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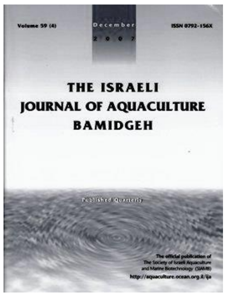

Effects of Dietary Organic Selenium on Growth, Survival, Physiological and Hematology Conditions of Snub-Nose Dart (*Trachinotus blochii* Lacepide, 1801)

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Abstract

This study aimed to determine the effects of dietary supplementation of organic selenium (OS) on growth, survival, as well as the physiological and hematological status of snub-nose dart, *Trachinotus blochii*. Five diets supplemented with OS-Selplex (Selplex, Alltech, USA) at 0, 0.1, 0.2, 0.3 and 0.4 g/kg were fed to fish (2.30 ± 0.28g, average wt) for 8 weeks. Survival was significantly highest ($P < 0.05$) when the fish were fed the 0.3 g/kg OS-Selplex supplemented diet. Growth, flesh protein, and liver lipids, were significantly higher ($P < 0.05$) in fish fed all OS-Selplex supplemented diets compared to the control group. Flesh moisture content was lower ($P < 0.05$) in fish fed 0.2, 0.3 and 0.4 g/kg OS-Selplex supplemented diets. Hematocrit values (Ht) of the fish fed 0.3 and 0.4 g/kg OS-Selplex supplemented diets were higher than in fish fed the other diets ($P < 0.05$). The highest proportions of monocytes were obtained in fish fed 0.2 and 0.3 g/kg OS-Selplex supplemented diets. The results suggest that feeding 0.3 g/kg of OS-Selplex in diets increases growth performance and health status of snub-nose dart.

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Introduction

Antibiotics have been used at sub-therapeutic concentrations to improve the growth and health of aquaculture organisms. However, the increasing global demand for safe seafood and the need to preserve an eco-friendly environment by minimizing the use of antibiotics that could encourage the increase of antibiotic-resistant pathogens and cause environmental deterioration has been questioned (Gatlin III et al., 2006). The ban on antibiotic usage in aquaculture has prompted interest in developing alternatives to antibiotics for growth and health improvement of non-aquatic animals (Rosen, 1996). Supplementation of the trace element selenium, for growth enhancement, survival, physiological status, and disease resistance, can reduce the use of antibiotics in aquaculture (Hunt et al, 2011, Abdel-Tawwab et al. 2007) and in crustaceans (Wang et al. 2009, Nugroho and Fotedar, 2013). Selenium (Se) is an essential trace element required for normal growth and physiological function of fish (Lin and Shiau, 2005). It serves as a component of the enzyme glutathione peroxidase known to protect cell membranes against oxidative damage (Rotruck et al., 1973). Two forms of Se have been used in fish culture, inorganic sodium selenite, and organic seleno yeast, or selenomethonine. Recently, there has been an increase in the use of organic forms of Se in fish culture (Abdel-Tawwab et al., 2007, Lin, 2014).

Snub-nose dart (*Trachinotus blochii* Lacepède 1801) is an important candidate for aquaculture in several production areas such as Hong Kong, Singapore, Taiwan, China, Malasia, and Vietnam. However, there is a lack of information regarding the use of organic selenium in the culture of this fish. Some indicators of the efficiency of feed additives on growth and survival, are moisture, crude protein, and total lipid content, in target organs (Torrecillas et al., 2007) as well as hematological parameters such as total blood cell count (TBCs), total leucocyte count (TLCs), differential leucocyte counts (DLCs) and hematocrit value (Ht) (Abdel-Tawwab et al., 2007). This study aims to investigate the role of dietary selenium in organic form (OS-Selplex) on the growth, survival, physiological, and hematological parameters of Snub-nose dart.

Materials and Methods

Fifteen composite rectangular tanks (500 x 800 x 800 mm), 360 L capacity were used as culture units in the experiment. Each tank was supplied with 300 L mechanically filtered and aerated seawater with an independent recirculating seawater system with a biological filter. The recycling rate of the water in each tank was maintained at approximately 5 L/min throughout the experiment. All experimental treatments were tested in triplicate in a random block design.

Juvenile fish from a commercial hatchery (Nha Trang, Vietnam) were shipped to the aquaculture station of the Institute of Oceanography, and acclimated to experimental conditions for one week before commencement of the experiment. During acclimation they were fed a basal, commercial diet (Nanolis C, Guyomarc'H, Vietnam) with 52% crude protein, and 8% lipid. The fish were fed twice daily at 8:00 and 16:00 at the rate of 5% of total biomass of the fish. Uneaten food and feces were siphoned out before each feed.

The experimental diets were supplemented with organic selenium (Selplex, Alltech, Kentucky USA) at levels of 0 g/kg (D1), 0.1 g/kg (D2), 0.2 g/kg (D3), 0.3 g/kg (D4) and 0.4 g/kg (D5). The mixed ingredients were then passed through a mince mixer with water to obtain 2.0 mm diameter pellets. The pellets were dried in direct sunlight for 6 h and allowed to cool at room temperature for half an hour before being packed in plastic containers and refrigerated at 4^0C until use. The concentration of Se in each diet was determined at 0.94; 1.81; 2.74; 3.61 and 5.52 mg/kg for D1, D2, D3, D4 and D5, respectively.

After acclimation, juvenile fish (2.30±0.28g, average weight) were randomly distributed into culture tanks at a density of 30 fish per tank and cultured for 8 weeks. Each random block of three tanks was independently assigned one of the diets D1, D2, D3, D4 and D5. Food was provided to the fish at 5% total biomass at 08:00 and 16:00. Uneaten food and feces were siphoned out before each feeding and the amount of siphoned water replaced with the same volume of water. Surviving fish were counted and weighed individually with an electronic scale (TE 412, Canada) every fortnight. Physiological and hematological parameters were determined after 8 weeks.

The survival rate of the fish in each culture tank was calculated using the formula: $S = 100x (nt/n0)$, where: S is the survival rate; nt is the number of the fish at time t and n0 is the number of fish at the commencement of the study (n=30)

Growth rate was calculated and expressed as specific growth rates (SGR) and average weekly gain (AWG) according to the following equation:

Specific growth rate in weight (%):

$SGR = 100 \times (\ln Wf - \ln Wo)/t$;

$AWG (g/week) = (Wf - Wo)/wk$.

Where: SGR is specific growth rate; Wf is weight of the animal at time t; Wo is weight at commencement; t and wk are the day and week number, respectively, in the growth period.

At the end of the culture period, ten fish from each tank were collected to determine the lipid accumulation in the liver, moisture content, and crude protein, in the flesh according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1995).

At the end of culture period, six fish from each tank were sampled for hematological tests. Differential blood cell counts (DBCs), leucocytes counts (DLCs), and hematocrit values (Ht), were determined by the established method of (Selvaraj et al., 2006). Fish blood samples were collected with a hypodermic syringe from the caudal vein and placed in Eppendorf tubes containing 20 U/L sodium heparinate/mL. After blood dilution with phosphate-buffered saline (pH 7.2), 100 blood cells were counted under a light microscope equipped with a Neubauer hemocytometer. The proportion of thrombocytes, leucocytes, and red blood cells, was calculated. For DLCs, blood smears were stained with Dip Quick (Lucerna Chem, Switzerland). 100 leucocytes were counted under the microscope and the percentage of different types of leucocytes calculated. Hematocrit values (Ht) were immediately determined after sampling by placing fresh blood in glass capillary tubes and centrifuged for 10 min in a microhematocrit centrifuge, and the packed cell volume was measured.

The data were statistically analyzed using SPSS for Windows (IBM Corporation, Armonk, NY, USA), version 15. All data are represented as mean ± standard error (SE) except for survival rates which are represented as mean ± standard deviation (SD). All percentage data were normalized using an arcsine transformation before analysis. The normality of data was assessed by the Shapiro–Wilk test prior to analysis. The homogeneity of variance was assessed by Levene's test prior to analysis. ANOVA (analysis of variance) and LSD (Least significant difference) post hoc tests were used to determine significant differences between growth, survival, physiological, and hematology parameters of fish fed different diets. Results were judged as significant at $P < 0.05$.

Results

Survival of fish fed D4 was highest ($P<0.05$) followed by fish fed D5 compared to fish fed the other diets. The fish fed D1 and D2 had lowest rates of survival ($P <0.05$). SGRs and AWG of fish fed all OS-Selplex supplemented diets were higher ($P < 0.05$) than SGR of fish fed the control diet. The highest SGRs and AWGs were observed in fish fed D5 followed by fish fed D4. SGRs and AWGs did not differ when fish were fed D2 and D3 (Table 1).

Table 1. Growth and survival of the fishes fed different OS supplemented diets.

Parameters	*Diets*				
	D1	*D2*	*D3*	*D4*	*D5*
Weight gain (g/wk)	1.26 ± 0.09^{a}	1.48 ± 0.07^{b}	$1.59 \pm 0.07b^{c}$	$1.60 \pm 0.03b^{c}$	1.80 ± 0.04^{c}
SGR (%/d)	3.00 ± 0.10^{a}	3.23 ± 0.07^{b}	$3.35 \pm 0.07b^{c}$	$3.36 \pm 0.03b^{c}$	3.54 ± 0.03^{c}
Survival (%)	75.56 ± 5.88^{ac}	73.33 ± 5.09^{a}	82.22 ± 5.88^{ab}	94.44 ± 1.11^{b}	90.00 ± 1.92^{c}

Different superscript letters in the same row indicate significantly different means at $P < 0.05$

Flesh protein and liver lipids were higher (P < 0.05) in fish fed D2, D3, D4 , D5 compared to fish fed the control diet (D1). Flesh protein and liver lipids were not significantly different (P > 0.05) between the fish fed any OS-Selplex supplemented diets. Moisture contents of liver were not different (P > 0.05) among the fishes fed any OS-Selplex supplemented diets. Flesh moisture content was lower (P < 0.05) in fish fed D3, D4, and D5 compared to D1 and D2 fed fish (Table 2).

Table 2. Physiological parameters of the fishes fed different OS supplemented diets.

Parameters	*Diets*				
	D1	*D2*	*D3*	*D4*	*D5*
Flesh protein (% wet weight)	8.99 ± 0.88^{a}	15.14 ± 0.58^{b}	12.81 ± 0.82^{b}	13.79 ± 0.61^{b}	13.51 ± 0.89^{b}
Liver lipid (% dry weight)	10.23 ± 0.79^{a}	16.95 ± 1.39^{b}	16.54 ± 1.10^{b}	17.47 ± 0.84^{b}	16.27 ± 1.51^{b}
Liver moisture content (%)	57.47 ± 2.33	54.73 ± 0.23	57.00 ± 2.66	58.10 ± 4.37	57.97 ± 2.82
Flesh moisture content (%)	75.20 ± 0.74^{a}	75.26 ± 0.53^{a}	73.61 ± 0.29^{b}	73.42 ± 0.13^{b}	73.54 ± 0.36^{b}

Different superscript letters in the same row indicate significantly different means at P < 0.05

Ht of the D4 and D5 fed fish was higher (P < 0.05) than that of fish fed D1, D2 and D3. The lowest (P < 0.05) Ht was observed in fish fed the control diet (D1). Leucocytes of fish fed 0.4 g/kg were the lowest (P < 0.05). The proportion of thrombocytes and red blood cells did not differ between fish fed different diets (Table 3).

Table 3. Differential blood cell count (DBCs) and hematocrit value (Ht) of the fish fed different OS supplemented diets.

Parameters	*Diets*				
	D1	*D2*	*D3*	*D4*	*D5*
Thrombocytes (%)	8.9 ± 1.1^{a}	7.2 ± 1.9^{a}	6.5 ± 0.9^{a}	10.9 ± 2.2^{a}	8.0 ± 2.0^{a}
WBC (%)	6.6 ± 1.5^{b}	4.9 ± 0.8^{ab}	4.1 ± 0.9^{ab}	6.4 ± 1.7^{b}	2.9 ± 0.6^{a}
RBC (%)	84.5 ± 2.1^{a}	87.9 ± 1.6^{a}	89.4 ± 1.7^{a}	82.7 ± 3.6^{a}	89.1 ± 2.3^{a}
Ht (%)	37.6 ± 1.9^{a}	39.0 ± 2.9^{ab}	40.1 ± 1.2^{ab}	41.0 ± 2.5^{b}	45.7 ± 2.6^{b}

Different superscript letters in the same row indicate significantly different means at P < 0.05

In the leucocytes count, the proportion of Basophil did not differ in fish fed any of the diets. However, neutrophils were highest in D5 fed fish. Eosinophil was highest in fish fed D3. Total proportion of granulocytes was lowest in fish fed D4. The highest proportions of monocytes were seen in the D3 and D4 fed fish. Lymphocyte proportions were lower D3 and D4 fed fish compared to fish fed D1, D2, and D5 (Table 4).

Table 4. Different leucocytes count (%) of the fish fed different OS supplemented diets.

Parameters	*Diets*				
	D1	*D2*	*D3*	*D4*	*D5*
Neutrophil	7.90 ± 0.95^{ab}	9.03 ± 0.43^{bc}	6.75 ± 0.88^{a}	5.95 ± 0.53^{a}	10.18 ± 0.59^{c}
Basophil	1.77 ± 0.27^{a}	3.07 ± 0.94^{a}	2.73 ± 0.53^{a}	1.8 ± 0.45^{a}	1.6 ± 0.70^{a}
Eosinophil	0.98 ± 0.05^{a}	1.15 ± 0.11^{ab}	1.57 ± 0.26^{b}	0.95 ± 0.14^{a}	1.00 ± 0.09^{a}
Lymphocyte	58.6 ± 2.3^{c}	57.5 ± 2.3^{bc}	48.1 ± 4.0^{a}	49.5 ± 3.2^{ab}	55.2 ± 0.9^{abc}
Monocyte	30.8 ± 2.2^{a}	29.2 ± 1.8^{a}	40.8 ± 4.3^{b}	41.8 ± 4.3^{b}	32.1 ± 1.2^{a}

Different superscript letters in the same row indicate significantly different means at P < 0.05

Discussion

This research demonstrated the effectiveness of supplemented Sel-Plex as a source of OS in the culture of Snub-nose dart, *Trachinotus blochii*. Sel-Plex is also known as selenoyeast and contains selenoprotein. It is a baker's yeast dried product, derived from Saccharomyces cerevisiae strain CNCM I-3060, cultivated in a Se-enriched fermentation medium to provide a high level of selenomethionine (Burdock and Cousins, 2010). This current study did not test the role of inorganic forms of Se on the performance of Snub-nose dart. However a previous study on other fish showed that the organic form of Se (OS) (selenoyeast or selenomethonine) may have benefits superior to those provided by the inorganic forms (Wang and Lovell, 1997, Bell and Cowey, 1989). Further study is needed to compare the specific effect of the two forms of selenium on the performance of the Snub-nose dart fish.

During the experiment, environmental conditions did not differ between the culture tanks. After the OS supplementation of 0.1 to 0.4 g/kg, the diets remained isocaloric and iso-nitrogenous. Thus, the results of the current study demonstrate only the results of the dietary OS inclusion. These suggested that the supplementation of 0.3 g/kg Selplex improved the survival, growth, physiology and hematology of the Snub-nose dart and provided additional information on the role of organic selenium in fish. Similar results were obtained with Nile tilapia, African catfish, grouper, and cobia (Abdel-Tawwab and Mohammed, 2008, Abdel-Tawwab et al., 2007, Lin, 2014, Lin and Shiau, 2007, Liu et al., 2010).

Although growth was highest in fish fed the 0.4 g/kg OS–Selplex diet, the survival, physiological, and hematological conditions were better in the fish fed 0.3 g/kg OS–Selplex diets. This suggests that 0.3 g/kg OS-Selplex supplemented in the diets is suitable for culture of the Snub-nose dart although 0.5 g/kg was found to be optimal for growth, survival, and feed utilization for tilapia (Abdel-Tawwab and Mohammed, 2008).

Physiological traits such as moisture content of flesh, moisture content of liver, liver lipid, and flesh protein have been used as indicators of the nutritional condition in fish. In the present study, higher lipid levels in the liver, protein levels in the flesh, and lower moisture in the liver and flesh, are indicators of better feed utilization, assimilation, and storage of energy by fish (Wang et al., 2014). Similar improvement in feed utilization of the fish was noted when OS-Selplex was supplemented in the diet of African catfish (Hunt, 2011; Abdel-Tawwab et al., 2007). In the current study, fish fed 0.3 g/kg OS-Selplex had a higher survival rate than fish fed 0.4 g/kg OS-Selplex, similar to the findings of Ahmad et al., (2006).

After enduring stress such as exposure to pollutants, diseases, metals, and hypoxia, hematology has been used as an index of fish health in a number of fish species (Sahu et al., 2007). In the current study OS supplementation improved the hematocrit value of the Snub-nose dart. A similar trend was observed in African catfish (Abdel-Tawwab et al., 2007). OS-Selplex did not affect the hematocrit of Yellowtail kingfish (*Seriola lalandi*) (Le et al., 2014) and rainbow trout (Rider et al., 2009), suggesting that Se requirements are different in different species of fish.

The red blood cell (RBC) count of an organism determines the carrying capacity of dissolved oxygen by hemoglobin from gills to tissues. RBC is a major and reliable indicator of various sources of stress (Rehulka, 2002). White blood cell (WBC) count is a vital index of non-specific immunity in fish (De Pedro et al., 2005). WBCs are mainly involved in phagocytic and immune responses to bacterial, viral and other challenges (Houstan, 1990). In the current study, red blood cell count (RBC) was not influenced by OS-Selplex supplemented in the diets. However, the inclusion of 0.1, 0.2 and 0.4 g/kg of OS-Selplex reduced the WBC while the inclusion of 0.3 g/kg OS-Selplex did not. Differences in differentiate leucocytes count of fish fed different OS-Selplex supplemented diets in the current study suggest that OS improves the non-specific immune response of the Snub-nose dart. This led to the in higher survival rates of fish fed 0.3 g/kg OS-selplex supplemented diet.

In conclusion, growth, survival, physiological, and hematological evidence from this study suggests that inclusion of 0.3 g/kg OS-Selplex in the diet is advisable for Snub-nose dart. Further research is needed to evaluate the role of OS-selplex on resistance of Snub-nose dart to bacterial infection and environmental metal toxicity.

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