kg) and linear regression between thrombocytes and selenium levels.

	Thrombocytes
F value	6.07
p value	0.004
CV (%)*	38.4
Linear regression	R^2=0.87 p=0.019
Treatment means	(10^3 cel. µL^{-1})
0.0 mg/kg	1.85 b
0.25 mg/kg	2.23 ab
0.5 mg/kg	2.19 ab
1.0 mg/kg	3.16 a
1.5 mg/kg	3.14 a

Mean in columns followed by the same letter did not differ significantly by Tukey test at 5% probability; F = Calculated F values from ANOVA;
* Coefficient of variation (CV)

Table 6 Prevalence and mean intensity of parasite *Cichlidogyrus tilapiae* in Nile tilapia after feeding with dietary selenium levels (mg/kg).

	Prevalence	Mean intensity
F values	1.0	3.29
P value	0.56ns	0.018*
CV (%)*	3.8	39.0
Linear regression	ns	R^2=0.94, P=0.005
Treatment/Mean		
0.0 mg/kg	100.0	68.70 a
0.25 mg/kg	91.6	75.66 ab
0.5 mg/kg	100.0	84.83 ab
1.0 mg/kg	100.0	116.83 ab
1.5 mg/kg	100.0	172.90 b

Mean in columns followed by the same letter did not differ significantly by Tukey test at 5% probability;
F = Calculated F values from ANOVA; * Coefficient of variation (CV); ns = No Significant

performance of tilapia, as noted in the present study with concentrations between 0.25 to 1.50 mg/kg. Other studies obtained similar results for pacu (*Piaractus mesopotamicus*) with selenium supplementation 0.72 to 2.51 mg/kg (Takahashi et al. 2017), and for rainbow trout (*Oncorhynchus mykiss*), at concentrations 0.25 to 4.0 mg/kg (Pacitti et al. 2015).

Possibly, the selenium levels in the basic diet for this study, reached the needed requirement for tilapia juvenile. Thus, the small initial concentration (0.11 mg/Kg) in the basic diet probably contributed to the growth in the control group discarding any significant differences between supplemented groups.

Although it has no effect on growth, the addition of selenium in the diet can affect the amount of blood cells, but it depends on the cell function and the cytosolic peroxidase (Brown et al. 2000). The pronounced response on thrombocytes in higher selenium concentration can be related to minor half-life of the circulating thrombocytes which responding more readily to increased selenium than other blood

cells from longer half-life (Dalir-naghadeh et al. 2015). In others studies changes in the total number of thrombocytes also were observed in tilapia fed with selenium (Seriani et al. 2012). However, studies must be carried out to clarify whether this is beneficial or not for the fish health.

Although the thrombocytes possess defense action in the body (Matushima and Mariano 1986) they may not directly influence the *Cichlidogyrus tilapiae* load. Thus, the responses of thrombocytes and parasitic load in the present study were caused by other problem. Thrombocytes of fish have similar functions to those of mammal's platelets, being responsible for process of blood clotting (Lemly 2002). Therefore, the increases of these cells would be related to response of internal injuries in organs as kidney and liver, or external injuries on the gills provoked by the higher levels of selenium probably due to the toxic effect (García-Cortés et al. 2016).

In the previous studies, on the same species, concentrations of 1.0 mg/kg of Se cause hepatotoxicity with hepatocyte derangement, necrosis and fatty degeneration (Tashjian et al. 2006). This organ has high capacity for bioaccumulation of this mineral, being influenced by its levels in the diet (Pacitti et al. 2016; Gomes et al. 2016; Pan and Huang 2013). Therefore, metabolic and immunological processes may have been altered in response to long supplemented time (90 days) at the highest level of selenium (1.5 mg/kg), which may have contributed indirectly to the increases of parasites number on fish, but this hypothesis, must be studied.

Contrary to expectations, in this study it was not possible to suggest an ideal level of selenium for tilapia. However, the excess and the deficiency of selenium in the diet promote changes in the liver, provoking lipid degeneration (Gomes et al. 2019). The deficiency of selenium compromises the concentration of glutathione peroxidase, which prevents the free radical formation, inducing the lipid peroxidation in liver (Pan and Huang 2013), thus the supplementation is essential for normal growth. Thus, the selenium concentration presented in the basic diet (0.11 mg/kg) was sufficient to normal growth of tilapias, being similar to the concentrations reported by Steffens et al. (1989) for other species. In addition to increased parasitic load with dietary supplementation of 1.5 mg/kg, it results agree with the literature about the selenium toxicity. In conclusion, supplemented diets with organic selenium have no effect on the growth performance for tilapias. However, 1.5 mg/Kg of organic selenium provides deleterious effects to the tilapia health.

Conflict of interest The authors declare no conflicts of interest.

Authors' contribution (Rodrigo) coordinate of project, supervision, review, text edition and draft the paper; (Gabriela) conducted the bioassay and laboratorial analysis; (Róberson) supervision and review; (Claudinei) supervision and review; (Natal) conducted the bioassay and laboratorial analysis; (Márcia) conducted the bioassay and laboratorial analysis; (Peterson) conducted the bioassay and laboratorial analysis; (Juliana) conducted the bioassay and laboratorial analysis; (Paulo) review and statistic; (Alexandre) review and statistic.

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References

Amato JFR, Boeger WA, Amato SB (1991) Protocolos para laboratório- coleta e processamento de parasitos do pescado. Universidade Federal do Rio de Janeiro, Brazil

APHA (American Public Health Association) (1998) Standard methods for the examination of water and wastewater. Washington DC 20: 2005-2605

Arn'er ESJ, Holmgren A (2000) Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 267: 6102–6109

Brown KM, Pickard K, Nicol F, Beckett GJ, Duthie GG, Arthur JR (2000) Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphocytes, granulocytes, platelets and erythrocytes. Clin Sci 98: 593-599

Bush AO, Laffertty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms. J Parasitol 83: 575-583

Castagnolli N, Cyrino JE (1986) Piscicultura nos trópicos. São Paulo Manole, 152 p

Dalir-naghadeh B, Bahrami Y, Rezaei S. A, Anassori E, Janalipour A, Khosravi V (2015) Platelet indices of selenium status in healthy and selenium-deficient sheep: a comparison with selenium indices in plasma, whole blood, and red blood cells. Bio Trace Elem Res 168: 74-81

Ferreira KS, Gomes JC, Bellato CR, Jordão CP (2000) Concentrações de selênio em alimentos consumidos no Brasil. Rev Panam Salud Públ 11: 172-177

García-Cortés M, Robles-Díaz M, Ortega-Alonso A, Medina-Caliz I, Andrade R (2016) Hepatotoxicity by dietary supplements: a tabular listing and clinical characteristics. Int J mol Sci 17: 537

Goldenfarb PB, Bowyer FP, Hall E, Brosious E (1971) Reproducibility in the hematology laboratory: the microhematocrit determination. Am J Clin Pathol 56(1): 35-39

Gomes GR, Cruz CD, Sakabe R, Ramos FM, Fujimoto RY (2016) Histopathological alterations in liver of Nile tilapia supplemented with selenomethionine. Pesq Agropec Bras 51: 288-291

Hamilton SJ (2004) Review of selenium toxicity in the aquatic food chains. Sci Total Environ 326: 1-31

Lemly AD (2002) Symptons and implications of selenium toxicity in fish: The Bellews Lake case example. Aquat Toxicol 67: 39-49

Matushima ER, Mariano M (1996) Kinetics of the inflammatory reaction induced on the swimbladder of *Oreochromis niloticus* (Nile tilapia). Braz J Vet Res An Sci 33: 5-10

Pacitti D, Lawan MM, Feldmann J, Sweetman J, Wang T, Martin SAM, Secombes CJ (2016) Impact of selenium supplementation on fish antiviral responses: a whole transcriptomic analysis in rainbow trout (*Oncorhynchus mykiss*) fed supranutritional levels of Sel-Plex®. BMC Genomics 17: 116

Pacitti D, Lawan MM, Sweetman J, Martin SAM, Feldmann J, Secombes CJ (2015) selenium supplementation in fish: A combined chemical and biomolecular study to understand Sel-Plex assimilation and impact on selenoproteome expression in rainbow trout (*Oncorhynchus mykiss*). PloS One 10(5): e0127041

Pan D, Huang H (2013) Selenium levels in hepatic steatosis patients. Bio Trace Elem Res 152: 305-309

Rosenfeld G (1947) Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rápido. Mem Inst Butantan 20: 329-334

Seriani R, Ranzani-paiva MJT, Gonçalves A, Siqueira SR, Lombardi JV (2012) Determination of selenium toxicity to *Oreochromis niloticus* based on hematological parameters. Acta Sci Biol Sci 34(2): 125-131

Steffens W (1989) Principles of fish nutrition. New York: Ellis Horwood, 384 p

Takahashi LS, Biller-takahashi JD, Mansano CFM, Urbinati EC, Gimbo RY, Saita MV (2017) Long-term organic selenium supplementation overcomes the trade-off between immune and antioxidant systems in pacu (*Piaractus mesopotamicus*). Fish Shellfish Immun 60: 311-317

Tashjian DH, Teh SJ, Sogomonyan A, Hung SSO (2006) Bioaccumulation and chronic toxicity of dietary L-selenomethionine in juvenile white sturgeon (*Acipenser transmontanus*). Aquat Toxicol 79: 401-409

Vallada EP (1999) Manual de técnicas hematológicas. São Paulo: Atheneu, 423 p

Watanabe T, Kiron V, Satoh S (1997) Trace minerals in fish nutrition. Aquaculture 151: 185-207

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