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Original Article

Effect of dietary vitamin E and nano-selenium and stocking density on growth and activity of AMP deaminase in Rainbow trout, *Oncorhynchus mykiss*

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Abstract: The present study aimed to evaluate the effects of vitamin E, selenium nanoparticles, and their combination on growth as well as the activity of the AMP deaminase in Rainbow trout kept in high density. A total of 1275 fish with an average weight of 42.6±2.3 g were acclimatized and distributed into 15 tanks at two stocking densities (low stocking density, 20 kg m^{-3}, and high stocking density, 80 kg m^{-3}). The fish were divided into five experimental groups according to stocking density and diet: Control (low stocking density; basal diet), Dense (high stocking density; basal diet), Vitamin E (high stocking density; 500 mg kg^{-1} vitamin E supplemented diet), Nano-selenium (high stocking density; 1 mg kg^{-1} nano-selenium supplemented diet) and Combination (high stocking density; 500 mg kg^{-1} vitamin E and 1 mg kg^{-1} nano-selenium supplemented diet). The best growth performance and health status of fish under high stocking density conditions were observed in fish-fed vitamin E-supplemented diets (Vitamin E and combination groups). The combination of nano-selenium with vitamin E improved the performance of the fish; nano-selenium had no significant effects on the growth performance of fish under high stocking density conditions. The results showed that the stocking density did not affect the activity of AMP deaminase, since its activity did not show a significant difference between experimental groups. In addition, AMP deaminase enzyme activity did not show significant differences among groups fed with vitamin E, nano-selenium and combination.

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Introduction

Due to the reduction of water resources and suitable land for aquaculture, as well as the increase in human population, which leads to increased demand, fisheries experts are looking for suitable and practical solutions to deal with this. One proposed solution is to increase production relative to the surface to increase stocking density. The strategy is to increase profitability in aquaculture to increase stocking density (Millan-Cubillo et al., 2016). Fish density is an important factor in aquaculture, so increasing stocking density may lead to adverse results in aquaculture and fish welfare by disturbing the physiological balance (Aksakal et al., 2011; Hasanalipour et al., 2013; Eagderi et al., 2016). Fish require a lot of energy to counteract metabolic regulation during high-density rearing, such as activating gluconeogenesis and glycolysis pathway enzymes (Vijayan et al., 1997). Increased energy demands in fish exposed to high-density stress are compensated by metabolic regulation (Vijayan et al., 1990; Wedemeyer, 1996).

AMP deaminase (EC 3.5.4.6) is isolated in different plant and animal tissues. This enzyme belongs to the group of hydrolytic enzymes that are involved in several physiological processes, such as the conversion of adenine nucleotide to hypoxanthine or guanine nucleotide, adenylate energy production, the metabolism of nitrogen amino acid derivatives, and reactions from the purine nucleotide cycle. This enzyme plays a key role in the purine nucleotide cycle, although its primary function in muscle may not be part of the main cycle (Mommsen, 1988). An association between anaerobic metabolism and AMP deaminase activity in white muscle has been reported.

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In vertebrates, the known oxidative capacity in white muscle is limited, and the production of ATP within it is highly dependent on anaerobic metabolism. Therefore, the amount of AMP deaminase is high, which is probably the role of this enzyme in stabilizing and regulating metabolism. This can be done by stabilizing the energy cost of adenylate production and the ratio of ATP to ADP and phosphorylation (Van Waarde, 1988).

Vitamin E and selenium (via glutathione peroxidase) are part of a multi-component antioxidant defense system. This system protects the cell against the harmful effects of reactive oxygen species and other free radicals (NRC, 1993). Decreased dietary vitamin E leads to decreased tissue vitamin E levels and generally higher activity of liver antioxidant enzymes and lipid peroxides in fish (Tocher et al., 2002). Previous studies have shown that vitamin E and selenium can enhance each other's effects (Fonseca et al., 2013) and affect many biological processes (El-Shenawy et al., 2015). Based on the background mentioned above, this study evaluated the effects of enriching diets with vitamin E and selenium nanoparticles and their combination on growth and activity of the AMP deaminase in Rainbow trout, *Oncorhynchus mykiss* kept at high stocking density.

Materials and Methods

Experimental design: The fish density was selected based on the results of McKenzie et al. (2012), which showed that densities between 75 and 100 kg/m^3 negatively affect the growth of Rainbow trout. A total of 1275 fish with an average weight of 42.6±2.3 g were acclimatized and distributed into 15 tanks at two stocking densities (low stocking density, 20 kg m^{-3}, and high stocking density, 80 kg m^{-3}). The fish were divided into five experimental groups according to stocking density and diet: Control (low stocking density; basal diet), Dense (high stocking density; basal diet), Vitamin E (high stocking density; 500 mg kg^{-1} vitamin E supplemented diet), Nano-selenium (high stocking density; 1 mg kg^{-1} nano-selenium supplemented diet) and Combination (high stocking density; 500 mg kg^{-1} vitamin E and 1 mg kg^{-1} nano-selenium supplemented diet). Each group had three replications. Fish were fed manually *ad libitum* three times a day (8:30, 12:00, and 15:30) for 70 days with experimental diets. To evaluate fish feeding, the amount of food consumed was recorded daily so that the food used for each tank was weighed before the feeding. After that, the difference was considered as food consumed daily in each repetition. Uneaten food was collected one hour after each feeding, dried at 70℃, and weighed to calculate the feed conversion ratio.

After 20 and 40 days of rearing to maintain the initial density (20 kg/m^3 in the control group and 80 kg/m^3 in the other groups), the fish in each tank were weighed, and the density was adjusted by randomly removing the required number of fish (Trenzado et al., 2009). Water with a flow of one L/min/ kg of fish was prepared during the study period. Water quality parameters were as follows: temperature 11±1.5°C, oxygen 7.9±0.6 mg /L, and pH 7.7±0.3. Water quality parameters were recorded every other day. No hazardous amount was recorded for ammonia concentration (less than 0.02 mg / l, AQUAPAA ammonia assay kit, Arvand Payam Aquatic Company, Iran). After 70 days, all the fish in each tank were weighed to evaluate the weight gain (WG), specific growth coefficient (SGR), and survival rate (Ahmadivand et al., 2013).

Biochemical analysis: For sampling, fish fasted for 24 h. They were anesthetized with 200 mg/L clove powder, and blood samples were captured from the caudal vein of fish (5 fish from each tank) by 2 ml heparinized syringes. The plasma was separated after centrifugation (Eppendorf, Germany) at 5000 × g for 10 min at 4°C. Serum biochemical parameters, including glucose, cholesterol, and triglyceride, were analyzed by a biochemical autoanalyzer (Mindray, China) using Pars Azmoon commercial kits (Karaj, Iran) based on the company protocol.

AMP deaminase assay: White skeletal muscles were washed in normal saline and homogenized in homogenate buffer containing 80 mM KCl, 2 mM EDTA, 54 mM KH_2PO_4, 35 mM K_2HPO_4, and pH 6.5 with a few crystals of PMSF (Sigma Aldrich, USA) to

Table 1. Effects of dietary vitamin E, Nano Selenium and their combination on growth performance and survival of rainbow trout under high rearing density for 70 days (means ± SD, n = 3).

Treatments / Growth parameters	Control	Crowded	Vit E	Nano Se	Mix
Final weight(g)	115.85±0.45[b]	105.31±0.95[c]	124.5±2.19[a]	109.35±2.45[c]	127.61±1.56[a]
Weight gain (g)	73.25±0.45[c]	62.71±0.95[c]	82.35±2.19[a]	66.75±2.45[c]	85.01±1.56[a]
Specific growth rate (%/day)	1.42±0.01[b]	1.29±0.01[c]	1.53±0.02[a]	1.34±0.03[c]	1.56±0.01[a]
Survival (%)	94.50±2.59[a]	95.78±1.26[a]	97.24±1.02[a]	96.86±0.66[a]	96.44±0.69[a]

Table 2. Effects of dietary vitamin E, Nano Selenium and their combination on serum metabolic responses of rainbow trout under high rearing density for 70 days (means ± SD, n = 3).

Treatments / Parameters	Control	Dense	Vit E	NanoSe	Mix
Cholesterol (mg/dL)	360.3±12.8[b]	423.1±3.4[a]	383.0±15.7[b]	367.0±9.6[b]	365.6±15.0[b]
Triglyceride (mg/dL)	321.7±8.1[c]	381.6±6.1[a]	338.7±3.1[b]	348.0±7.2[b]	340.0±8.0[b]
Glucose (mg/dL)	128.3±3.1	126.7±4.5	123.2±2.5	124.0±4.3	122.3±3.5

inhibit protease. The homogenate was centrifuged at 12,000 g for 10 min (Eppendorf, Germany), and the supernatant as a crude extract was removed. Enzyme purification was performed according to affinity chromatography with some modifications based on Smiley's (1967) method. Phosphocellulose (Sigma Aldrich Chemical Co. USA.), as resin, was kept for 24 h at 4°C for equilibration; then, it was added to the supernatant, and the suspension was stirred for 2 h on ice. This made the enzyme to attach to phosphocellulose. Again, the slurry was centrifuged at 12000 g for 10 min, and the supernatant was discarded. To delete the remaining proteins, the pellet was washed with homogenate buffer. Finally, the slurry was poured into a column and washed with a progressive concentration of KCl (Sigma Aldrich, USA), including 0.4, 1, 1.5, and 2 M (pH= 7), facilitating the binding between the enzyme and AMP. Finally, AMP deaminase was purified with 0.4 M KCl. AMP deaminase activity was measured using a Microplate reader (Power Wave Xs2, Biotek) at 285 nm for 5 minutes. The protein content was analyzed using the Bradford method using bovine serum albumin (Merck, Germany) as the standard (Bradford, 1976).

Statistical analysis: Data are presented as means±standard error (SE). The data's normality and homogeneity of variances were checked with Shapiro-Wilk and Levene tests, respectively. One-way ANOVA was used to analyze the data, and Tukey's post hoc was used to compare the means of groups. Statistical analysis was conducted by SPSS software (version 25), and the acceptable significance value in statistical tests was $P<0.05$.

Results

The effects of dietary vitamin E and nano-selenium on growth indices (final weight, weight gain, SGR) and survival of Rainbow trout are shown in Table 1. According to the results obtained after 70 days of rearing, growth factors, including final weight, weight gain, and SGR, were lower in fish kept in high stocking density and fed with basal diet (dense group) compared to fish reared in low stocking density and fed basal diet ($P^{<}0.05$). However, in higher stocking density treatments, growth performance improved in fish-fed experimental diets. In higher stocking density groups, the SGR improved in groups fed experimental diets, so the highest SGR was recorded in the vitamin E and Vit E±nano selenium treatments, and the lowest SGR was recorded in the higher stocking density treatment. After 70 days of rearing, fish survival did not show significant differences between the experimental groups, and the results showed that density and feeding did not affect fish survival (Table 1).

Blood cholesterol increased in treatment with higher stocking density compared to the control, but

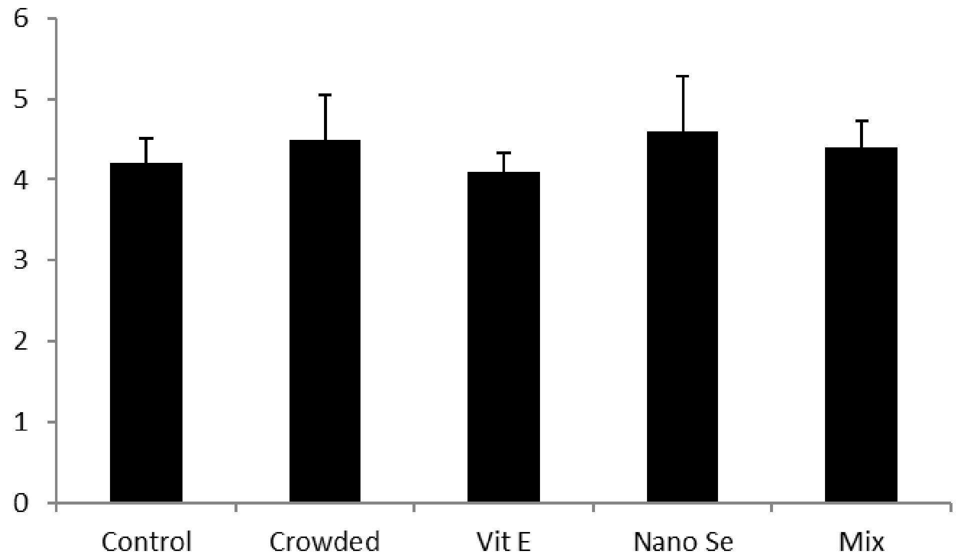

Figure 1. Effects of dietary vitamin E, Nano Selenium and their combination on AMP deaminase activity (mU/mg protein) of rainbow trout under high rearing density for 70 days

its value decreased significantly in fish-fed experimental diets ($P^{<}0.05$, Table 2). Triglyceride was affected by density as its value was higher in the higher stocking density group than the control ($P^{<}0.05$). Its value in fish-fed experimental diets decreased compared to the dense group but was higher than in the control ($P^{<}0.05$). Stocking density and feeding do not affect *O. mykiss* blood glucose in this study.

The results showed that density did not affect the activity of AMP deaminase, as its activity did not show a significant difference in the experimental groups. Also, in the groups fed vitamin E, nano-selenium, and both, the activity of the AMP deaminase enzyme did not show a significant change ($P>0.05$, Fig. 1).

Discussions

This study showed that high density (dense group) reduces the final weight, weight gain, and specific growth rate compared to the control group. Similar results have been reported in Rainbow trout kept in high stocking density (Ellis et al., 2002; Suárez et al., 2015). In general, high stocking density conditions reduce feeding in fish (Ellis et al., 2002), so low weight gain could be associated with lower feeding, as previously reported in this species (Küçükbay et al., 2009; Suárez et al., 2015). On the other hand, energy consumption increases in high stocking density conditions, depleting the body's reserves and thus leading to reduced growth (Vijayan et al., 1990).

In the present study, at high stocking density conditions, the best growth performance was observed in fish-fed diets supplemented with vitamin E (vitamin E and combination groups). Vitamin E is an essential nutrient for physiological functions in fish (Hamre, 2011). These results are consistent with previous studies (Trenzado et al., 2007; Naderi et al., 2019) that showed that supplementing the basal diet with vitamin E could improve the growth performance of Rainbow trout under dense conditions. Chronic stress suppresses the immune system (Yin et al., 1995; Ndong et al., 2007), causing physiological defects, increased susceptibility to infection, and even death (Ojolick et al., 1995). Therefore, reducing the harmful effects of stress is one of the important aims of aquaculture research. Proper nutrition is essential for achieving the desired growth rate and maintaining fish health. In the last two decades, efforts have been made to understand the relationship between nutrition, immune response, and disease resistance in farmed fish, and a variety of nutrients have been used to reduce stress or enhance fish immunity (Ebrahimi et al., 2015; Arani et al., 2021; Maniat et al., 2023).

While the combination of nano selenium and vitamin E improved fish growth performance, supplementing the diet with nano selenium had no significant effects on the reduced growth performance of Rainbow trout at high stocking densities. These indicate that in conditions of high stocking density, fish selenium demands may not be met through a diet supplemented with 1 mg of nano selenium per kg. These results differ from the study of Küçükbay et al. (2009), which reported the positive effects of dietary supplementation with selenium on this species in dense conditions. Therefore, it seems that the improvement in the growth performance of fish in the combination group is only due to vitamin E in the diet.

In the present work, blood cholesterol and triglyceride increased in treatment with higher stocking density. Although the exact mechanism of the effect of antioxidants on lipids in fish is not clear but it seems that antioxidants (for example, selenium), by increasing the amount of Glutathione peroxidases and HDL, decrease the formation of platelet bodies and fatty acid oxidation (Dhingra and Bansal, 2006). On the other hand, a decrease in selenium causes the production of oxidized LDL, which leads to the rapid destruction of glutathione. This situation is caused by an increase in the function of γ-glutamylcysteine synthetase (GCS) that restricts the Glutathione synthesizing enzyme (Shen and Sevanian, 2001). In mice, the relation between Selenium and Vitamin E and expression of LDL receptors had been shown as feeding mice with diets containing low amounts of selenium resulted in decreased LDL receptor expression following increased blood cholesterol (Dhingra and Bansal, 2006). An increase in blood cholesterol can be caused by an increase in the need for antioxidants in stressful situations. Sarma et al. (2009) found that the need for vitamin C increases under stress conditions. Contrary to our findings, Suárez et al. (2015) reported lower cholesterol, LDL, HDL, and triglyceride levels in Rainbow trout reared at high stocking density. Harsij et al. (2020) reported increased blood triglycerides and cholesterol in Rainbow trout kept at high density and fed by antioxidants. They concluded that increased triglyceride levels in fish-fed diets supplemented with antioxidants could be related to their possible roles in providing energy during stress. In the current study, crowding or experimental diets did not affect blood glucose. It has been shown that chronic stress does not affect blood glucose. This condition is caused by a new homeostasis that maintains blood glucose levels within the normal range (Naderi et al., 2017).

In the current study, the effects of high stocking density and supplementation of diets with vitamin E and nano selenium diets on fish muscle AMP deeaminase activity were evaluated, and AMP deaminase enzyme activity was not significantly different between different experimental groups. AMP deaminase catalyzes the reversible reaction of AMP to IMP and is the main source of ammonia production in fish muscle (Lowenstin, 1972; Lushchak et al., 1998). When ATP levels decrease (such as hypoxia), most AMP deaminase activity is usually seen in skeletal muscle (Kaletha et al. 1991; Raffin et al. 1993; Lushchak et al. 1998), which is not in accordance with our findings, which showed that the enzyme activity did not change under conditions of stocking density stress.e The activity of AMP deaminase increases due to the consumption of phosphagens, which is caused by different factors, such as stress. Because blood glucose did not decrease during the study, but on the other hand, blood TG values increased, the body may supply the energy demands through lipolysis and does not need to consume phosphagens, so no difference in the activity of AMP deaminase was recorded between the experimental groups.

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