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Effect of vitamin E (Dl-all-rac-α-tocopherol acetate) and nano particle of selenium on growth, survival, body composition and whole body Glutathione peroxidase (GPx) and malondialdehyde (MDA) in Caspian kutum (Rutilus kutum (Kamensky, 1901))

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Abstract: Iranian Fisheries Organization releases millions of kutum fingerlings to the sea annually to restock its population. The survival rate of the fish at releasing time depends on their resistance. If they are feeding on a suitable diet, they will get adequate resistance. Vitamins and minerals are fundamental components of a diet. Therefore, the effect of vitamin E (100 mg kg-1) and nano-selenium (1 mg kg-1), which have nutritional relationship, separately and in combination with together was investigated on growth, survival, carcass composition, whole body glutathione peroxidase activity and whole body malondialdehyde content of Caspian kutum. Results showed that vitamin E is capable to improve growth, FCR and WG in Kutum fingerlings; however, nano-selenium is not. Glutathione peroxidase activity was higher in the fish fed on the diets containing nano-selenium (T-2 and T-3) than the fish fed on the diets without nano-selenium supplementation (control and T-1). There was no significant difference in the fish carcass composition and whole body malondialdehyde among the treatments. According to this study, vitamin E can improve the growth and selenium can improve the glutathione peroxidase activity in kutum larva.

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Keyword: Vitamin E, nano-selenium, Growth performance, Glutathione peroxidase, Malondialdehyde.

Introduction

Caspian Kutum is one of the endemic species of the Caspian Sea. It is a popular fish, particularly in north Iran, due to meat quality and marketability (Ebrahimi et al., 2012). It is a valuable species for fishing industry (Ouraji et al., 2011) because of its distribution along the Caspian coastline (Heyrati et al., 2007).To restock the population of the Caspian Sea species, Iranian fisheries organization produces and releases a huge number of fingerlings, annually, which, Caspian kutum comprises 89% of total bony fishes produced fingerlings (Abdolhay et al., 2011). In restocking center, Caspian Kutum larva feed on zooplanktons and formulated diet (Ebrahimi et al., 2012). The formulated diet should be balanced with respect to all dietary components to allow the larvae to reach higher weight and to have adequate resistance to enter seawater.

Vitamin E is predicated to all materials acting as alpha-tocopherol (Parker & Parker, 2011). Tocopherols protect tissue against the damages caused

by lipid oxidation (Hamre, 2011). Vitamin E is necessary to maintain meat quality, immune system, natural stability of blood cells against hemolysis, capillary permeability and heart muscles (Halver & Hardy, 2002).

Selenium is one of the elements necessary in the life natural cycle of all organisms including fish (Köhrle, 2004). Selenium is important because it is involved in glutathione peroxidase (GPx) structure. This enzyme protects cells and tissues against oxidative damages (Watanabe et al., 1997).

Vitamin E and selenium has an obvious nutritional relationship. This relationship has been studied in a number of fish species. The results shows that the symptoms of combined vitamin E and selenium shortage are muscular dystrophy, presence of muscular proteins in plasma and anemia (Bell et al., 1985 ; Bell et al., 1986 ; Bell et al., 1987 ; Gatlin et al., 1986 ; Poston et al., 1976).

Nano-materials have special properties. Recently, nano-selenium has attracted attention due to high bioavailability and low toxicity (Wang et al., 2007a). The aim of the present study was to investigate the singular and combined effects of vitamin E and nano-selenium on growth, survival, carcass composition, whole body GPx activity and malondialdehyde content of Caspian kutum fingerlings, which was performed at the aquaculture laboratory of Gorgan University of agricultural sciences and natural resources, Gorgan, Iran, over 6 weeks.

Materials and methods

Nano-selenium used in this study was provided from Nanosani Co. (Mashhad, Iran). Average particle size of this product was 50 nm. Also, Dl-all-rac-α-tocopherol acetate (Sigma Chemical Co. Steinheim, Germany) was used as vitamin E source, because of high stability and bioavailability to fish (NRC, 1993). Basal diet composition is presented in table 1. Four diets were used in this study: 1- control diet without nano-selenium and vitamin E supplementation (control), 2- the control diet supplemented with 100 mg/kg vitamin E (T-1), 3- the control diet supplemented with 1 mg/kg nano-selenium (T-2), and 4- the control diet supplemented with 100 mg/kg vitamin E and 1 mg/kg nano-selenium (T-3). To produce the diets, dry ingredients were mixed and then oil and water was added to the mixture. Nano-selenium, being a solution, was added to the diets in combination with water, whereas, Dl-all-rac-α-tocopherol acetate was added in combination with oil. The resultant dough was then passed through a mesh to form threads. The threads were air-dried and crushed into appropriate size before use.

Table 1: Formulation and proximate composition of experimental diets

Ingredients	%
Soybean meal	34
Fish meal	30
Corn flour	13
Wheat flour	12
Fish oil	7
Vitamin mix*	2
Mineral mix**	2
Proximate composition (%)	
Moisture	11.3
Crude protein	38.8
Lipid	8.4
Ash	10.5

* Vitamin mixture was manually provided according to feed requirements of the fish:(each kg^{-1} diet): vitamin A, 10,000 IU; vitamin D_3 2000 IU; vitamin K, 20 mg; vitamin B_1, 400 mg; vitamin B_2, 40 mg; vitamin B_6 20 mg; vitamin B_{12}, 0.04 mg; choline chloride, 1200 mg; folic acid, 10 mg; niacin, 200 mg.
** Mineral mixture (mg g^{-1}) : $CaCO_3$ 36; KH_2PO_4 502; $MgSO_4.7H_2O$ 162

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Fish and experimental conditions

Caspian kutum fingerlings, with the average weight of 250 ± 0.03 mg, were provided from Teleost Propagation Center of Sijaval, Bandar Torkman, Iran. The fish were allowed to acclimatize to experimental condition in two 500 l tanks for 2 weeks. During the acclimation period, the fish were fed with commercial feed (kutum starter feed, protein = 30%, fat = 10%; Mazandaran Feed Co.). After the acclimation, 480 fish were stocked into 12 tanks (100 l). Each of the aforementioned diets was offered to three tanks.

During the trial, fish were fed with their corresponding diet (12% body weight per day) thrice a day (08:00, 12:00 and 16:00). Wastes were daily siphoned from the tanks. 50% of the tanks' water content was daily replaced with fresh water. Water quality was periodically monitored. Temperature was 20.3-24.8, pH was 7.3-7.6 and oxygen was higher than 7.8 ppm. The fish were maintained under natural photoperiod during the experiment.

The diets and fish carcass composition was determined by standard methods suggested by (AOAC, 2002). Moisture content was determined with oven at 105 ºC until reaching to a constant weight. Ash was determined by combustion at 550 ºC for 12 h. Protein was determined by Kjeldahl method after acid digestion. Fat was determined by Soxhelt apparatus using ether petroleum for 6 h. The fish weight was recorded at the experiment initiation and fortnightly thereafter to adjust feed amount. The feed conversion ratio (FCR) and weight gain (WG%) were evaluated by the following formulas:

Weight gain (WG%) = $[(W_F-W_I)/W_I]\times100$

Feed conversion ratio (FCR) = [Total feed intake(g) / Total wet weight gain(g)]

To determine GPx and MDA, the fish whole body was first washed in sterile phosphate buffered saline (137mM NaCl, 2.7mM KCl, 4.3mM $Na_2HPO_4.7H_2O$, pH 7.3) and then 1 g sample was homogenized in 10 ml in same buffer. The homogenized was centrifuged (5000 g) and supernatant was separated for assay. The supernatant was stored at -70 ºC until analyses.

GPx was determined according to Bell et al. (1986) and expressed as U mg^{-1} protein. The supernatant total protein was determined according to Peterson (1977). Bovine serum albumin was used as standard. The sample MDA was determined by Kei (1978)method.

Statistics were performed using SPSS program version 16. One way ANOVA was used to determine the significant (P< 0 .05) difference among the groups.

Results

Growth and carcass composition

T-1 and T-3 groups had a significantly ($P < 0.05$) higher final weight and weight gain as well as lower FCR compared to the control and T-2 groups (Table 2). There was no significant ($P > 0.05$) difference in survival among the treatments (Table 2). There was no significant difference ($P > 0.05$) in carcass moisture, protein, lipid and ash content among the treatments (Table 3).

Table 2: Effect of vitamin E and nano-selenium of growth parameters of Caspian Kuttum.

Group/treatment	control	T-1	T-2	T-3
Initial weight (g)	0.27±0.02[a]	0.23±0.02[a]	0.26±0.03[a]	0.22±0.04[a]
Final weight (g)	0.86±0.02[b]	0.94±0.01[a]	0.85±0.02[b]	0.92±0.03[a]
Survival rate (%)	95±2.5[a]	96.6±1.4[a]	95[a]	97.5±2.5[a]
WG	212±21[b]	299±37[a]	223±26[b]	321±61[a]
FCR	4.2±0.2[b]	3.6±0.2[a]	4.3±0.2[b]	3.6±0.2[a]

Table 3: Whole-body proximate compositions (% live weight basis) of kutum fingerlings after feeding trial

Composition	Control	T-1	T-2	T-3
Moisture	73.1± 0.4[a]	73.6± 0.6[a]	73.5± 0.6[a]	73±0.9[a]
Crude protein	12.4± 0.3[a]	12.5± 0.2[a]	12.5± 0.2[a]	12.7± 0.1[a]
Crude lipid	11.2± 0.4[a]	10.9± 0.3[a]	11.4± 0.3[a]	11.1± 0.2[a]
Ash	2.4± 0.2[a]	2.4± 0.1[a]	2.3± 0.2[a]	2.5± 0.1[a]

Whole body GPx and MDA

GPx activity of the control and T-1 groups were similar and significantly ($P < 0.05$) lower than T-2 and T-3 groups (Figure 1). There was no significant difference ($P > 0.05$) in GPx activity between T-2 and T-3 groups (Figure 1). There was no significant difference ($P > 0.05$) in MDA content among the treatments (Figure 2).

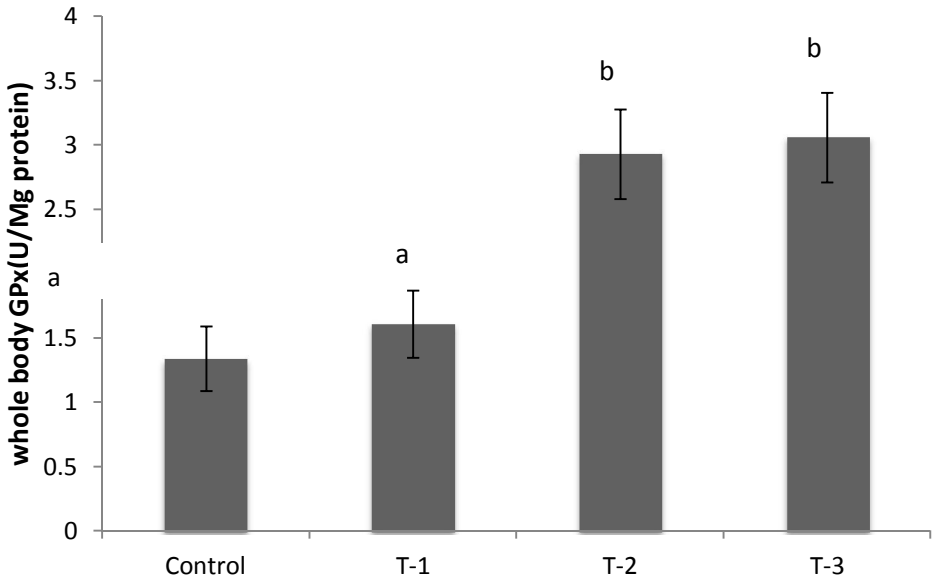

Figure 1: Effect of vitamin E and nano-selenium on whole body GPx activity of Caspian Kutum.

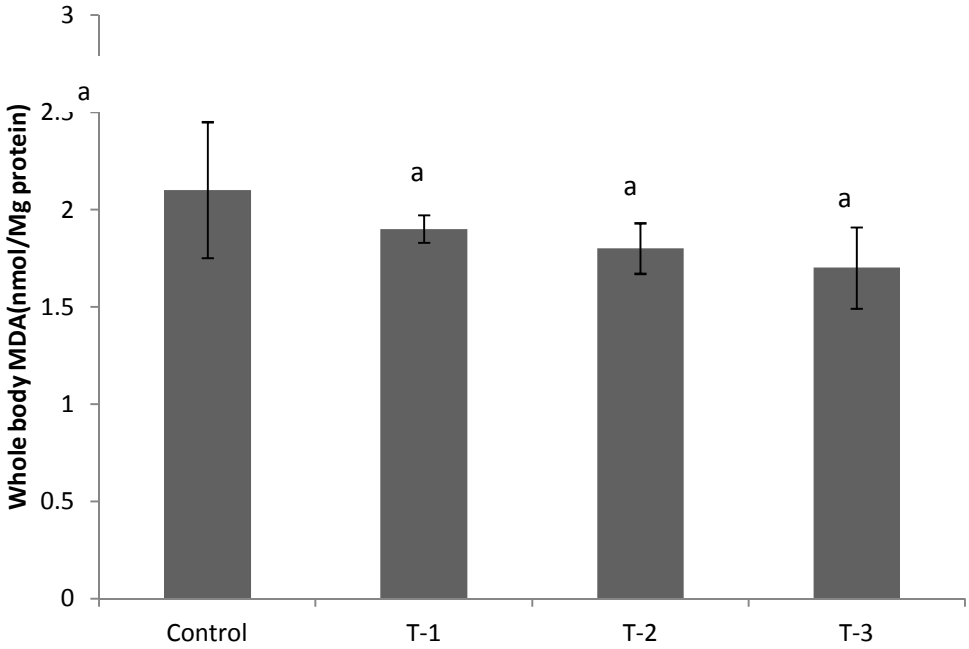

Figure 2: Effect of vitamin E and nano-selenium on whole body MDA content of Caspian Kutum

Discussion

The results of this study showed that the treatments containing vitamin E were significantly different from other treatments with respect to final weight, FCR and WG. Thus, it can be stated that vitamin E was the factor responsible for growth promotion, furthermore previous studies revealed the effect of vitamin E on growth promotion (Huang & Huang, 2004 ; Peng *et al*., 2009 ; Amlashi *et al*., 2011). According to the results obtained in the present study, it can be expressed that nano-selenium had no effect on growth, FCR and GW of Caspian Kutum.

This result is not in line with Zhou *et al.* (2009) (Zhou *et al*., 2009), which showed that nano-selenium improved growth and FCR in *Carassius auratus gibelio*. The reasons for this contradiction could be due to lower FCR in Caspian Kutum compared to *C. auratus gibelio*, the presence of selenium in the diets' ingredients, and presence of vitamin E or other antioxidants, such as vitamin C, in the diets covering the role of selenium. However, feeding Senegalese sole (Solea senegalensis) larvae on live food supplemented with organic selenium (Sel-Plex containing selenomethionin) had no beneficial effects on growth and survival (Ribeiro *et al*., 2012). Also, in another study on *Oncorhynchus mykiss*, growth was not affected by different levels of Se-yeast and selenite(Rider et al., 2009). which is in line with the present study.

Selenium is a structural component of glutathione peroxidase (Rotruck *et al*., 1973). In the present study, the whole body GPx activity was measured. The fish receiving the diet containing nano-selenium had higher GPx activity than the other fish, confirming the effect of nano-selenium on GPx activity. Previous studies on the effect of nano-selenium (Zhou *et al*., 2009) and inorganic or organic selenium (Wang *et al*., 2007b ; Ribeiro et al., 2012 ; Bell *et al*., 1985) on fish showed that plasma or liver GPx activity increased because of selenium supplementation. In the present study, GPx activity of the control and T-1 groups were approximately similar. Therefore, it can be stated that vitamin E, without selenium presence, cannot modulate GPx activity. Previous studies on rainbow trout (Bell *et al.,* 1985) and channel catfish (Wise *et al*., 1993) showed similar results.

MDA, produced as a result of lipid peroxidation, is measured as oxidative stress indicator (Esterbauer *et al*., 1991). In the present study, whole body MDA among the treatments was not significantly different, which could be due to the presence of other antioxidants such as vitamin C in the diets or lack of oxidative conditions during the experiment. Similar to the present study, Zhu *et al*. (2012) reported an increase in liver GPx activity and no change in MDA content in *Micropterus salmoides* as a result of feeding on selenium-supplemented diet.

Oxidative stress can be induced by a large variety of conditions, including nutritional imbalance, exposure to chemical and physical agents in the environment, strenuous physical activities, injury, and hereditary disorders (Chow, 1991), Caspian Sea is polluted with heavy metals, oil, industrial wastewater and etc, this pollutions can induced the oxidative stress and increased mortality at the time of entering the kutums larva into the sea; according to previous studies (Özkan-Yılmaz *et al*., 2013 ; Mittler, 2002) GPx by decreasing free radicals and finally MDA can increase the resistance of fish against the oxidative stress. On the other hand presence of vitamin E on diet can improve the final weight, that increase in final weight of fish can improve the resistance of fish against unsuitable conditions.

Overall, the results of the present study showed that nano-selenium could not affect growth performance without the presence of vitamin E. Likewise, vitamin E failed to increase GPx activity, without the presence of selenium. Therefore, it is concluded that both nano-selenium and vitamin E should be added to Caspian Kutum diet to increase growth performance and antioxidant enzyme, particularly GPx, activity. Such condition can lead to increase in resistance of Caspian Kutum fingerling while entering the sea and afterwards.

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2/12/2022