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Comparative Effects of Organic and Inorganic Selenium Supplementation on Growth Performance, Feed Utilization, Hemato-Biochemical Parameters, and Hepatic Antioxidant Enzyme Activity in Nile Tilapia

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Abstract

A feeding trial was conducted to investigate the effect of dietary supplementation of selenium forms (inorganic and organic) on growth performance, feed utilization, hemato-biochemical parameters and hepatic antioxidant enzymes activity of Nile tilapia, *Oreochromis niloticus* for 70 days. Three isonitrogenous and isocaloric diets (306.6 g kg^{-1} crude protein and 20.04 MJ kg^{-1} gross energy) were formulated. Each diet was supplemented with selenium forms; control, organic selenium and inorganic selenium. After 70 days, the highest final body weight, weight gain, average daily gain, protein efficiency ratio and best feed conversion ratio were recorded in fish-fed organic selenium. No significant differences ($P \geq 0.05$) were found in haemoglobin, hematocrit, and red blood cells compared with the control diet. On the other hand, adding selenium significantly ($P \leq 0.05$) decreased the ALT and AST values where the lowest values of ALT and AST were recorded in fish-fed diet supplemented with inorganic and organic selenium. While adding selenium significantly ($P \leq 0.05$) increased serum total protein, the best value was recorded in fish-fed organic selenium. Also, the addition of organic selenium ($P \leq 0.05$) enhanced the hepatic superoxide dismutase activity and total antioxidant capacity level while; malondialdehyde concentration was significantly ($P \leq 0.05$) decreased with addition of organic selenium. In conclusion, the addition organic selenium in tilapia diet improved the growth performance, feed utilization hemato-biochemical parameters and hepatic antioxidant enzymes activity.

Key words: Growth; feed utilization; hematology parameters; antioxidant activity and Nile Tilapia

Introduction

Nile tilapia (Oreochromis niloticus) has become one of the most extensively farmed tropical fish species globally, contributing significantly to the aquaculture industry with a global production of 5.5 million metric tons in 2018 (WHO, 2020). This species is highly profitable in aquaculture due to its fast growth, adaptability, and high market demand.

Minerals are essential for maintaining the physiological and metabolic functions in animals, including fish. Deficiencies in key minerals can lead to various health issues. Fish, like other animals, require an array of micronutrients crucial for their survival, growth, health, and reproduction (Aliko et al., 2018).

Selenium (Se) is a trace mineral that has garnered significant attention in animal nutrition due to its critical role in various biological processes. Selenium is a constituent of the enzyme glutathione peroxidase (Rotruck et al., 1973), which is vital for catalyzing reactions that convert hydrogen peroxide and fatty acid hydroperoxides into water and fatty acid alcohols using reduced glutathione. This conversion helps protect cellular membranes from oxidative damage. Beyond its antioxidant properties, selenium has been shown to regulate inflammation, enhance immune responses, exhibit antitumor activity, and play a role in the metabolism of thyroid hormones in animals (Köhrle et al., 2000).

For fish, selenium is an indispensable nutrient. Its essentiality and dietary requirements for growth have been established for various species, including rainbow trout (Hilton et al., 1980), channel catfish (Gatlin and Wilson, 1984), grouper (Lin and Shiau, 2005), and yellowtail kingfish (Le and Fotedar, 2013). Research on selenium supplementation has covered different fish species, such as crucian carp (Carassius carassius; Wang et al., 2007), hybrid striped bass (Morone chrysops × Morone saxatilis;

Cotter et al., 2008), crucian carp (Carassius auratus gibelio; Zhou et al., 2009), rainbow trout (Oncorhynchus mykiss; Hunt et al., 2011), gilthead sea bream (Sparus aurata; Saleh et al., 2014), and Wuchang bream (Megalobrama amblycephala; Long et al., 2017).

The outcomes of these studies indicate that organic selenium is generally more digestible and biologically active than inorganic selenium, with the dietary selenium requirements differing among species and ranging from 0.2 to 12 mg per kg of diet (Prabhu et al., 2016). Given this context, the current study aimed to compare the effectiveness of different forms of selenium (organic and inorganic) in meeting the dietary selenium needs of Nile tilapia. This was assessed through an evaluation of growth performance, hematological indices, and antioxidant enzyme activities in the fish.

Material and methods

1. Fish rearing technique

O. niloticus were obtained from private farm (Kafer-Elsheikh Governorate, Egypt). Fish after arrival were acclimated to the experimental conditions for two weeks at the laboratory of fish at the Faculty of Agriculture, Benha University and kept in two concrete ponds (2×4×1 m) for prior acclimatization. During the acclimation period, fish were fed a commercial feed (30 % crude protein). After the acclimatization, the experimental fish were randomly distributed into the experimental cylindrical plastic tanks (0.5 m^3 for each) representing the six treatments studied. A set of 225 fish of *O. niloticus* L. mono-sex male fingerlings with an average initial weight of 3.07 ± 0.09 g were used in this trail. Twenty-five fish were randomly stocked into each cylindrical tank with three replications for each treatment. Underground water was supplied to each tank housed within green house. A photoperiod of 12-h light, 12-h dark (08:00 – 20:00 h). About 20% of water volume in each tank was daily replaced by aerated fresh water after removing the accumulated excreta. During the 70-day experimental period, all groups of fish were hand-fed with the respective diet to apparent satiation three times daily at 09:00 am, 12:00 am and 3:00 pm. Feed intake was calculated and expressed as the total feed intake in whole period of experiment per fish. Water temperature was recorded daily at 1.00 pm using a mercury thermometer. Dissolved oxygen (DO) was measured at 07.00 am using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia and nitrite were measured twice weekly using a DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA). A pH was estimated on morning by using a pH meter (Orion pH meter, Abilene, Texas, USA). Water temperature ranged from 27.20 to 29.25°C; dissolved oxygen (DO) ranged between 5.32 and 6.81 mg l^{-1}; pH values ranged between 8.04 and 8.30 and total ammonia ranged from 0.18 to 0.2 mg l^{-1} for the different treatments during the entire experimental period (84-day) of the study. All tested water quality criteria (temperature, pH value, DO and total ammonia) were suitable and within the acceptable limits for rearing Nile tilapia *O. niloticus* fingerlings (Boyd, 1990).

2. Preparation of experimental diets

Three isonitrogenous and isocaloric diets (306.6 g kg^{-1} crude protein and 20.04 MJ kg^{-1} gross energy) were formulated. A 0.4 mg Se/kg diet was supplemented to each diet with different forms (organic selenium and inorganic selenium). Using a pelleting hand noodle maker, all the components were thoroughly combined with the two selenium sources (organic and inorganic) before being formed into pellets with a diameter of 2 mm. These pellets were then allowed to dry overnight at room temperature and then kept at 4°C. Gross energy was determined as reported by Brett (1973) and is shown in Table 1 along with the proximate analysis of the ingredients and diets as analyzed by the AOAC (2012) method.

Table 1. Formulation and proximate composition of the experimental diet (g kg^{-1} diet, dry matter)

Ingredients	%
Soybean meal 44%	490
Corn gluten 62%	120
Yellow corn 8.5%	220
Wheat bran 14%	80
Fish oil	50
Premix[1]	40
Total	
Chemical composition (g kg^{-1})	
Protein	306.6
Lipid	61.75
Ash	45.32
Fiber	48.82
Nitrogen free extract[2]	53.75
Gross energy[3] (MJ kg^{-1})	20.04

[1]Vitamin and mineral mixture kg^{-1} of a mixture contains 4800 I.U. Vit A, 2400 IU cholecalciferol (Vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B12, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, Pantothenic acid, 8.0 g Nicotinic acid, 400 mg Folic acid, 20 mg Biotin, 200 gm Choline, 4 g Copper, 0.4 g Iodine, 12 g Iron, 22 g Manganese, 22 g Zinc, 0.04 g Selenium. Folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamine. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3077 mg; ferrous sulphate ($FeSO_4.7H_2O$, 20% Fe), 65 mg; manganese sulphate ($MnSO_4$, 36% Mn), 89 mg; zinc sulphate ($ZnSO_4.7H_2O$, 40% Zn), 150 mg; copper sulphate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I).
[2]NFE (Nitrogen free extract) = 100 − (crude protein + lipid + ash + fibre content).
[3]Gross energy was calculated using gross calorific values of 23.63, 39.52, and 17.15 kj/g for protein, fat, and carbohydrate, respectively, according to **Brett (1973).**

3. Growth and feed efficiency

Before the feeding trial and following it, each tank was counted and the number of fish was recorded. At the end of experiment (84 days), all the formulae employed to determine the growth parameters and feed utilization efficiency are listed as follow:

- Weight gain (WG) = final body weight (g) – initial body weight (g)
- Specific growth rate (SGR) = LnW2 - LnW1/t (days)*100, where Ln = the natural log; W1 = initial fish weight, W2 = final fish weight in grams, t = period in days
- Feed conversion ratio (FCR) = feed intake (g)/weight gain (g)
- Protein efficiency ratio (PER) = weight gain (g) / protein ingested (g).

2.4. Hemato-biochemical indices

At the end of the experiment, five fish (n = 5) were randomly selected from each tank and euthanized with tricaine methane sulfonate 1 g L^{-1} for 5 minutes to collect the blood samples from the caudal vein of fish in all treatments and were divided into two portions. The first portion was collected with anticoagulant 10% ethylene diamine tetraacetate (EDTA) to determine the hematocrit (Htc), hemoglobin (Hb), and red blood counts (RBCs) according to standard methods as described by Rawling *et al.* (2009).

The second portion of the blood sample was allowed to clot at 4°C and centrifuged at 3000 rpm for 10 min. The non-hemolyzed serum was collected and stored at −20 °C until used for measuring the serum biochemical parameters. Levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to the method described by Reitman and Frankel (1957). Serum total protein was determined according to Henry (1964).

2.5. Assessments of the liver's antioxidant activity

Hepatic samples (livers from three fish per replicate) were weighed, homogenized, and rinsed with ice-cold phosphate buffer (1:10; phosphate buffer: pH 7.4, 0.064 M) after anesthetizing the fish with 3-aminobenzoic acid ethyl ester (MS 222, 100 mg/L, Sigma, St. Louis, MO). Following the Peskin and Winterbourn (2000) method, the homogenate was centrifuged for 10 minutes at 4°C and 4000 g, and the supernatant was used to assay the activity of superoxide dismutase (SOD). The total antioxidant capacity (T-AOC) was estimated according to the method of Benzie and Strain (1996). The concentration of malondialdehyde (MDA) was assessed according to Dogru et al. (2008)..

Results

3.1. Growth and feed utilization parameters

Table 2 presents the growth performance and feed utilization of fish fed diets supplemented with different forms of selenium compared to the control. Dietary selenium in various forms significantly ($P < 0.05$) increased the final body weight (FBW), weight gain (WG), average daily gain (ADG), and protein efficiency ratio (PER), with the highest values recorded in fish fed organic selenium. No significant ($P > 0.05$) difference was observed in specific growth rate (SGR) among all treatments. The best feed conversion ratio (FCR) was observed in fish fed the diet supplemented with organic selenium.

Table 2. Effect of dietary containing different forms of selenium on growth performance and feed utilization of Nile tilapia, *O. niloticus* for 70 days

Items	Experimental Diets			± SE	P value
	Control	Organic Se	Inorganic Se		
Initial body weight (g $fish^{-1}$)	2.92^{b}	3.15^{a}	3.15^{a}	0.07	0.0386
Final body weight (g $fish^{-1}$)	16.68^{c}	19.29^{a}	17.87^{b}	0.37	0.0011
Weight gain (g $fish^{-1}$)	13.77^{b}	16.14^{a}	14.72^{b}	0.37	0.0023
Average daily gain	0.19^{b}	0.23^{a}	0.21^{b}	0.01	0.0018
Specific growth rate (% day^{-1})	2.21	2.33	2.19	0.53	0.18
Feed intake (g $fish^{-1}$)	21.82^{a}	21.23^{b}	21.80^{a}	0.09	0.0007
Protein efficiency ratio	2.57^{c}	3.03^{a}	2.76^{b}	0.05	0.0002
Feed conversion ratio	1.32^{a}	1.11^{c}	1.24^{b}	0.03	0.0004

Values (± SEM, n = 3). Means in the same row sharing the different superscript are significantly different (P < 0.05). Weight gain (WG) = final weight (g) – initial weight (g); Specific growth rate (SGR) = $LnW_2 – LnW_1/t$ (days), Where, Ln=the natural log; W_1= initial fish weight, W_2 = the final fish weight in grams and t =Period in days; Feed conversion ratio (FCR) was calculated according to by the equation: FCR = Feed intake (g)/weight gain (g); Protein efficiency ratio (PER) = Weight gain (g)/protein ingested (g).

3.2. Hematological indices

The effect of dietary supplementation of selenium forms on the haematological parameters of Nile tilapia (O. niloticus) fed inorganic and organic selenium are presented in Table 3. No significant differences in haematological parameters, including haemoglobin, hematocrit, and red blood cells, were found in fish-fed diets supplemented with inorganic and organic selenium compared to the control.

Table 3. Effect of dietary containing different forms of selenium on hematology of Nile tilapia, *O. niloticus* fingerlings for 70 days

	Experimental Diets			± SE	P value
Items	Control	Organic Se	Inorganic Se		
Hemoglobin (g dL^{-1})	9.25	9.45	9	0.31	0.5946
Hematocrit (%)	14.12^{c}	16.80^{a}	15.38^{b}	0.40	0.0018
RBC's† (×10^6 µl)	2.42	2.6	2.4	0.07	0.1229

Values (± SEM, n = 5). Means in the same row sharing the different superscript are significantly different (P < 0.05). †RBCs: Red blood cell counts.

3.3. Biochemical indices

The effects of dietary supplementation of selenium forms on alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total protein levels in Nile tilapia (O. niloticus) are presented in Table 4. The addition of selenium significantly (P < 0.05) decreased ALT and AST values, with the lowest levels recorded in fish fed diets supplemented with inorganic and organic selenium. Conversely, selenium supplementation significantly (P < 0.05) increased serum total protein, with the highest value observed in fish fed organic selenium.

Table 4. Effect of dietary containing different forms of selenium on biochemical blood parameters of Nile tilapia, *O. niloticus* fingerlings for 70 days

	Experimental Diets			± SE	P value
Items	Control	Organic Se	Inorganic Se		
ALT* (UL^{-1})	73.35^{a}	66.30^{b}	69.22^{ab}	1.46	0.0168
AST (UL^{-1})**	29.37^{a}	24.40^{b}	24.00^{b}	0.50	<.0001
TP* (gdL^{-1})**	1.57^{c}	3.09^{a}	2.52^{b}	0.06	<.0001

Values (± SEM, n = 3). Means in the same row sharing the different superscript are significantly different (P < 0.05). *ALT: Alanine aminotransferase; **AST, Aspartate aminotransferase; ***TP: Total protein

3.4. Hepatic antioxidant activity

Table 5 shows the effects of dietary selenium supplementation on hepatic antioxidant enzyme activities, including superoxide dismutase (SOD), total antioxidant capacity (TAC), and malondialdehyde (MDA) concentration in Nile tilapia (O. niloticus) fingerlings. Fish fed diets with organic selenium showed significantly elevated (P < 0.05) hepatic SOD activity and TAC levels. Conversely, MDA concentrations were reduced in fish supplemented with organic selenium.

Table 5. Effect of dietary containing different forms of selenium on liver antioxidant enzymes of Nile tilapia, *O. niloticus* fingerlings for 70 days

	Experimental Diets			± SE	P value
Items	Control	Organic Se	Inorganic Se		
SOD*	144.25^{b}	182.67^{a}	180.83^{a}	2.59	<.0001
TAC**	9.05^{c}	13.93^{a}	11.05^{b}	0.45	<.0001
MDA***	263.89^{a}	186.50^{b}	197.00^{c}	1.80	<.0001

Values (± SEM, n = 3). Means in the same row sharing the different superscript are significantly different (P < 0.05). *SOD: Superoxide dismutase; **TAC: Total antioxidant capacity; *** MDA: Malondialdehyde

Discussion

To promote the health and growth of fish, and thereby support the sustainable production of aquatic animals, aquafeeds need optimal supplementation with micronutrients and functional elements like selenium (Se). Selenium (Se) is a vital micronutrient and acts as a cofactor for deiodinases, which are enzymes that regulate the activation and deactivation of thyroid hormones (Hefnawy and Tortora-Perez, 2010). In this study, supplementation with both

organic and inorganic selenium enhanced the growth rate of Nile tilapia, with organic selenium proving more effective in promoting superior growth compared to inorganic selenium. This increased efficacy suggests that organic selenium may be more readily absorbed in the digestive tract compared to its inorganic counterpart (Küçükbay et al., 2009). Organic forms such as L-selenomethionine can substitute for methionine when it is limited or undergoing catabolism. The bioavailability of selenium varies with its chemical form, influencing how it is absorbed and metabolized in the gut, and thus its conversion into biologically active forms (Fairweather-Tait et al., 2010; Schrauzer et al., 2000; Yu et al., 2022).

Selenium in different forms has been shown to improve hematological indices in various fish species. For instance, hybrid tilapia demonstrated enhanced hematocrit percentages (Hct%) (El-Hammady et al., 2007), Nile tilapia showed increased red blood cell (RBC) counts (Neamat-Allah et al., 2019), and African catfish exhibited improvements in RBC counts, hemoglobin (Hb), and Hct% values (Abdel-Tawwab et al., 2007).

In the present study, the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased with the supplementation of both organic and inorganic selenium forms. These findings are consistent with those of Hao et al. (2014), who reported reductions in ALT, AST, and alkaline phosphatase (ALP) levels in loach supplemented with 0.39–0.50 mg/kg Se methionine. Similarly, Saffari et al. (2017) observed the lowest ALT, AST, and ALP levels in fish supplemented with Se methionine. The increase in serum protein levels associated with selenium supplementation enhances the innate immune response, contributing to stronger immunological defenses (Sahu et al., 2007).

Moreover, the current study found that the levels of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were modulated by dietary supplementation of selenium and probiotics, either alone or in combination. The cellular antioxidant system operates on three primary levels of defense: prevention of free radical formation through the activity of enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase, and metal-binding proteins (Combs & Combs, 1986).

Conclusion

The present study highlights the importance of including selenium with different forms supplemented to tilapia diet enhanced the performance of fish as well as oxidative stress. Se provided as organic form has relatively better efficiency than inorganic form.

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