The effects of different dietary levels of organic and inorganic selenium on some growth performance and proximate composition of juvenile rainbow trout (*Onchorhyncus mykiss*)

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Abstract

Selenium, a trace mineral complement is used as cofactor of antioxidant enzymes that protects fishes against environmental stress agents and enhances performance in fishes. In this study the different levels of organic and inorganic source of selenium were included in juvenile rainbow trout diet to evaluate feed conversion ratio (FCR), specific growth rate (SGR), weight gain percent (WG), condition factor (CF), survival rate (SR) and proximate analysis of the fillet during 60 days of the experiment. The fishes were allotted to 9 treatment groups including, Tc the fishes were fed diet without any selenium, control group, To1, To2 ,To3 and To4 the fishes were fed different dosages of inorganic selenium and Ti1, Ti2, Ti3 and Ti4 those were fed different dose of inorganic Se in their diet. Results showed that To4, showed the highest level of WG from 50±2.8 to 168.54± 25.56 g in comparison to Tc (134.38± 27.26g) (p<0.05). Average initial total length of fishes (19± 1.12 centimeters) increased to 21.1± 1.12 cm in Tc and 22.46± 1.25 cm in To4 significantly (p<0.05). Among all treatments, FCR, SGR, WG, CF and SR were improved in To3 group. Also carcass protein increased in To4 (32.58±1.22%) on the contrary of Ti4 (22.43±1.51%) (p<0.05). As a general conclusion, dietary incorporation of organic selenium at 0.45 mg/kg showed satisfactory results in some growth parameters and was a useful supplement in salmonid fish diets.

Keywords: Organic selenium, Inorganic selenium, Rainbow trout, Proximate composition, Growth

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Introduction

In our country a viable way to increase production of fish and other aquatic organisms is the widely developed intensive farming systems, technique confirmed with high performances in Western Europe (Bud *et al*., 2007). In these systems the role of additional food is crucial for achieving good performance economically (Esmaeili et al., 2014), quantitatively and qualitatively, in order to solve one of the biggest problems in the fish diet: anti-oxidation. A solution is to use organic selenium (Se). In nature selenium can be found in two forms: inorganic and organic (Surai, 2006) and as a part of multiple components. It is generally accepted that selenium in biological systems is part of the amino constituent of proteins: cysteine, methionine and derivatives, seleno-cystein (seleno-methionine) (Jacob *et al*., 2003). Organic selenium which is now the main form of selenium supplementation for livestock in many countries and had been tested in fishes (Alina *et al*., 2010), sheep (Davis *et al*., 2008), goats (Kachuee *et al*., 2013), pigs (Horky *et al*., 2013), horse (Jancikova *et al*., 2013) or rats (Sochor *et al*., 2012), and its effect on selenium in blood serum. The objective of this study was to compare the effects of supplemented organic and inorganic selenium to juvenile rainbow trout on measures of selenium status in the rainbow trout muscle. When juvenile trout (*Salmo salar*) was given seleno-methionine, its compounds were present in muscles and all over the body, unlike selenite which is treated almost entirely in the liver (Lorentzen *et al*., 1994) Selenomethionine is assimilated more easily in the body because it is absorbed as amino acid, and methionine is similar (Schrauzer, 2000). Some is used immediately for synthesis of seleno-methionine and another part of organic selenium is incorporated into newly synthesized proteins. Dissimilar, the selenite is passively absorbed in the intestine as mineral used for the synthesis of seleno-protein and the remainder is excreted in faeces and urine (Surai, 2004) selenium stored during the process of protein turnover is recovered from tissues. A protein is of good quality when its chain is complete: incorporates in its constitution the nine amino acids. These nine amino acids are called essential because they cannot be synthesized. But they are of insufficient quantities in the fish body. Unlike protein amino acids cannot be stored in the fish body, and when there is a need for a certain amino acid first this is consumed from blood, but after that proteins are destroyed in order to deliver amino acids (Guillamune, 2007). Consequently, amino acids supplementation is needed. In the present research methionine supplementation was made through the management of organic selenium, improving the fodder with Sel-Plex. Selenium binds methionine and fish treats the body as amino acid resulting in a better assimilation. The most

important peroxidases (important enzymes for living organisms) also depend on selenium (Sibonani, 2003).Therefore peroxidases have to be present in fish food in an amount of 0.1-0.5 mg/g food, as it is an extremely important enzyme (Lenzi *et al*., 2000; Sen and Paker, 2000) found in all tissues where oxidative processes occur. Peroxidase it is also considered the ''emergency enzyme'' responsible for preventing oxidative stress (Pierce *et al*., 2004). The elimination of oxidative stress trough the administration of organic selenium was also among the concerns of this research: the organic selenium will keep the balance of oxidative stress. Symptoms in case of selenium deficiency in fish are multiple: reduced growth, anemia, muscular dystrophy and even mortality (Bell and Cowey, 1989). Dietary intake varies selenium in fish flesh ranging between 1.32 and 4.6μg per 100μg. Selenium may affect meat quality due to degradation of lipids (Surai, 2006). The chemical composition of fish meat varies according to age, season, type of food, body region, Administration of 0.25 ppm selenium in the form of Sel-Plex, to Atlantic salmon (*Salmo salar*) may lower the amount of fat to 6.93%, (De-lyons, 1998) Regardless of the accuracy of recommendations, it appears that the RDI (Recommended Daily Intake) is greater than the optimum levels of intake. This means deficiency of selenium. Approximately 40 diseases and physiological states have been associated with considerable deficiencies from country to country, but the overall intake of Selenium is low. This led to establishing a standard of reference for selenium daily intake in different countries (Surai, 2007). A study was conducted on 11 species of fish in Turkey and the amount of selenium, among which: arthritis, cancer, cardiovascular disease, cataracts, cystic fibrosis, diabetes, (Reilly, 1998). Thus increasing demand for higher quality food provides an excellent opportunity to produce Selenium enriched functional food.

Material and method

The experiment was carried out for a period of 60 days at the Fisheries Research Station, Khojir, Iran. A total of 1080 juvenile rainbow trout (50 ± 2.8 g) were randomly divided into 9 treatment groups with three replicates of 30 fish per replicate. Treatments were Tc: 0 mg/kg selenium in basal diet (BD); To1: 0.15 mg/kg organic selenium; To2: 0.3 mg/kg organic selenium; To3: 0.45 mg/kg organic selenium and To4; 0.6 mg/kg organic selenium also; Ti1: 0.15 mg/kg inorganic selenium; Ti2: 0.3 mg/kg inorganic selenium; Ti3: 0.45 mg/kg inorganic selenium and Ti4: 0.6 mg/kg inorganic selenium in similar basic diet. The juveniles were distributed into 800 liter concrete ponds at a stocking rate of 30 fish per pond. During the experiment, the water temperature was 16± 1°C, dissolved oxygen was 8.3±0.81 mg/L and pH 6.3± 0.15. Basal

diet was prepared according to National Research Council recommendation for rainbow trout (Table 1).

Table 1: Ingredients of Basal diet contain and Selenium content.

Ingredients	Percent
Fish Meal	34.9
Soybean Meal	20.5
Wheat	20
Canola oil	9.9
Corn	0.8
DCP	2.05
Carboxy Methyl Cellulose	2
$Caco_3$	1.89
Mineral premix	0.25
Vitamin premix	0.4
Vitamin C	0.08
Colin	0.15
Digestible Energy	3.8 Kcal/g
Protein	38
Fish Meal Protein	25
Calcium	2
Phosphorous	1.2
Arg	2.2
Lys	2.6
Leu	2.8
Tre	1.52
Metionin+Cystein	1.3

To prepare experimental diets, at first, all ingredients were pulverized and then mixed to homogenize. Then they were mixed again with some 60°C water for 30 min. At the end of the trial, dry pellets, 6 mm in diameter, were made by pellet-making machine (Mitsubishi Mr-720, Japan). The feeding ratio was 2.5% Kg^{-1} of the fish body weight at 16°C water temperature.

To investigate the growth and survival rates of the fishes and also to determine feeding size, biometry parameters were carried out twice per a month during the trial. In each biometry, all of the fish were captured, anesthetized by clove powder (220 ppm) and then some parameters including average weight and total length, specific growth rate (SGR), weight gain (WG), condition factor (CF), body weight gain (BWG), daily growth rate (DGR), feed conversion ratio (FCR) and survival rate (SR) were calculated as follows:

$$SGR = \frac{Ln\ BWF - lN\ BWI}{T} \times 100$$

(Helland *et al.*, 1996)

Where BWF refers to the final weight of rainbow trout, and BWI refer to the initial weight of rainbow trout.

$WG = BWF - BWI$ (Ghosh *et al*., 2003)

BWG= BWF- BWI / BWI × 100

$$BWG = \frac{BWF-BWI}{BWI} \times 100$$

(Ghosh *et al*., 2003)

$CF = \frac{BW}{TL^3} \times 100$ (Ghosh *et al*., 2003)

Where BW refers to the weight of rainbow trout, and TL refers to the total length of rainbow trout.

$FCR = \frac{F}{WF-Wi}$ (Helland *et al*., 1996)

Where F refers to the value of consumed food

$$SR = \frac{total\ number\ of\ alive\ fish}{total\ number\ of\ fish} \times 100$$

(Helland *et al*., 1996)

$DGR = \frac{BWF-BWI}{T}$ (Helland *et al*., 1996)

Where BWF refers to the final weight of rainbow trout, and BWI refers to the initial weight of rainbow trout.

Body composition assays

After 60 days of the experiment, 4 samples from each treatment were selected randomly for analysis of body composition and total selenium. Body chemical composition parameters were carried out according to procedures described by AOAC (1995). Total crude protein was performed by Kjeldahls method with the multiplier 6.25; lipid content by Soxhlet method, with petroleum ether as solvent for 8h; total ash content was measured by the mineralization of sample at temperature of 550°C for 8 hours (Linn Electro-thermal Furnace, Germany). All data were replicated 4 times and subjected to one way analysis of variance (ANOVA) and means were separated by Duncan's tests at 95% level by using SPSS software version 20.

Results

There were significant differences between experimental treatments and control group in terms of weight (Fig. 1), Length (Fig. 2), DGR (Fig. 3), BWG (Fig. 6), and FCR (Fig. 8). Accordingly, weight and total length average during 2 months were statistically higher in To4 with significant difference (p<0.05). In this regard, the values of SR, CF were statistically higher in organic treatments. The FCR values were statistically different between To3, 4 Ti2 with control group (p<0.05) (Fig. 8). There is no significant differences observed in carcass protein content, lipid, ash and moisture between treatments and control group (Table 2) (p>0.05). There were significant differences between selenium content (Fig. 9) of To1, 2, 3 and control (p<0.05). It should be noted that the allowance of selenium is 3.5 mg/kg meat (FDA, 2003).

Discussion

The present research confirms some of the positive effects of organic selenium compared with inorganic selenium in trout. Moreover, there is not enough information about adding Selenium in trout species as a growth stimulator. The research is valuable from both technological and economical point of view (Bud, 2007), because it allows an inside view on the influence of organic selenium on the fish body (Surai, 2006) helped to test Sel-Plex on carp; and (Lemly, 2008) demonstrates that organic selenium enriched fodder leads to higher fish production and economical effectiveness (functional food, a food conversion ratio lower, increasing body mass in less time and better quality at lower costs). The results showed that the growth performances of fish fed with selenium is higher than that in the control group. Because selenium is a structural component of the physiological antioxidant properties (Talas *et al.*, 2007; Ates *et al.*, 2008; Orun *et al.*, 2011) and it acts as a growth promoter, it caused extensive tissue lipid peroxidation in fish (Berntssen *et al.*, 2003).

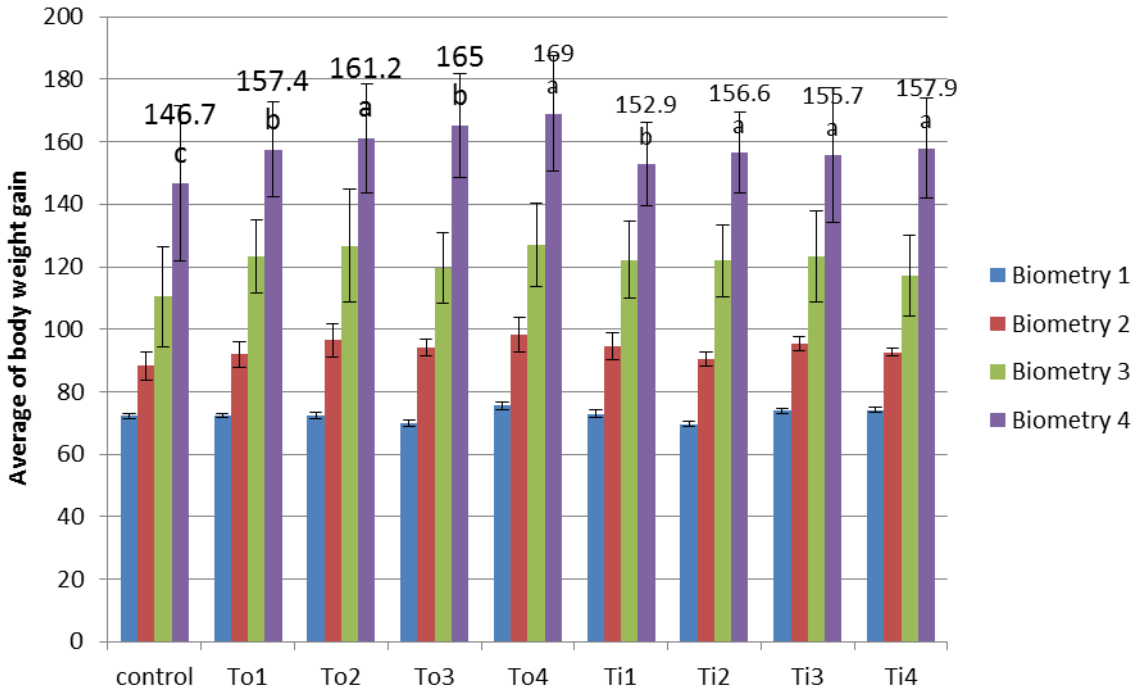

Figure 1: Comparison of weight values in treatment groups fed by various levels of organic and inorganic selenium during 2 months. Bars (Mean±SD) with different letters are significantly different (p<0.05).

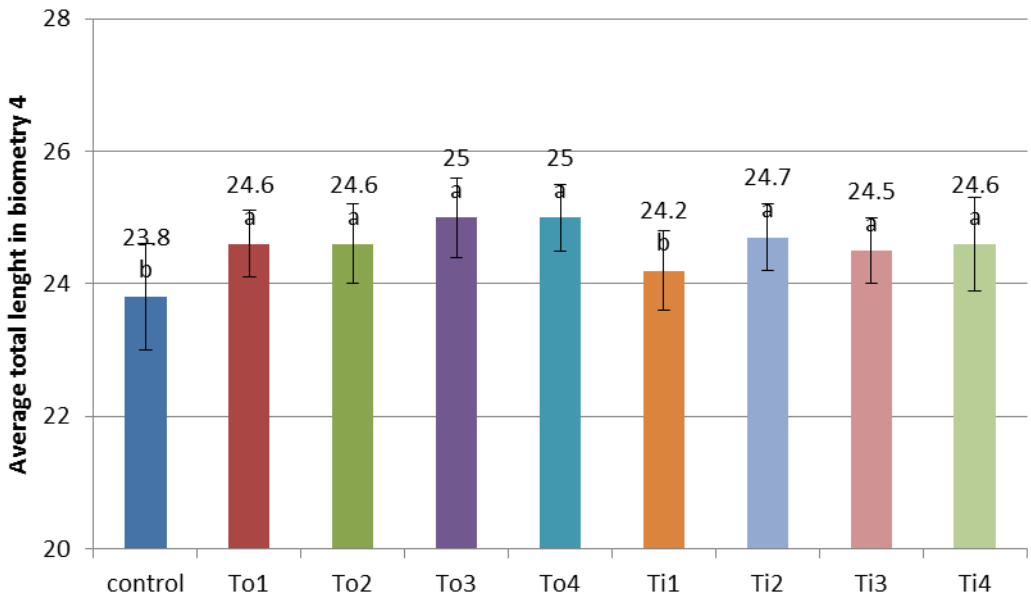

Figure 2: Comparison of total length values between experimental fish groups fed by various levels of organic and inorganic selenium during 2 months. Bars (Mean±SD) with different letters are significantly different (p<0.05).

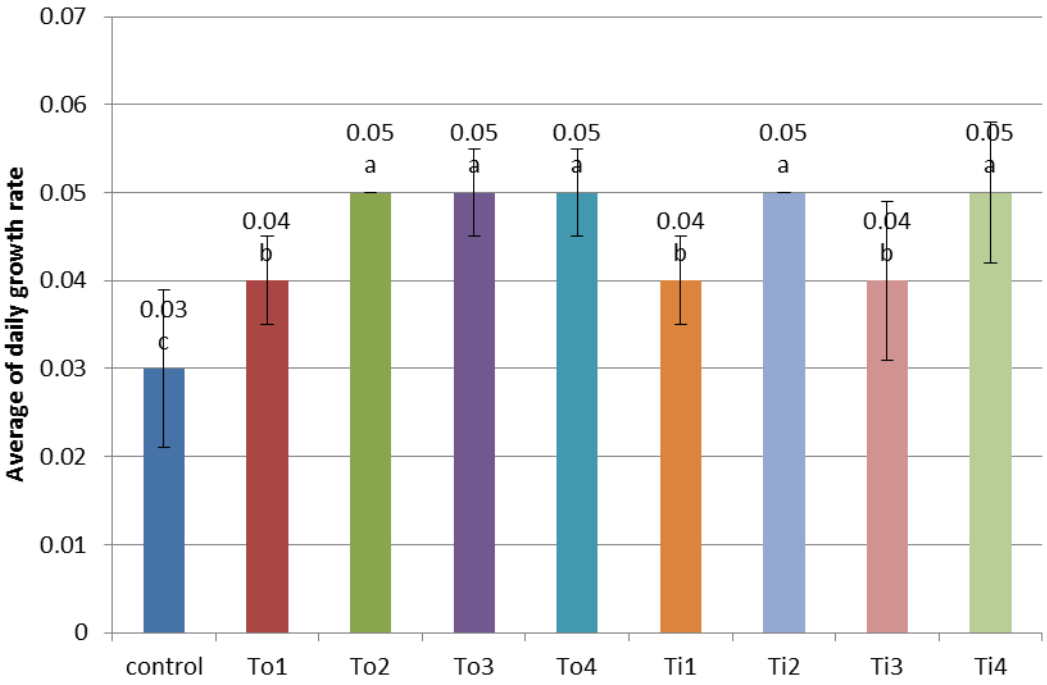

Figure 3: Comparison of DGR values between experimental fish groups fed by various levels of organic and inorganic selenium. Bars (Mean ± SD) with different letters are significantly different (p<0.05).

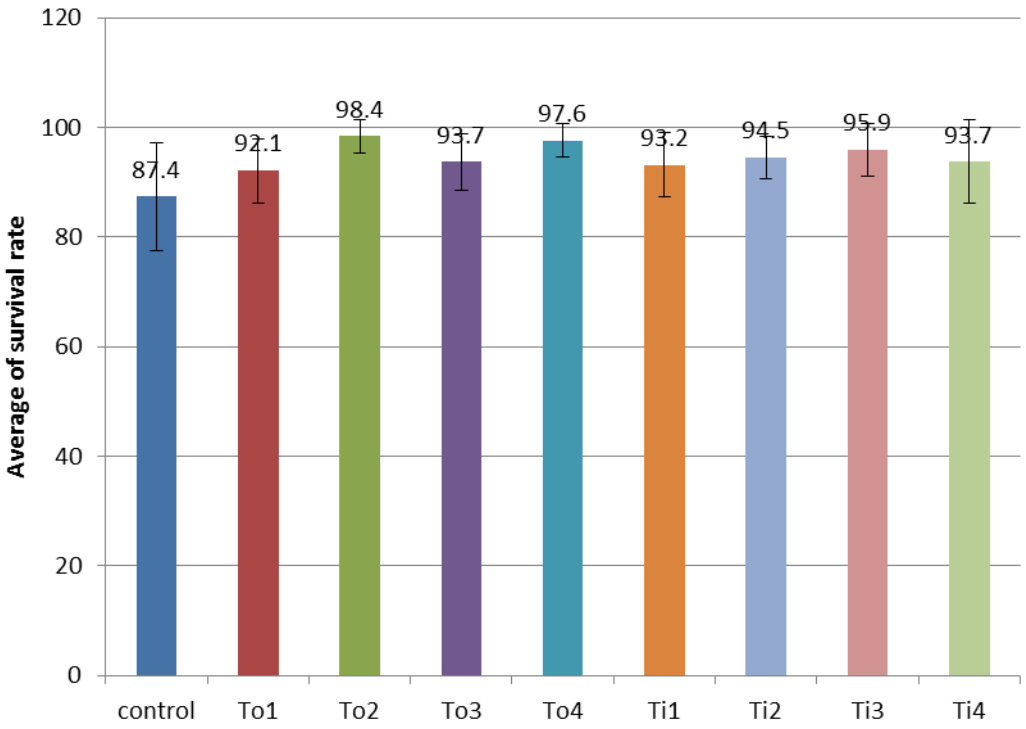

Figure 4: Comparison of SR values between experimental fish groups fed by various levels of organic and inorganic selenium. Bars (Mean ± SD) with different letters are significantly different (p<0.05).

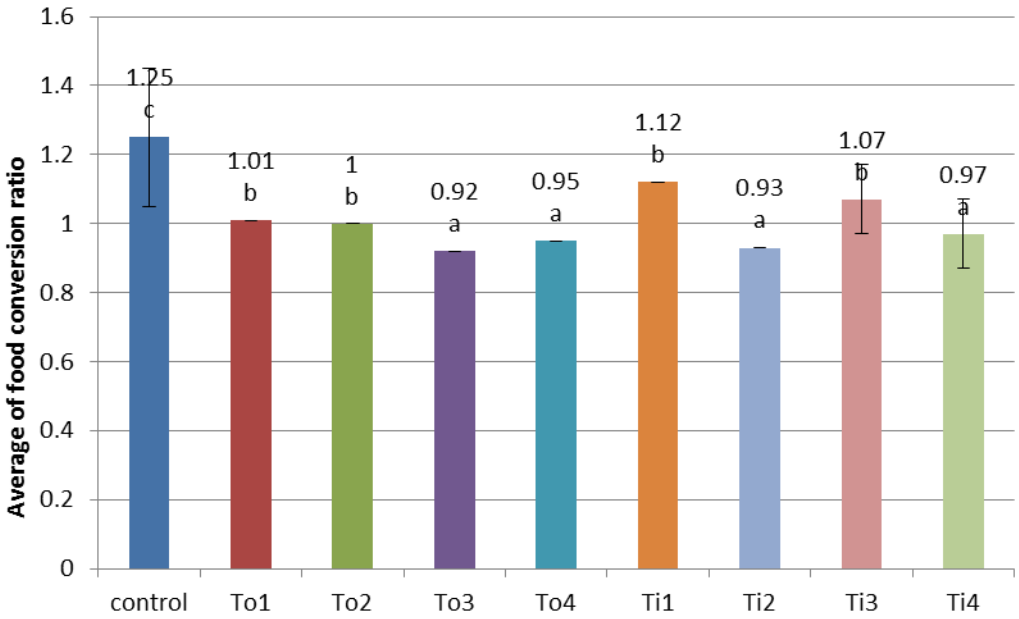

Figure 5: Comparison of FCR values between experimental fish groups fed by various levels of organic and inorganic selenium. Bars (Mean ± SD) with different letters are significantly different (p<0.05).

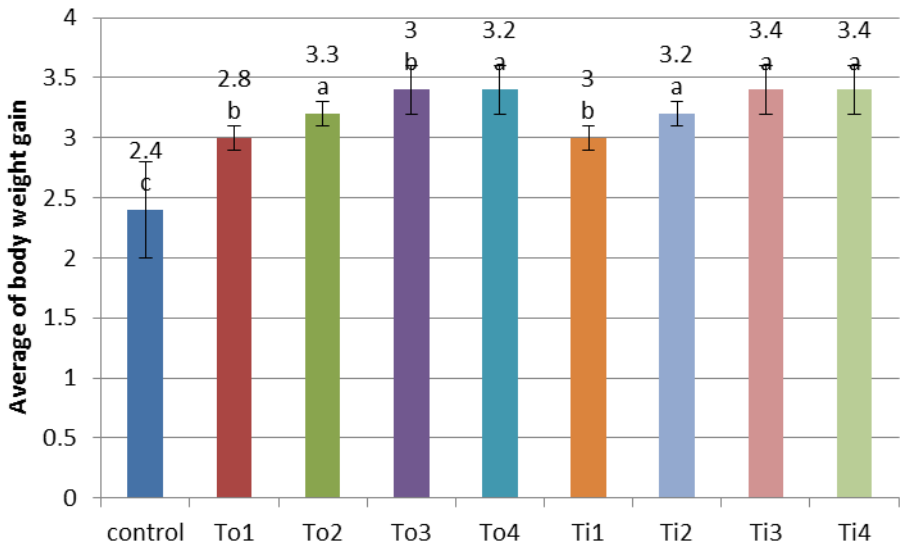

Figure 6: Comparison of BWG values between experimental fish groups fed by various levels of organic and inorganic selenium. Bars (Mean ± SD) with different letters are significantly different (p<0.05).

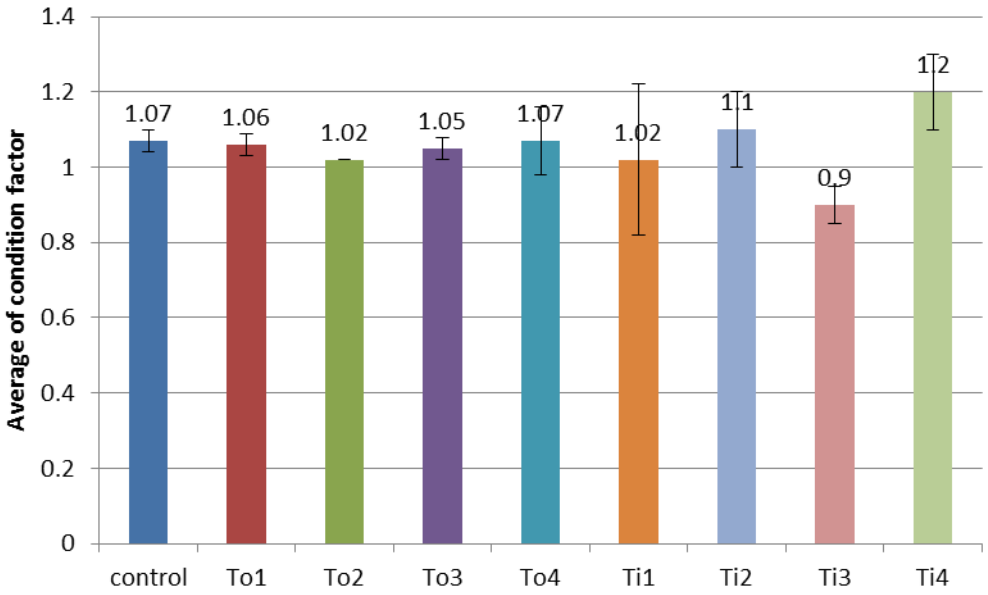

Figure 7: Comparison of CF values between experimental fish groups fed by various levels of organic and inorganic selenium. Bars (Mean ± SD) with different letters are significantly different (p<0.05).

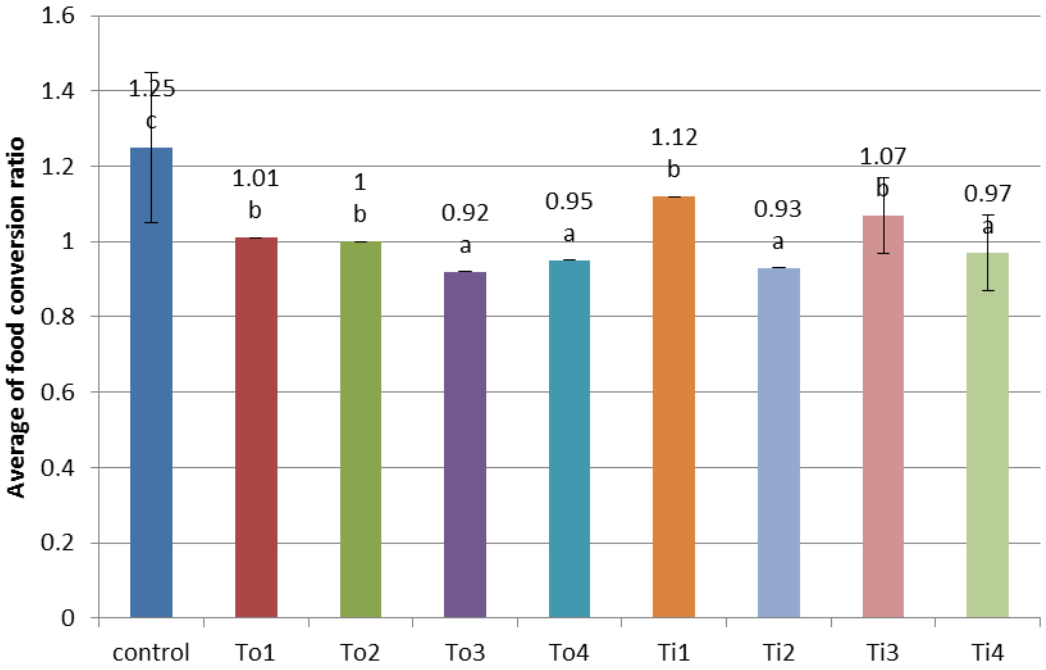

Figure 8: Comparison of SGR values between experimental fish groups fed by various levels of organic and inorganic selenium. Bars (Mean ± SD) with different letters are significantly different (p<0.05).

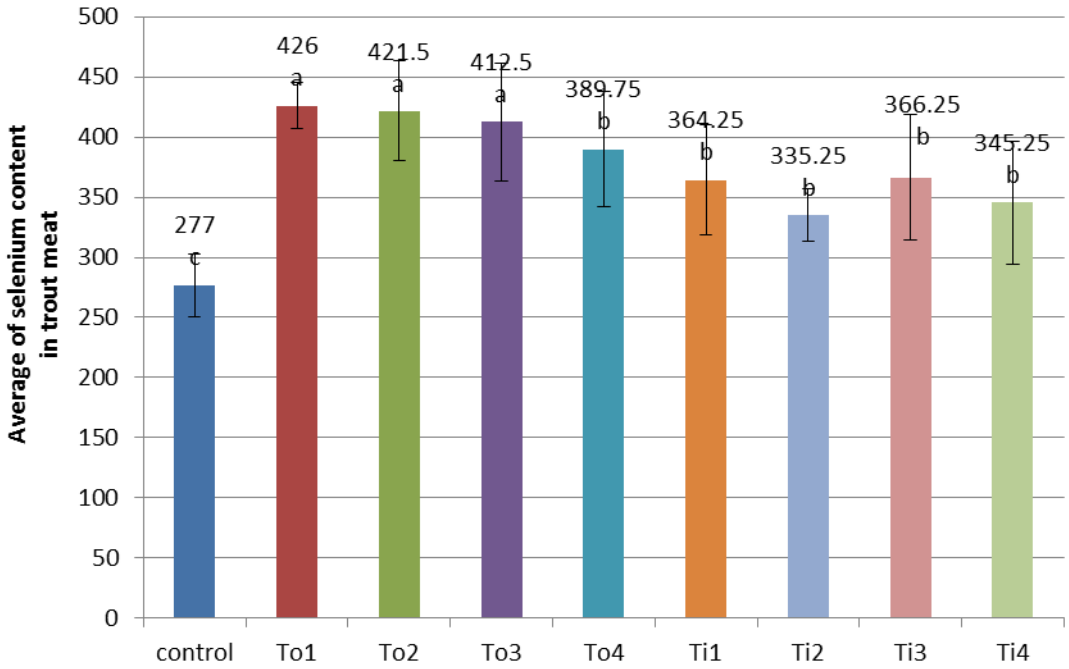

Figure 9: Comparison of Selenium content between experimental fish groups fed by various levels of organic and inorganic selenium. Bars (Mean±SD) with different letters are significantly different (p<0.05).

Table 2: Proximate composition of rainbow trout at the end of experiment. Means within each column with different letters differ significantly (p< 0.05).

	Lipid	Moisture	Protein	Ash
Tc	6.98±0.55^{a}	71.28±1.48^{a}	22.43±1.51^{b}	1.40±0.10^{a}
To1	6.24±0.96^{a}	70.19±1.55^{a}	22.47±1.08^{b}	1.34±0.09^{a}
To2	6.46±0.85^{a}	72.01±0.94^{a}	22.72±0.29^{b}	1.36±0.05^{a}
To3	5.91±0.51^{a}	69.86±1.36^{a}	32.58±0.90^{a}	1.40±0.42^{a}
To4	6.36±0.42^{a}	70.48±1.32^{a}	29.49±1.22^{a}	1.37±0.09^{a}
Ti1	6.33±0.49^{a}	71.52±0.96^{a}	20.99±0.52^{b}	1.38±0.03^{a}
Ti2	6.36±0.85^{a}	71.55±0.90^{a}	20.83±1.33^{b}	1.35±0.07^{a}
Ti3	6.55±0.93^{a}	71.27±0.65^{a}	21.93±0.42^{b}	1.40±0.12^{a}
Ti4	6.30±0.80^{a}	71.45±0.56^{a}	21.40±1.02^{b}	1.39±0.02^{a}

Similar results were obtained by Alina *et al* (2010) that indicate Protein (18.72±0.12), Fat (7.71±0.17), selenium (174.6±12.25), length (43±0.9), Weight (1510 g), FCR (1.79) in common carp. Selenium is a component of several seleno-proteins with essential biological functions (Van Cauwenbergh *et al*., 2004). This element acts as a cofactor of the GPx family of enzymes which protect against oxidative stress. Specifically, Se-dependent GPx enzyme recycles glutathione, reducing lipid peroxidation by catalyzing the reduction of peroxides, including hydrogen peroxide. In general all these enzymes at their reduced state catalyze the breakdown of lipid hydroperoxides

and hydrogen peroxides in human cells (NavarroAlarcon and López-Martínez, 2000; Van Cauwenbergh *et al*., 2004; Hartikainen, 2005; Navarro-Alarcon *et al*., 2005). From all these associated enzymes, GPx and selenoprotein P are also involved in the regulation of the inflammatory response (Van Cauwenbergh *et al*., 2004). Moreover, the antioxidative function of selenium can help to ameliorate the damage induced by the ultraviolet-β radiation in humans. In farm animals diseases associated with selenium deficiency have been an important problem. White muscle disease is a nutritional muscular dystrophy that is the most common selenium deficiency disease (Peter and Costa, 1992). Usually actively growing animals suffer from this disease, showing symptoms weakness, problems with feeding, and cardiac implications that very often produce death. On the other hand, subclinical deficiency levels are associated with poor growth, impairment of animal production, and decrease in immune efficiency (Peter and Costa, 1992; Chahardeh Baladehi and Hedayati, 2017). On the other hand, the selenoprotein P is a plasma protein whose source is the liver and kidney. This protein constitutes the main plasma selenium carrier carrying more than 60% of plasma selenium. Besides, it is known that the protein levels depend on the body's selenium status, such that it has been used as a biomarker of body selenium content. Particularly, the selenoprotein P acts as an extra cellular antioxidant associated with the vascular endothelium which diminishes the peroxinitrile level that represents reactive nitrogen species (Orun *et al.,* 2005; Orun *et al.,* 2008; Li *et al*., 2007; Ates *et al*., 2008).

Thus increasing demand from consumers for higher quality foods provides an excellent opportunity to produce functional foods rich in selenium. Functional foods will give the most benefits because they can be adapted to the needs and lifestyle of each country. Modernization and intensification in fisheries production lead to the need to focused attention to use with maximum efficiency the production capacity, to adopt new technologies for mining, scientific organization of production and labor in order to increase their while lowering cost price.

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