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RESEARCH PAPER

Effects of α-Tocopherol (vitamin E) and Ascorbic Acid (Vitamin C) and Their Combination on Growth, Survival and Some Haematological and Immunological Parameters of Caspian Brown Trout, Salmo Trutta Caspius juveniles

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Abstract

We examined the effects of different dietary levels of ascorbic acid (vitamin C) and α- tocopherol (vitamin E) and their combinations on growth, survival; and some haematological and immunological parameters of Caspian brown trout, *Salmo trutta caspius* juveniles. The experiment was designed as 15 experimental treatments and one control group. 480 fish (35±5.5 g) were fed by experimental diets containing different levels of Vit C and E and control diet separately for a period of 2 months. According to the results, Vit C and E improved survival and growth parameters including specific growth rate (SGR), percent weight gain (WG) and biomass. The highest values of these parameters obtained in T8 (Vit E (30 mg.kg^{-1}) + Vit C (300 mg.kg^{-1}) and T9 (Vit E (40 mg.kg^{-1}) + Vit C (300 mg.kg^{-1}). The lowest FCR obtained in T8. The haematological parameters including red blood cells (RBCs), white blood cells (WBCs), haematocrit (%Hct) and haemoglobin (Hb) were higher in vitamin treated groups than control group with highest values in T8. In T13 (Vit E (20 mg.kg^{-1}), WBC values were higher compared to other experimental groups. The immunological parameters (lysozyme activity, Immunoglobulin (IgM) and total immunoglobulin (TIg)) were found to be higher in vitamin supplemented groups than in control group with highest values in T12 (Vit C (300 mg.kg-1). In conclusion, our results show that a combination of 30 mg.kg diet $^{-1}$ Vit E+ 300 mg.kg^{-1} Vit C or 40 mg.kg^{-1} Vit E + 300 mg.kg^{-1} Vit C could be a good option for obtaining appropriate growth and survival in Caspian brown trout juveniles.Caspian brown trout.

Keywords: Vitamins E, vitamins C, growth, survival, haematological parameters, immunological parameters.

Introduction

The Caspian brown trout, *Salmo trutta caspius*, is a critically endangered anadromous species that has been considered for a biological conservation program in the southern part of the Caspian Sea (Kiabi *et al*., 1999; Niksirat and Abdoli, 2009). During last decade, aquaculture and production of cultured brooders from larvae obtained from artificial propagation is carried out to compensate brooder shortage in nature. It is obvious that use of diet with high quality especially in terms of vitamin content is vital for aquaculture enhancing in particular for Caspian brown trout. Vitamins are organic substances that are necessary for health growth and maintenance of animals including fish (Lin and Shiau, 2004). Ascorbic acid (Vit C) and α-tocopherol (Vit E) as antioxidant are very essentials for most cultured fish species. It has also been recognized that these vitamins are among the most important nutrients that affect various aspects of fish including immune system, growth and survival (Durve and Lovell, 1982; Blazer and Wolke, 1984; Li and Lovell, 1985; Verlhac & Gabaudan, 1994; Waagbo, 1994; Verlhac *et al*., 1993; Anbarasu & Chandran, 2001; Chen *et al*., 2003; Lin and Shiau, 2004; Yamaguchi *et al*., 1995). Vit C stimulate immune responses such as macrophage activities, cell proliferation, natural killer cell activity, complement activity, lysozyme level, leucocyte phagocytic activity, cytokine production and antibody levels (Li and Lovell, 1985; Navarre and Halver, 1989). Also, Vitamin E depleted diets have been reported to reduce immune responses in several fish species (Blazer and Wolke, 1984; Hardie *et al*., 1990; Verlhac *et al.* 1993; Wise *et al*., 1993; Montero *et al*., 2001). High dietary levels of Vit E enhanced the natural cytotoxic activity of leucocytes (Cuesta *et al*., 2001) and innate immune responses in gilthead sea bream, *Sparus aurata* (Ortuno *et al.* 2000). Vitamin E in immune cell membranes (Beharka *et al*., 1997) protects macrophage membranes from peroxidative damage by free radicals and thus has a key role in the fish immunity (Waagbo, 1994). The antioxidantory role of Vit C and E against oxidation

by free radicals have been documented well (Hunter and Willet, 1994). The synergistic effects of Vit C and E have also been studied in some fish species, such as Nile tilapia, *Oreochromis niloticus* (Kim *et al*., 2003), hybrid tilapia (Shiau and Hsu, 2002), rainbow trout, *Oncorhynchus mykiss* (Verlhac *et al.*, 1993; Wahli *et al*., 1998), gilthead seabream (Marrero *et al*., 1999; Ortuno *et al*., 2001) and Atlantic salmon, *Salmo salar* (Hamre *et al*., 1997). In the present study, we examined the effects of different dietary levels of Vit C and E and their combinations on growth, survival and some haematological and immunological parameters of Caspian brown trout juveniles.

Material and Methods

480 Caspian brown trout (35±5.5 g) were considered for 2 months experiment. After one week adaptation period, fish were distributed in 48 polyethylene tanks (10 fish per tank) containing 300 L of dechlorinated and gentle aerated water. During the experiment, fish were fed daily in 3% of body weight (Mahmoudi *et al.*, 2009) by commercial diet (EX-TG2) with various dietary levels of vitamins E, C and their combinations. In this respect, 15 experimental treatments with three replicates including: T1: Vit E (20 $mg.kg^{-1}$) + Vit C (100 $mg.kg^{-1}$), T2: Vit E (30 $mg.kg^{-1}$) + Vit C (100 $mg.kg^{-1}$), T3: Vit E (40 $mg.kg^{-1}$) + Vit C (100 $mg.kg^{-1}$), T4: Vit E (20 $mg.kg^{-1}$) + Vit C (200 $mg.kg^{-1}$), T5: Vit E (30 $mg.kg^{-1}$) + Vit C (200 $mg.kg^{-1}$), T6: Vit E (40 $mg.kg^{-1}$) + Vit C (200 $mg.kg^{-1}$), T7: Vit E (20 $mg.kg^{-1}$) + Vit C (300 $mg.kg^{-1}$), T8: Vit E (30 $mg.kg^{-1}$) + Vit C (300 $mg.kg^{-1}$), T9: Vit E (40 $mg.kg^{-1}$) + Vit C (300 $mg.kg^{-1}$), T10: Vit C (100 $mg.kg^{-1}$), T11: Vit C (200 $mg.kg^{-1}$), T12: Vit C (300 $mg.kg^{-1}$), T13: Vit E (20 $mg.kg^{-1}$), T14: Vit E (30 $mg.kg^{-1}$) T15: Vit E (40 $mg.kg^{-1}$) and one group without vitamin supplement were considered as the control group. Before adding of vitamins E and C to EX-TG2, the vitamin E and C content of EX-TG2 were measured by HPLC. Then, the values of experimental vitamin supplements were regulated on the basis of their values in EX-TG2. Vitamin E (α-Tocopherol acetate) and C (L- ascorbyl- 2- poly phosphate) supplement were provided from RSHT-DANEH Company, Gorgan, Iran. To prepare experimental diets, the basal diet (EX-TG2) was mixed thoroughly and made into dough with the addition of distilled water in a mixer. After adding vitamin supplements to dough, it was extruded using meat chopper machine (MG-1400R-Pars Khazar, Iran) in 4.0 mm diameter size and was dried using an electric fan at room. The pellets were stored at -20°C until used. During the experiment, water quality parameters including pH, temperature and dissolved oxygen were checked daily by a pH meter (EUTECH DO6+). The ranges of these parameters were 6.9 ± 0.02, 7.9 ± 0.1 $mg.L^{-1}$ and 17.1 ± 1.5 respectively.

Assessment of Growth Parameters

To measure growth parameters, the biometry carried out monthly. Before biometry, fish were anaesthetized in 100 ppm of MS222 (tricaine methane sulphonate). The growth parameters of fish including growth rate (SGR), Weight Gain Percent (WG), Conditon Factor (CF), Feed Conversion Ratio (FCR) were measured after experiment as follow:

$$SGR = \ln BWt - \ln BWi / t \times 100$$ (Helland *et al*., 1996)

Where BWt refers to the final weight of Caspian brown trout, and BWi refer to the initial weight of Caspian brown trout.

$$WG = BWt - BWi / BWi \times 100$$ (Helland *et al.*, 1996)

$$CF = BW/TL3 \times 100$$ (Lagler *et al.*, 1962)

Where BW refers to the weight of Caspian brown trout, and TL refer to the total length of Caspian brown trout.

$$FCR = F/BWt\text{-}BWi$$ (Helland *et al.*, 1996)

Where F refers to the value of consumed food. Also, BWt refers to the final weight of Caspian brown trout, and BWi refer to the initial weight of Caspian brown trout.

Assessment of Hematological Parameters

To investigate the haematological parameters of serum, the blood samples were obtained from caudal peduncle using a heparinized syringe at the end of 2 months experiment. Immediately after blood sampling, the blood samples were delivered to lab for RBCs, WBCs, Hct, Hb, IgM, TIg and Lysozyme assays.

The MCV and MCHC values were calculated as follows:

$$MCV\ (fl) = (\text{haematocrit value}) / \text{total number of RBCs (million. } mm^{-3}) \times 10$$

$$MCHC\ (g.dl^{-1}) = (\text{haemoglobin concentration}) / (\text{haematocrit value}) \times 100$$

The microhaematocrit capillary tubes were used for measurement of Hct values according to Rehulka (2003). The Hb values were determined by Cyanmethemoglobin according to Blaxhall and Daisley (1973). In this regard, amount of 20 µL uncoagulated blood was mixed with 50 µl Drabkin's solution and then placed in dark environment for 5-10 min. Then, the Hb concentration was measured by spectrophotometry in wave-length of 540 nm. RBC and WBC values were determined with chamber

method using Neubauers haemocytometer (Drabkin, 1945). Serum concentrations of IgM were measured nephelometrically by Binding Site Nephelometry kit. To measure the concentration of total immunoglobulin, 100 mL of serum was mixed with 100 mL of 12% polyethylene glycol (PEG, 10,000 MW; Sigma Chemical, St. Louis, MO, USA). The samples were incubated for 2 h to precipitate the immunoglobulin molecules, which were then removed by centrifugation at 5000 *g* for 10 min. The concentration of total immunoglobulin was calculated subtracting the serum protein treated with 12% polyethyleneglycol solutions of the total serum protein (Amar *et al*., 2000).

Lysozyme activity of serum was measured using a modified turbidimetry method described by Ellis (1990). Briefly, a standard suspension of 0.375 $g.mL^{-1}$ *Micrococcus lysodeikticus* (Sigma) was prepared in 1 mL phosphate-buffered saline (pH 5.8). Serum (25 mL) was added to ml of bacterial suspension, and the optical density was measured after 15 and 180 seconds by spectrophotometer at 670 nm. One unit of lysozyme activity was defined as reduction in absorbance of 0.001/min. The units of lysozyme present in serum were obtained from a standard curve made with hen egg white lysozyme (Sigma).

Statistical Analysis

All data were analyzed by SPSS software (version 16; 2007). The data normality was investigated by The Shapiro–Wilk test. The non-parametric test of Kruskal–Wallis was used for non-normal distributed data. One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify level of significance at 0.05.

Results

There were no significant differences in values of MCV and MCHC (Table 1) compared to control group (P>0.05). Other haematological parameters including RBCs, WBCs, Hct and Hb were higher in vitamin treated groups than control group (Table 1, P<0.05). The highest values of RBCs, Hct and Hb were observed for T8 (P<0.05). In T13, WBC values were higher compared to other experimental groups (P<0.05). The immunological parameters including lysozyme activity, IgM and TIg were significantly higher in vitamin supplemented groups than in control group (Table 2, P<0.05). In this regard the highest values of these parameters were found in T12 (P<0.05). The lowest values of TIg and lysozyme activity were observed in control group and fish fed by only vitamin E i.e. T13, T14 and T15 (P<0.05). Also, fish fed control diet had lower IgM compared to other experimental treatments (P<0.05). Fish fed vitamin incorporated diets were showed better performances by all growth parameters than that of fish fed control diet. In this regard, more SGR (Figure 1), WG (Figure 2) obtained in T9 (P<0.05). The lowest SGR, Survival rate (SR) (Figure 3) and WG were recorded by fish fed control diet (P<0.05). Also, the lowest and highest FCR (Figure 4) were observed in T8 and control group respectively (P<0.05). There were significant differences in terms of CF between experimental groups (Figure 5, P<0.05). Almost vitamin treated groups had higher CF compared to control group (P<0.05). Also, in T6, T7, T8 and T9, fish yielded more biomass compared to other experimental groups (Figure 6, P<0.05).

Discussion

Studies on vitamin requirements of Caspian brown trout have not been made so far. In the present study, the growth parameters improved significantly in vitamin supplemented groups compared to control group. In this regard, the highest SGR, WG, FCR and CF have obtained in fish fed 30 $mg.kg^{-1}$ Vit E+ 300 $mg.kg^{-1}$ Vit C and 40 $mg.kg^{-1}$ Vit E + 300 $mg.kg^{-1}$ Vit C. It seems that the requirement of Caspian brown trout to Vit C and E is higher than Atlantic salmon and less than rainbow trout. NRC, (1993) recommends 50 mg.kg-1 Vit C for an optimal performance of juvenile fish based on analyses made on young rainbow trout. In Atlantic salmon, a dietary Vit C of 79 $mg.kg^{-1}$ improved more the growth parameters, whereas levels as low as 33 $mg.kg^{-1}$ had no detrimental effect on growth (Li *et al.*, 2007). Also, Vit E requirements of 120 $mg.kg^{-1}$ have been evaluated for Atlantic salmon (Hamre *et al.*, 1994) and rainbow trout (Cowey *et al.*, 1981). Similar to other fish species, vitamin requirements of salmonids especially Vit C and E is species-specific (Miar *et al*., 2013) and is depending water temperature, fish density and other cultural conditions (Arab and Rajabi Islami, 2015). Our results were in coincident with other studies that have shown a reduced growth in relation to reduced dietary Vit E and C for other fish species (Baker and Davies, 1997; Tocher *et al.*, 2003; Huang and Huang, 2004). Ascorbic acid is an essential coenzyme in Tyrosine amino acid oxidation and phenylalanine (Brander and Pugh, 1977) which can increase growth rate and weight gain though protein synthesis (Brander and Pugh, 1977; Andrade *et al*., 2007; Faramarzi, 2012) such as collagen (as a structural protein) (Smedsrød *et al.*, 1993; Terova *et al.*, 1998). Kaushik *et al.* (1998) observed an interaction of vitamin C level and protein source of protein utilization in salmonids. Also, some studies indicate that Vit E exerts its role on growth though preventing muscle atrophy and damages to collagen tissues (Fracalossi *et al.*, 2001; Montero *et al.*, 1999; Wang *et al.*, 2003; Chen *et al.*, 2004). Also, it was demonstrated that Vit E affects fish growth by controlling or reducing metabolic costs resulting in reduced tissue damage during stress. The

Table 1. Values of haematological components in Caspian brown trout juveniles after 2 months feeding with diet containing various levels of Vit c and E. Bars (Mean ± SD) with different letters are significantly different (P<0.05)

Experimental groups	WBC	RBC	Hb	Hct	MCV	MCH	MCHC
Control	6500±100 ^{a}	690000±2000 ^{a}	8.3±0.1 ^{a}	32±1 ^{a}	463.3±6.5 ^{a}	120±2 ^{a}	25.8±0.15 ^{a}
T_1	9133.3±251.6 ^{b}	710000±1000 ^{a}	8.7±0.1 ^{a}	34.6±1.5 ^{a}	491.6±3.5 ^{a}	121.6±3.5 ^{a}	24.7±0.15 ^{a}
T_2	7900±200 ^{a}	719000±1000 ^{a}	8.7±0.15 ^{a}	35.3±1.5 ^{a}	486±4 ^{a}	121.3±3.05 ^{a}	25.03±0.2 ^{a}
T_3	10033.3±152.7 ^{b}	789666.7±1527.5 ^{b}	9.8±0.05 ^{b}	39.6±1.5 ^{a}	505.6±5.5 ^{a}	125±3 ^{a}	24.7±0.2 ^{a}
T_4	9466.6±57.7 ^{b}	835000±5000 ^{b}	10.4±0.05 ^{b}	41±1 ^{b}	511.3±22.2 ^{a}	123±2 ^{a}	24.9±0.05 ^{a}
T_5	9100±100 ^{b}	760000±2000 ^{a}	9.8±0.1 ^{ab}	40.6±1.5 ^{b}	540.6±3.5 ^{a}	129±2 ^{a}	23.8±0.15 ^{a}
T_6	9133.3±152.7 ^{b}	769666.7±1527.5 ^{a}	9.56±0.15 ^{ab}	40.6±1.5 ^{b}	545.6±4.5 ^{a}	126.3±3.5 ^{a}	24.7±0.15 ^{a}
T_7	12033.3±251.6 ^{c}	915000±2000 ^{c}	11.7±0.2 ^{c}	45±1 ^{b}	563.3±6.5 ^{a}	128.6±1.5 ^{a}	25.9±0.1 ^{a}
T_8	12200±100 ^{c}	930333.3±2516.6 ^{c}	11.9±0.1 ^{c}	47±2 ^{bc}	573.3±6.1 ^{a}	130.6±3.05 ^{a}	26.7±0.1 ^{a}
T_9	12100±100 ^{c}	920666.7±4041.4 ^{c}	11.8±0.2 ^{c}	46±1 ^{c}	565±5 ^{a}	129.3±2.5 ^{a}	26.2±0.75 ^{a}
T_{10}	8800±100 ^{b}	740666.7±5033.2 ^{a}	9.4±0.15 ^{a}	38±2 ^{c}	513±7 ^{a}	127.6±2.5 ^{a}	25±3 ^{a}
T_{11}	10833.3±152.7 ^{ab}	909666.7±1527.5 ^{b}	11.5±0.1 ^{c}	45.3±2.5 ^{a}	496±6.2 ^{a}	125.6±3.5 ^{a}	25.5±0.3 ^{a}
T_{12}	9800±100 ^{b}	769666.7±6506.4 ^{a}	9.7±0.15 ^{a}	39.3±2.5 ^{b}	505±6.5 ^{a}	126.3±4.5 ^{a}	24.8±0.3 ^{a}
T_{13}	12966.6±57.7 ^{c}	908333.3±1527.5 ^{c}	11.3±0.1 ^{bc}	43.6±2.5 ^{a}	484.6±9 ^{a}	125.6±5.03 ^{a}	25.9±0.25 ^{a}
T_{14}	11800±100 ^{c}	890000±3000 ^{c}	10.4±0.15 ^{b}	41±2 ^{b}	462±7.5 ^{a}	118±4 ^{a}	24.5±0.2
T_{15}	12266.6±115.4 ^{c}	896666.7±7637.6 ^{c}	11.1±0.15 ^{bc}	40.6±3 ^{bc}	450.6±10.9 ^{a}	126±4.3 ^{a}	27.7±0.2 ^{a}

Table 2. Values of Immunological components in Caspian brown trout juveniles after 2 months feeding with diet containing various levels of Vit c and E. Bars (Mean ± SD) with different letters are significantly different (P<0.05)

Experimental groups	Lysozyme activity	IgM	TIg
Control	10.6±1.5 ^{a}	5.1±0.4 ^{a}	7.3±0.4 ^{a}
T_1	34.3±2.08 ^{b}	9.5±0.1 ^{b}	12.3±0.5 ^{b}
T_2	34.3±1.5 ^{b}	9.4±0.2 ^{b}	12.2±0.1 ^{b}
T_3	34±1.7 ^{b}	9.3±0.2 ^{b}	12.25±0.2 ^{b}
T_4	46.6±1.15 ^{c}	13.7±0.3 ^{c}	14.1±0.2 ^{c}
T_5	47.3±2.08 ^{c}	13.65±0.3 ^{c}	14±0.4 ^{c}
T_6	41.6±2.5 ^{c}	13.6±0.4 ^{c}	14±0.3 ^{c}
T_7	43.3±2.08 ^{c}	14.2±0.4 ^{d}	16.2±0.4 ^{c}
T_8	43.6±2.5 ^{c}	14±0.2 ^{d}	16.2±0.3 ^{c}
T_9	43.3±1.5 ^{c}	13.9±0.4 ^{c}	16±0.1 ^{c}
T_{10}	39.6±5.03 ^{b}	11.1±0.15 ^{ab}	12.3±0.2 ^{b}
T_{11}	42.6±2.5 ^{bc}	14±0.3 ^{c}	15.1±0.4 ^{c}
T_{12}	49.3±4.16 ^{d}	16.1±0.5 ^{c}	16.4±0.25 ^{d}
T_{13}	11±2 ^{a}	8.8±0.2 ^{ab}	7.1±0.3 ^{a}
T_{14}	10.6±1.5 ^{a}	7.2±0.4 ^{a}	7±0.1 ^{a}
T_{15}	10.6±0.5 ^{a}	6.7±0.3 ^{a}	6.9±0.2 ^{a}

haematological parameters in fish are used usually as indicators of health status (Serpunin *et al.*, 1998). In our study, the values of WBCs, RBCs, Hb and Hct were higher in vitamin supplemented fish compared to control group. In rainbow trout, hematocrit values for trout fed without vitamins were very low (21.58%) and were significantly less than trout fed with vitamins (Miar *et al.*, 2013). Again in rainbow trout, Hct, MCV, MCH and MCHC were higher in fish fed with different levels of vitamin E than those fish fed

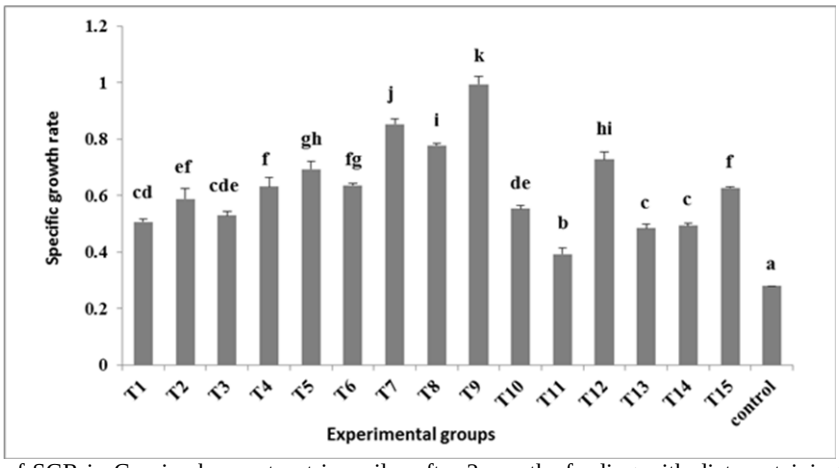

Figure 1. Values of SGR in Caspian brown trout juveniles after 2 months feeding with diet containing various levels of Vit c and E. Bars (Mean ± SD) with different letters are significantly different (P<0.05).

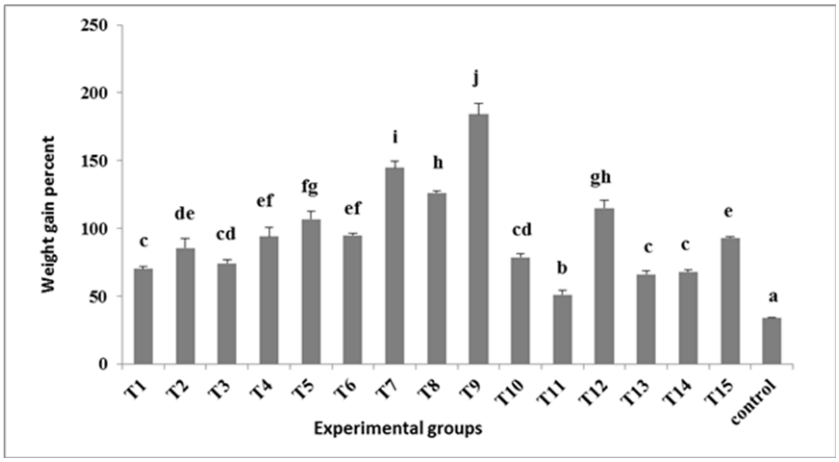

Figure 2. Values of WG% in Caspian brown trout juveniles after 2 months feeding with diet containing various levels of Vit c and E. Bars (Mean ± SD) with different letters are significantly different (P<0.05).

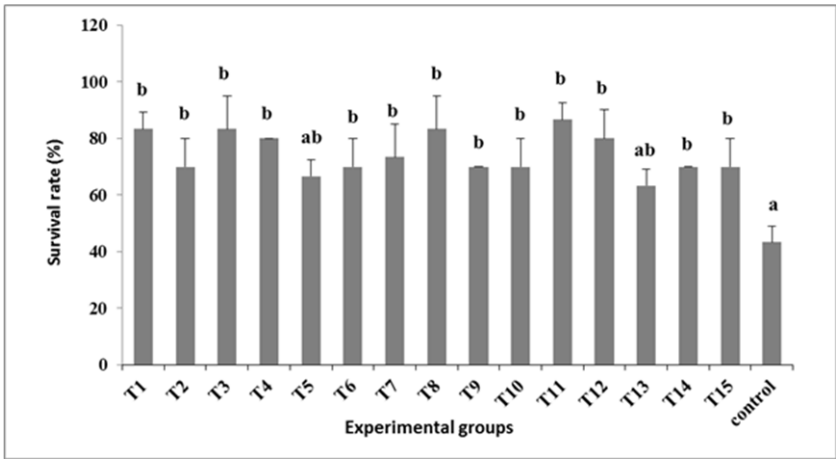

Figure 3. Values of SR in Caspian brown trout juveniles after 2 months feeding with diet containing various levels of Vit c and E. Bars (Mean ± SD) with different letters are significantly different (P<0.05).

with the control diet (Esmaeili and Khara, 2014). The increase in vitamin E and C content seemed to provide some protective effect to the trout preserving high hematocrit. Bai and Lee (1998), were showed in Korean rockfish, *Sebastes schlegeli*, hematocrit of fish fed control group was lower than that of fish fed

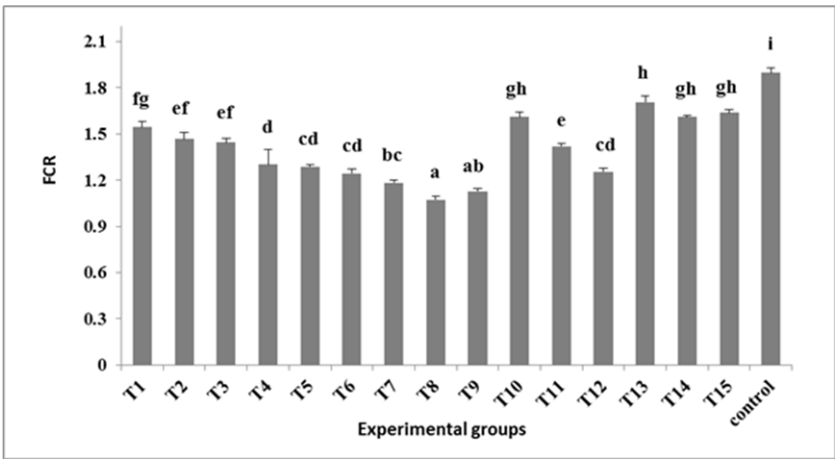

Figure 4. Values of FCR in Caspian brown trout juveniles after 2 months feeding with diet containing various levels of Vit c and E. Bars (Mean ± SD) with different letters are significantly different (P<0.05).

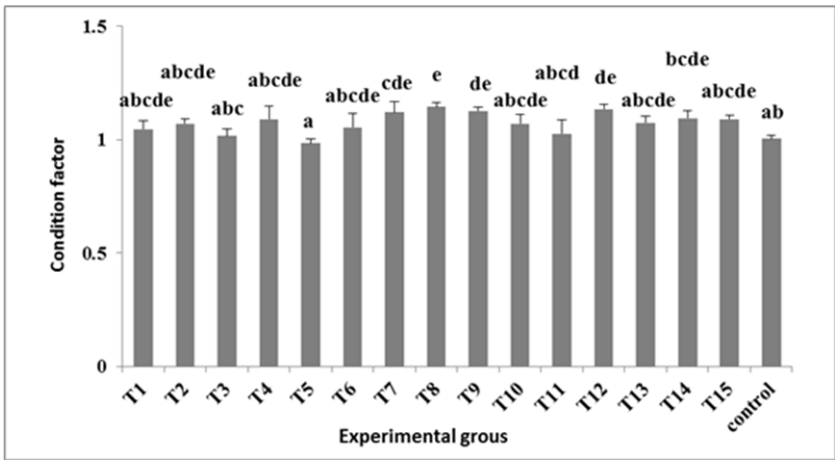

Figure 5. Values of CF in Caspian brown trout juveniles after 2 months feeding with diet containing various levels of Vit c and E. Bars (Mean ± SD) with different letters are significantly different (P<0.05).

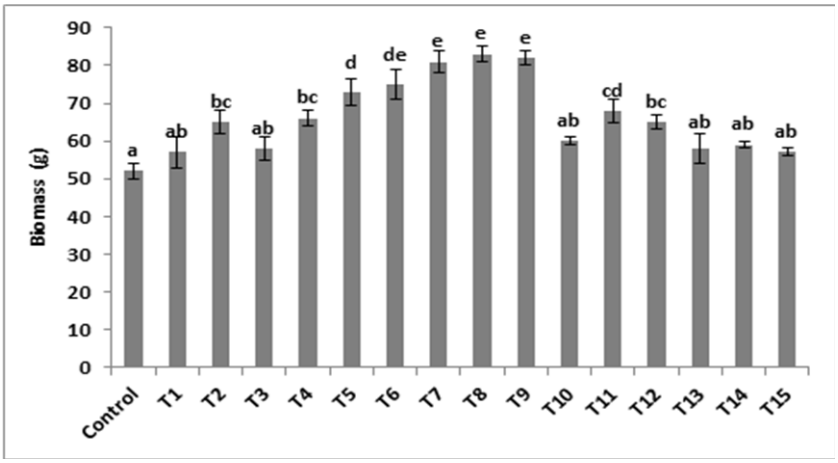

Figure 6. Values of biomass in Caspian brown trout juveniles after 2 months feeding with diet containing various levels of Vit c and E. Bars (Mean ± SD) with different letters are significantly different (P<0.05).

high level of vitamin E. Adham *et al*. (2000) demonstrated that vitamin C insufficient feeds cause macrocytic anemia, characterized by a decrease in the hemoglobin, reduction in number of erythrocytes and hematocrit. In general, the enhancing action of Vit C on fish haematology has been attributed to its role in

releasing liver ferritin and its utilization for erythropoiesis process (Dinning, 1962; Cox, 1968). Also, Vit C helps to absorption of iron from the gastrointestinal tract. Thus, the increases in RBCs, Hct and Hb in vitamin supplemented groups may be due to this action. Vitamin C is a powerful antioxidant protecting against oxidative damage to various tissues of fish including red blood cells (Sahoo and Mukherjee, 2003). Similar to Vit C, decreases in RBCs, Hct and Hb was reported in Amago salmon, *Oncorhynchus masou* fed Vit E deficiency diet (Taveekijakam *et al*., 1996). It was reported that Vit E as antioxidant protects cell membrane including red blood cells against oxidative damages (Sahoo and Mukherjee, 2003). In our study, it seems that use of Vit C and E for Caspian brown trout stimulates immunity since elevations were observed in WBCs, lysozyme activity, IgM and TIg in vitamin supplemented groups compared to control group. The immune system in fish includes humoral/cellular and specific/nonspecific immune responses. The immunostimulatory effects of Vit E and C were documented in other fish species such as rainbow trout (Clerton *et al.*, 2001) and gilthead seabream (Ortuno *et al.* 2000; Cuesta *et al.* 2001). In immunity system, several roles were attributed to dietary Vit C including: macrophage activities, cell proliferation, natural killer cell activity, complement and lysozyme activities (Anbarasu and Chandran, 2001; Chen *et al.* 2003; Lin and Shiau, 2004). In conclusion, our study showed that dietary Vit C and E improve growth and immunity of Caspian brown juveniles. In this regard, a combination of 30 mg.kg diet $^{-1}$ Vit E+ 300 mg.kg^{-1} Vit C or 40 mg.kg^{-1} Vit E + 300 mg.kg^{-1} Vit C could be a good option for obtaining appropriate growth and survival in Caspian brown trout juveniles.

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