Blood parameters of Caspian brown trout (*Salmo trutta caspius*) fingerlings affected by dietary L-ascorbyl-2-polyphosphate

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Abstract

This study was aimed to evaluate the effect of L-ascorbyl-2-polyphosphate as a dietary ascorbic acid source on blood parameters of Caspian brown trout (*Salmo trutta caspius*), including red blood cell (RBC) count, white blood cell (WBC) and WBC differential. A total number of 600 Caspian brown trout (9.6±0.6 g) fingerlings were randomly distributed in triplicates among five treatments each containing 40 specimens. Experimental diets were also prepared by adding 0, 50, 100, 200 and 400 mg kg^{-1} L-Ascorbyl-2-Polyphosphate to the basal diet. Feeding was done for nine weeks in each treatment. The survival rate in all treatments was 100%. The results showed a significant increase in RBC, hemoglobin, hematocrit, WBC and lymphocyte (p<0.05) by supplementing L-ascorbyl-2-polyphosphate compared to the control treatment. The fish fed by 200 mg ascorbic acid kg^{-1} diet had the maximum hemoglobin and hematocrit in comparison with the other treatments. The current research showed that dietary L-ascorbyl-2-polyphosphate influences the complete blood count of Caspian brown trout while fingerlings fed with the optimum amounts of 200 mg ascorbic acid kg^{-1} diet for a period of 9 weeks trail.

Keywords: Ascorbic acid, Growth, Hematological parameters, *Salmo trutta caspius*.

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Introduction

Caspian brown trout (*Salmo trutta caspius*, Kessler, 1877) is an endangered anadromous species distributed in southern region of the Caspian Sea which migrates to rivers which connected to the Caspian Sea such as Sefid-Rud, Gorgan-Rud, and Cheshme Kile rivers for breeding (Armantrout, 1980; Kiabi *et al*., 1999; Coad, 2000).

Artificial propagation and releasing fingerlings to natural water is an approach to prevent brown trout's extinction, but achieving this goal is faced problematic from which feeding and mortality in fry and fingerlings are the most important ones. Caspian brown trout is sensitive to stress and disease like many other species. Powerful defense mechanisms like specific and non-specific immune are needed against pathogens to improve fish health. Non-specific immune in fish is more important than mammalians (Magnadóttir, 2006).

Hematology, a valuable tool for determination of fish physiological condition and blood parameters, is commonly used as a physiological index against internal and external changes (Xiaoyun *et al*., 2009; Satheeshkumar *et al*., 2011). One of the solutions to improve health, increase resistance to stress, and rise immune system efficiency is feed additive supplementation (Ai *et al*., 2004; Cerezuela *et al*., 2009) such as vitamin C. Feed quality is an important factor to fish health preservation. Immune system efficiency will be increased by consuming immune stimulant such as antioxidant like vitamin C, carotenoids and other feed additives (Magnadóttir, 2006; Orun *et al*., 2008a). Results of different researches have shown that vitamin C has an effective Immunostimulant for increases resistance against stresses (Hardie *et al*., 1991; Mulero *et al*., 1998; Kumari and Sahoo, 2005; Ai *et al*., 2006; Tewary and Patra, 2008). Vitamin C is easily obtained from the gut and distributes widely through the body (Secombes and Ellis, 2012).

Although, many studies have been done on feeding Caspian brown trout (Saber *et al*., 2005; Ramezani, 2009; Sotoudeh *et al*., 2011), few study has been carried out on its blood parameters. The present study was conducted to examine RBC and WBC's changes in Caspian brown trout fed by dietary supplementation of L-ascorbyl-2-polyphosphate as an immunostimulant.

Materials and methods

Diet preparation

The basal practical diet was formulated (Table 1) to contain about 49.60% crude protein and 14.96% crude lipid, which is suitable for the growth of Caspian brown trout fingerlings (Saber *et al*., 2005). For preparing the experimental diets, different levels (50, 100, 200 and 400 mg) of ascorbic acid in the form of L-ascorbyl-2-polyphosphate (Tiger, China) were added per kilogram of diet and the basal diet was used as a control.

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Table 1: Ingredient and chemical proximate composition of experimental diets suggested by Saber *et al* (2005) for Caspian brown trout, *Salmo trutta caspius* (% dry matter).

Ingredient	%
Fish meal	66.54
Soybean meal	8.00
Wheat meal	3.84
Fish oil	7.00
Dried cheese	3.00
Gammarus powder	2.00
Mineral premix	2.00
Vitamin premix	2.00
Proximate analysis (Average)	
Crude protein %	49.60
Crude lipid %	14.96
Ash %	17.50
Moisture %	5.64

Mineral premix (mg kg^{-1} diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; Ca $(H_2PO_3)_2 \cdot H_2O$, 3000 mg; NaCl, 100 mg.
Vitamin premix (mg or g kg^{-1} diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCL, 20 mg; vitamin B_{12}, 0.1 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg, biotin, 1.20 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol, 120 mg; choline chloride, 2500 mg, ethoxyquin 150 mg, wheat middling 14.012 g. f L- 2APP, L-ascorbyl-2-polyphosphate (Tiger, China). L- 2APP was separately supplemented with 0.0, 50.0, 100.0, 200.0 and 400.0 mg ascorbic acid equivalent kg^{-1} diet. The analyzed levels of dietary ascorbic acid were 9.8, 43.8, 89.4, 188.5 and 384.2 mg kg^{-1} diets.

Feed preparing was done every three weeks due to prevent ascorbic acid degeneration. Feeds were stored at -12 °C in a freezer until used.

Proximate analysis of diets

The amount of dry matter was evaluated by drying an aliquot of each diet in a mechanical convection oven at 105 °C for 16 h to obtain a constant weight. Ash content was determined by heating an aliquot of the samples in a muffle furnace at 550 °C for 3 h and weighing the remained material. Protein content was also determined by converting the nitrogen content (N×6.25) based on the Kjeldahl method. Total lipid content was also extracted using a chloroform-methanol (2:1 by vol.) mixture. All measurements were done according to the AOAC (2000) and the result was expressed as g 100 g^{-1} diet.

Ascorbic acid content was also analyzed based on the Shiau and Hsu (1999). Approximately 3–5 g of grounded diet was treated with 25 mL chloroform and 100 mL distilled water, shacked for 25 min, settled for 25 min, and centrifuged for 5 min at 2739 ×g. One mL of the supernatant was buffered by 0.2 M acetic acid buffer (pH=4.8) and 0.2% DTT, kept in 37 °C bath for 2 h, and centrifuged for 5 min

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at 2739 ×g. Afterwards, 20 µL of supernatants were sieved through a 0.22-pore size syringe filter and subjected to ascorbic acid analysis. The ascorbic acid content in the diets were determined by reverse-phase HPLC (KNAUER pump 1000, German) with an ODS column (4.6×25 mm, German). Mobile phase (flow rate 0.6 mL min^{-1}) was an aqueous solution of 0.05 M KH_2PO_4 (adjusted to pH 3 with phosphoric acid) and the effluent was monitored by a UV-detector (254 nm wave length).

Fish bioassay

Six hundred Caspian brown trout fingerlings have been obtained from the Coldwater Fishes Research Center (CFRC), Tonekabon, Iran, and transferred to a local fish farm in Dohezar Road, Tonekabon. After fasting for initial adaptation to the experimental condition, the specimens were fed by artificial feed (Behparvar Co., Karaj, Iran). Fish were adapted with new conditions for 2 weeks and fed with control diet. The average weight of fish fingerlings at the beginning of the experiment was 9.6±0.6 g.

The water was supplied from a spring with flow rate of 6 L s^{-1}. The water temperature, dissolved oxygen, pH, and electrical conductivity were measured every week. Water temperature and dissolved oxygen measured by an oxygen meter WTW (Oxi 300i, Germany). Level of pH and electrical conductivity (µS cm^{-1}) were also analyzed by a pH meter (Metrohm 827, Germany). During the experiment, the temperature ranged between 9.6 to 10.2 °C, the pH ranged between 7.5-7.7, electrical conductivity ranged from 248.3 to 252.8, and the dissolved oxygen was approximately 10.2 mg L^{-1}. Accordingly, the water condition was appropriate for the growth of Caspian brown trout fingerlings.

The fish were divided into 15 raceways with dimensions of 1.5×1.5×0.8 m. The experiments were designed in 5 treatments with different levels of ascorbic acid (0, 50, 100, 200 and 400 mg ascorbic acid per 1 kg feed) and each treatment was set as 3 replications. There were 40 fish in each raceway, and they were fed to saturation, 3 times a day, for a period of nine weeks. Fish were fed quickly and no feed remained in the water.

Determination of growth and blood parameters

Feeding was stopped a day before tests. Every 3 weeks, 5 fish were selected from each treatment and anesthetized using 250 ppm of Cloves oil (Soltani *et al*., 2001). Final weight and the growth parameters including feed conversion rate (FCR) and specific growth rate (SGR, % d^{-1}) were calculated as follows:

$$FCR = \frac{\text{Total of feed consumed (g)}}{\text{Weight Gain (g)}} \times 100$$

$$SGR = \frac{(\text{Ln Final Weight (g)} - \text{Ln Initial Weight (g)})}{\text{t (Day)}} \times 100$$

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Blood samples were collected by cutting the caudal peduncle and 1 mL of blood was poured into a tube containing Ethylene diamine tetra-acetic acid (EDTA) (Svobodova *et al*., 1991) and moved to the laboratory for further analysis. Hematocrit (Hct) and Hemoglobin (Hb) were according to the Vázquez and Guerrero (2007) method. Red blood cell (RBC) and white blood cell (WBC) counts were also determined according to the methodology of Blaxhall and Daisley (1973). Other blood indices including mean corpuscular volume (MCV, femtoliter), mean corpuscular hemoglobin (MCH, picogram), and mean corpuscular hemoglobin concentration (MCHC, g dL^{-1}) were calculated by the following formulas:

1. $MCHC = \frac{100 \times Hb}{Hct}$

2. $MCV = \frac{10 \times Hct}{RBC\ (in\ milion)}$

3. $MCH = \frac{10 \times Hb}{RBC\ (in\ milion)}$

Statistical analyses

The data analysis was done using SPSS-16 software. The means were subjected to the one-way analysis of variances (ANOVA) after examination of normality by Kolmogorov-Smirnov test. Where the differences occurred, Tukey's HSD test was used to determine the difference points for growth parameters and LSD for hematological parameters, respectively. Results expressed as mean ± standard error and a p-value less than 0.05 was assigned as significant difference.

Results

Proximate composition of experimental diets

The results of the proximate analysis for each experimental diet were presented in Table 2. No significant difference was found between the treatments in the amount of moisture, protein, lipid, and ash contents.

Survival and growth rates

The survival rate of fish in all treatments was %100 and no symptom of ascorbic acid deficiency was observed in Caspian brown trout fingerlings during the 9 weeks of culture. The result showed significant increase in growth of fish fed by 200 mg ascorbic acid kg^{-1} compared to fish fed by control diet (p<0.05). Treatments with 100, 200 and 400 mg ascorbic acid kg^{-1} showed no significant differences in the fish growth after 9 weeks experiment (Fig. 1).

SGR had significant differences between treatments (p<0.05). The lowest SGR was recorded in the control group during all sampling times. SGR in 200 mg ascorbic acid kg^{-1} at third and ninth weeks was higher than other treatments. The highest SGR were observed in fish fed by 400 mg ascorbic acid kg^{-1} at sixth week, while no significant differences was found in fish fed by 50, 100 and 200 mg ascorbic acid kg^{-1} (Table 3).

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Table 2: Proximate composition of experimental diets (g 100 g^{-1}).

	Moisture (g 100 g^{-1} ww†)	Protein (g 100 g^{-1} dw‡)	Lipid (g 100 g^{-1} dw)	Ash (g 100 g^{-1} dw)	Ascorbic acid (mg kg^{-1} dw)
Control	5.23±0.35	49.6±0.25	15.01±0.57	17.40±0.13	9.8
50 mg AA$^{\clubsuit}$	5.57±0.32	49.7±0.24	14.80±0.48	17.33±0.09	43.8
100 mg AA	5.56±0.28	49.6±0.31	15.20±0.35	17.56±0.14	89.4
200 mg AA	5.62±0.30	49.5±0.23	14.88±0.43	17.43±0.18	188.5
400 mg AA	5.64±0.32	49.6±0.49	14.93±0.39	17.78±0.17	384.2

$^{\clubsuit}$Ascorbic acid, †Wet Weight; ‡Dry Weight

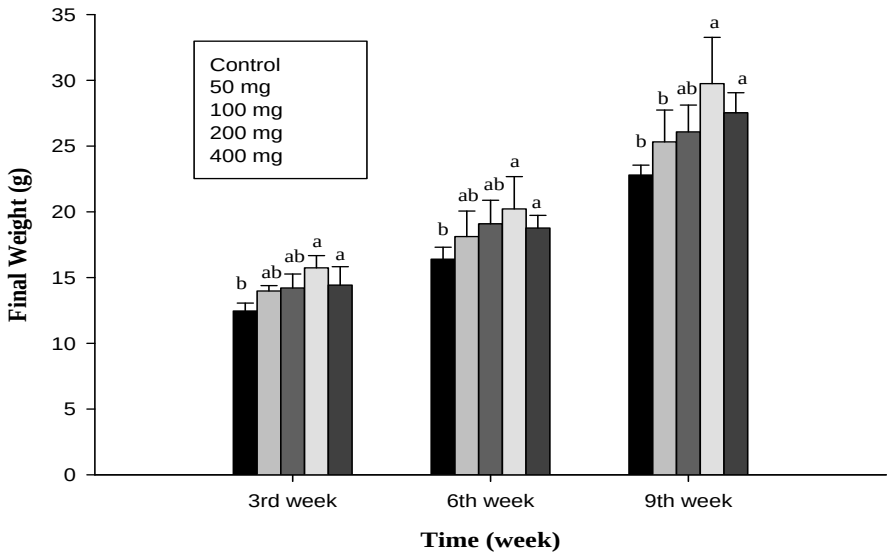


Figure 1: Effects of graded supplementary ascorbic acid on final weight of Caspian brown trout (*Salmo trutta caspius*) during nine weeks. Different letters at the same time indicate significant differences between the treatments (p<0.05).

Table 3: Specific growth rate (SGR, % d^{-1}) of Caspian brown trout (*Salmo trutta caspius*) fed diets with graded levels of ascorbic acid during 9 weeks experiment.

	3rd week	6th week	9th week
Control	0.52±0.03^{a}	0.54±0.09^{a}	0.60±0.75^{a}
50 mg AA$^{\clubsuit}$	0.60±0.13^{ab}	0.71±0.26^{b}	0.76±0.05^{b}
100 mg AA	0.67±0.14^{b}	0.68±0.14^{ab}	0.80±0.14^{b}
200 mg AA	0.71±0.28^{b}	0.72±0.38^{a}	1.01±0.28^{c}
400 mg AA	0.64±0.07^{b}	0.79±0.04^{b}	0.83±0.06^{b}

Similar letters at the same column show no significant difference between results for each treatment (p<0.05).

$^{\clubsuit}$Ascorbic acid

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Control treatments had the highest FCR compared to the other treatments containing ascorbic acid throughout the experiment (Table 4). In contrast, the lowest FCR, 0.78±0.03, was also observed on group of fish those fed 200 mg ascorbic acid kg^{-1} after the third week, while it increased in this treatment and reached to 1.18±0.09 at the end of bioassay. In the ninth week, the lowest FCRs were observed on group of fish those fed with 400 and 200 mg ascorbic acid kg^{-1} diet (1.11±0.09 and 1.18±1.44, respectively).

Blood parameters

The results of this study showed that nine weeks treatment of fish fed with 200 mg ascorbic acid kg^{-1} caused a significant increase in RBC compared to those fed the control diet (p<0.05), however no significant difference was observed in RBC after nine weeks (Fig. 2).

Caspian brown trout fingerlings fed with control diet had the lowest hemoglobin during the experiment (Fig. 3). The highest hemoglobin was found in fish fed with diet containing 200 mg ascorbic acid kg^{-1} (18.00±1.52 g dL^{-1}). Moreover, hemoglobin in control, 50, 100 and 400 mg ascorbic acid kg^{-1} treatments was lower than 200 mg ascorbic acid kg^{-1} treatment in the sixth week of the experiment. However, hemoglobin of these treatments was higher when compared to the control treatment.

Hematocrit was also significantly (p<0.05) different between treatments (Fig. 4). The hematocrit in treatment of 200 mg ascorbic acid kg^{-1} was higher than other treatments, although there were no significant differences between treatments of 50, 100 and 400 mg ascorbic acid kg^{-1}.

Table 4: Feed conversion rate (FCR) of Caspian brown trout (*Salmo trutta caspius*) fed diets with graded levels of ascorbic acid during 9 weeks.

	3rd week	6th week	9th week
Control	1.69±0.43[a]	1.83±0.62[a]	1.52±0.02[a]
50 mg AA♣	1.10±0.09[b]	0.93±0.38[a]	1.35±3.14[a]
100 mg AA	1.06±0.03[b]	1.20±1.23[b]	1.39±0.35[a]
200 mg AA	0.78±0.03[c]	1.16±0.27[b]	1.18±1.44[b]
400 mg AA	1.00±0.09[b]	1.31±0.07[a]	1.11±0.09[b]

Similar letters at the same column show no significant difference between results for each treatment (p<0.05).

♣Ascorbic acid

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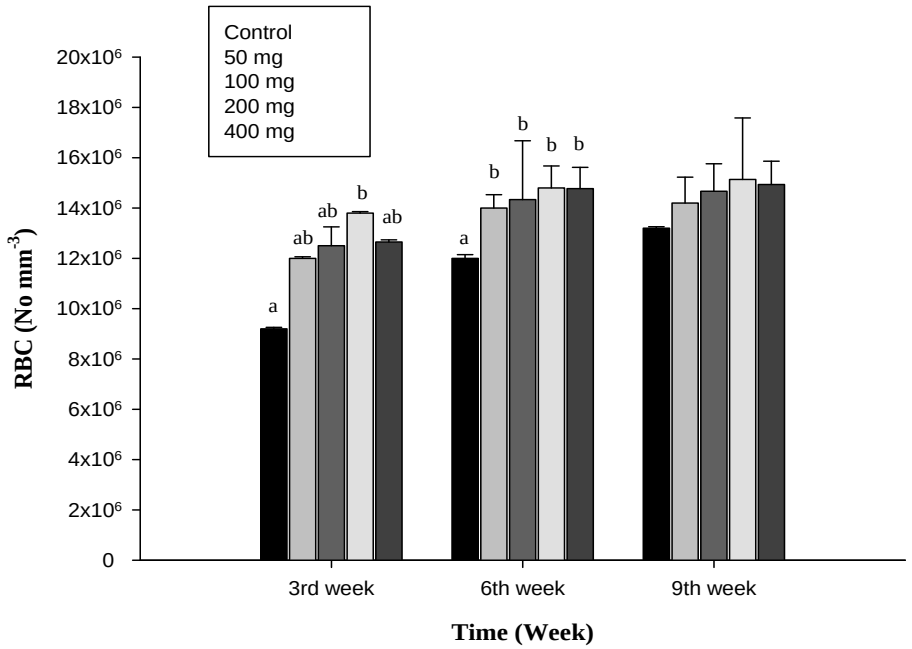


Figure 2: Effects of graded supplementary ascorbic acid on red blood cell (RBC) count of Caspian brown trout (*Salmo trutta caspius*) during nine weeks. Different letters indicate significant differences between the treatments (p<0.05).

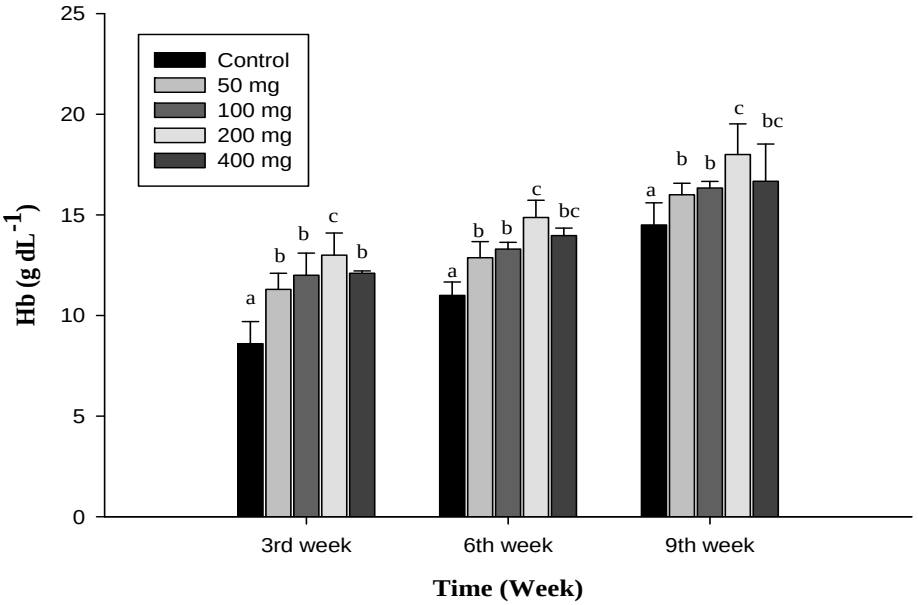


Figure 3: Effects of graded supplementary ascorbic acid on hemoglobin (Hb) of Caspian brown trout (*Salmo trutta caspius*) during nine weeks. Different letters indicate significant differences between the treatments (p<0.05).

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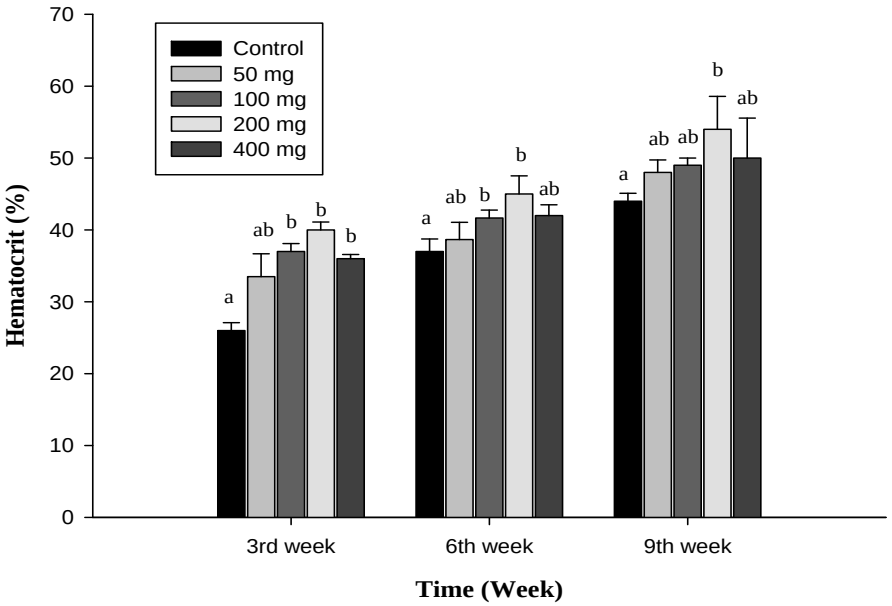


Figure 4: Effects of graded supplementary ascorbic acid on hematocrit of Caspian brown trout (*Salmo trutta caspius*) during nine weeks. Different letters indicate significant differences between the treatments ($p<0.05$).

Evaluation of MCH showed no significant difference between treatments at the third and ninth week of experiment (Fig. 5). The fish fed containing 100 mg ascorbic acid kg^{-1} diet had the highest amount (10.31±2.06 picogram) at the sixth week.

The results showed significant differences in MCHC between treatments at the third and sixth weeks (Fig. 6). The highest MCHC was observed in 50 mg ascorbic acid kg^{-1}, (33.95±0.80 g dL^{-1}), and 100 mg ascorbic acid kg^{-1}, (33.28±0.25 g dL^{-1}), at the third and sixth weeks, respectively. There were no significant differences between fish fed by 50 and 100 mg ascorbic acid kg^{-1} diet with those fed by 200 and 400 mg ascorbic acid kg^{-1} diet. Furthermore, no significant differences were observed in the ninth week.

Control treatments had the highest MCV compared to the diets containing dietary ascorbic acid at the third and sixth week (Fig. 7). The highest MCV (36.96±4.40 femtoliter) was observed on fish fed 200 mg ascorbic acid kg^{-1} diet at the ninth week, while it had no significant differences with other treatments. The lowest MCV (27.61±1.43 femtoliter) was also recorded on fish fed 50 mg ascorbic acid kg^{-1} diet after the sixth week.

There was a significant difference in WBCs during 9 weeks of experiment (Fig. 8).

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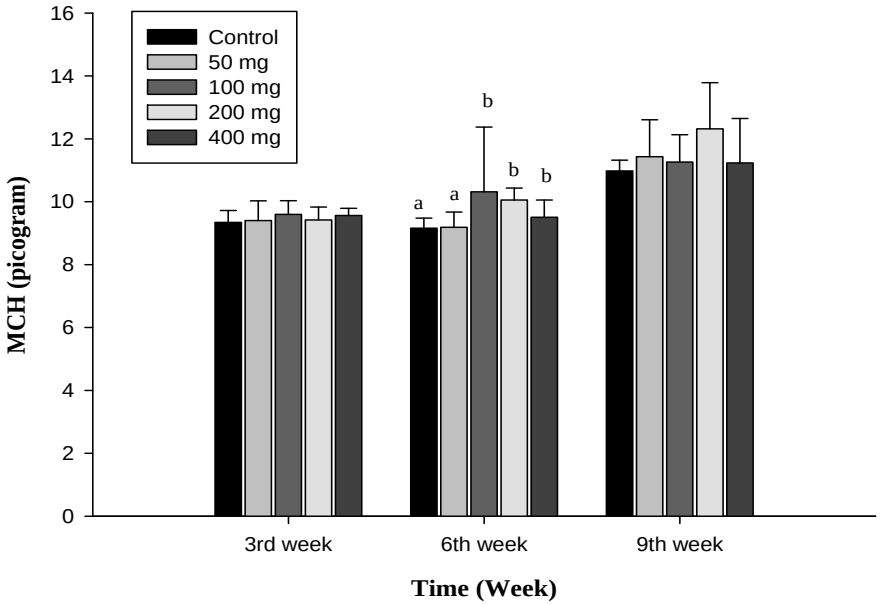


Figure 5: Effects of graded supplementary ascorbic acid on mean corpuscular hemoglobin (MCH) of Caspian brown trout (*Salmo trutta caspius*) during nine weeks. Different letters indicate significant differences between the treatments (p<0.05).

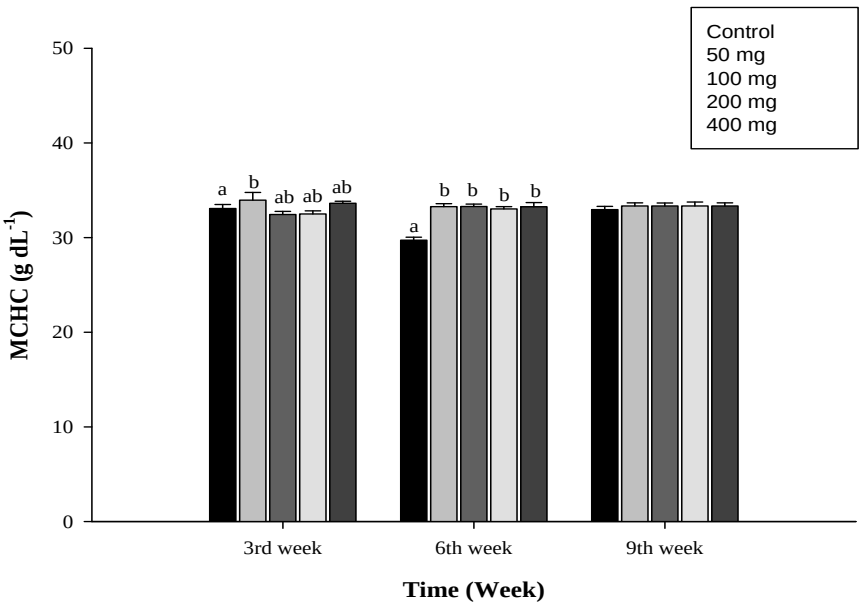


Figure 6: Effects of graded supplementary ascorbic acid on mean corpuscular hemoglobin concentration (MCHC) of Caspian brown trout (*Salmo trutta caspius*) during nine weeks. Different letters indicate significant differences between the treatments (p<0.05).

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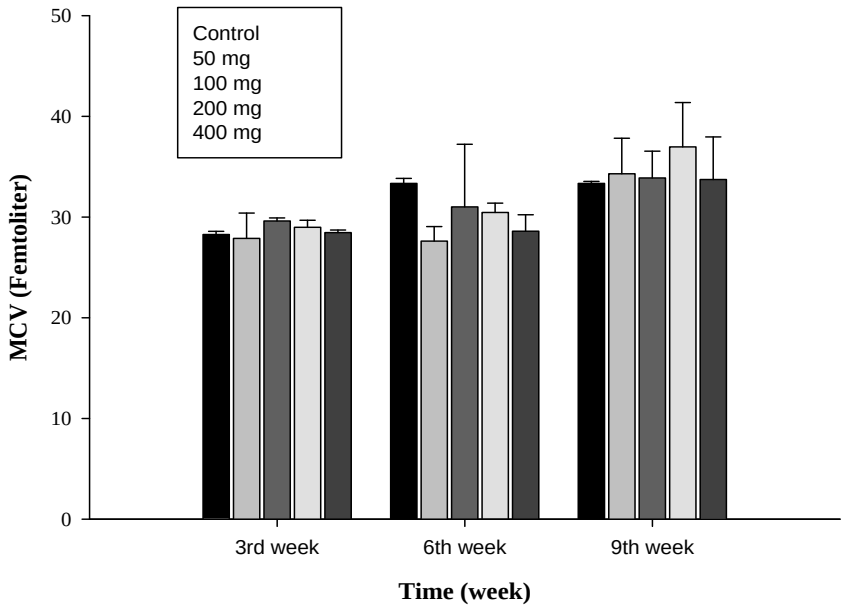


Figure 7: Effects of graded supplementary ascorbic acid on mean corpuscular volume (MCV) of Caspian brown trout (*Salmo trutta caspius*) during nine weeks. Different letters indicate significant differences between the treatments (p<0.05).

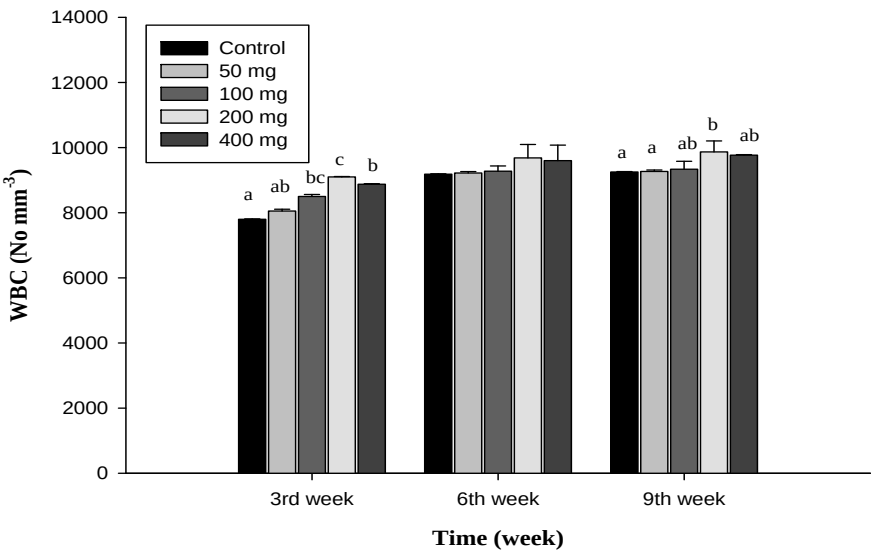


Figure 8: Effects of graded supplementary ascorbic acid on white blood cell (WBC) count of Caspian brown trout (*Salmo trutta caspius*) during nine weeks. Different letters indicate significant differences between the treatments (p<0.05).

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However, no significant differences were observed in the sixth week of the experiment. WBCs in Caspian brown trout fed the control diet were the lowest in comparison with other diets group. The highest number of WBCs was 9866.66±337.06 found in fish fed by 200 mg ascorbic acid kg^{-1} diet.

Evaluation of lymphocyte showed a significant proliferation in lymphocyte among different treatments by increase of dietary ascorbic acid level (Fig. 9). However, no significant differences was found in lymphocyte of fish fed with 200 mg kg^{-1} ascorbic acid diet after six and nine weeks of feeding trial except control treatment.

Neutrophil count decreased in the sixth and ninth week with other supplemented ascorbic acid treatment after six and nine weeks of feeding trial.

Neutrophil count decreased significantly by supplementing ascorbic acid to the diet during the experiment (Fig. 10). The highest number of neutrophil obtained in control treatment (31.00±1.20 %), which was significantly different with treatment containing 200 mg ascorbic acid kg^{-1} diet at the third week. Furthermore, there was a significant difference between control treatment with those supplemented by ascorbic acid at the sixth and ninth weeks. There was no significant difference in monocytes and basophiles counts between treatments throughout the experiment.

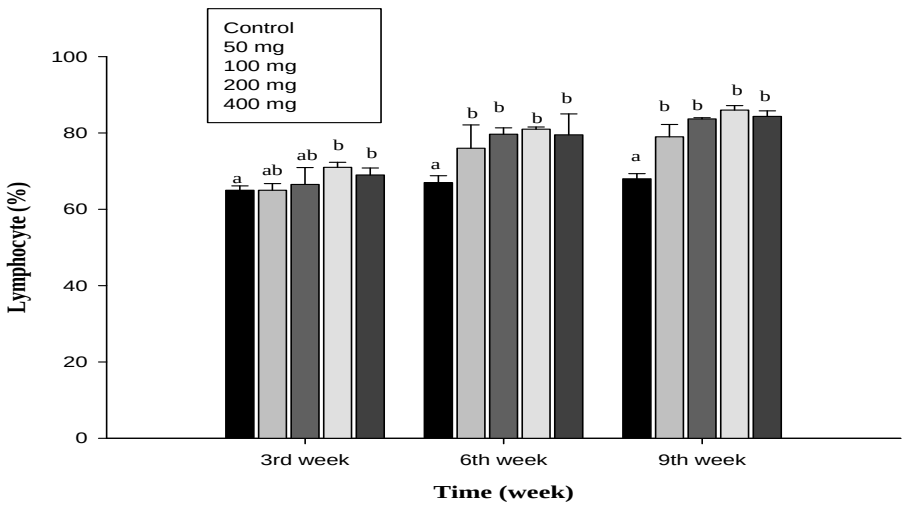


Figure 9: Effects of graded supplementary ascorbic acid on lymphocyte of Caspian brown trout (*Salmo trutta caspius*) during nine weeks. Different letters indicate significant differences between the treatments (P<0.05).

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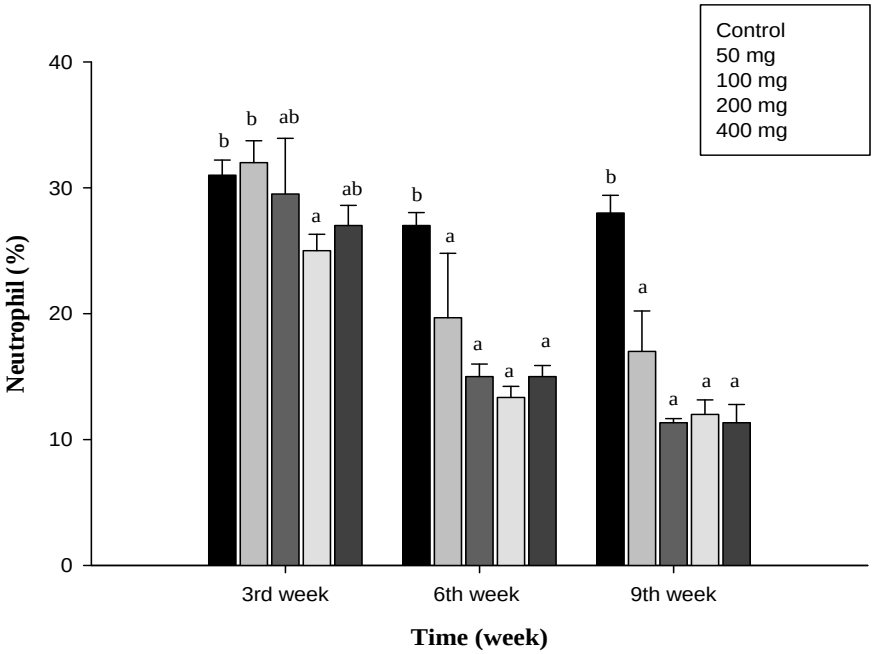


Figure 10: Effects of graded supplementary ascorbic acid on neutrophil of Caspian brown trout (*Salmo trutta caspius*) during nine weeks. Different letters indicate significant differences between the treatments (p<0.05).

Discussion

The Caspian brown trout is an economically valuable species which the recruitment activities affects their population in the southern area of the Caspian Sea. This study showed that ascorbic acid as an essential component of the diet increased the growing rate of Caspian brown trout fingerlings. Ascorbic acid is an essential coenzyme in tyrosine amino acid oxidation, phenylalanine (Brander and Pugh, 1977), lysine, proline and methionine. Therefore, more growth of Caspian brown trout fingerlings in fish fed by ascorbic acid concentrations could be confirmed by synthesis of more protein in muscle tissue (Arab *et al*., 2013). The higher SGR of Caspian brown trout fingerlings in this study can be due to the lower initial weight (9.6±0.6 g) and their potential to use supplementary factors like ascorbic acid in natural metabolisms and weight gain (Ibiyo *et al*., 2007; Adunni Adewolu and Aro 2009). Confirming the previous studies, this study showed that ascorbic acid can affect FCR (Ai *et al*., 2006; Adunni Adewolu and Aro, 2009). The lowest FCR in Caspian brown trout was found in 200 mg ascorbic acid treatment, although it had no significant difference with 400 mg ascorbic acid treatment.

Indeed, it is vital to use principles and tools to prevent fish against diseases and culture them healthy. White cells act as a defense barrier against pathogens and unwanted conditions. Any changes in blood cells number can be an appropriate index for evaluating fish immune conditions (Stoskopf, 1993; Hrubec *et al*., 2001).

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There are different factors causing improvement or weakness of defense system in fish which the most important one is feeding conditions (Houston *et al*., 1996; Kori-Siakpere *et al*., 2005; Orun and Talas, 2008b).

Ascorbic acid is known to perform numerous biochemical and physiological functions, especially in teleost fish. Ascorbic acid is an important nutrient in fish diets. In many types of fish, the biosynthesis of ascorbic acid does not occur. Accordingly, vitamin C must be added as a supplementary into the feed. The fish requirement is around 200 mg ascorbic acid kg^{-1}, although it is related to many factors like stress influence, growth rate, size of fish, temperature of water and other nutrients in the diet. The result of the present study showed that use of 200 mg ascorbic acid kg^{-1} before creating stress condition brings about improvement of fish immune system and their resistance to stress and diseases.

Different species of fish need different levels of ascorbic acid in their diet. For instance, rainbow trout (*Oncorhynchus mykiss*) is one of the most common species which was studied, associated with requirements for vitamin C. It is noted that an amount of 100 mg kg^{-1} dry weight would be enough for rainbow trout. In trout with muscular injury, the normal dose of 500 mg kg^{-1} dry weight should be given five times to cope with this situation. Also, a high level of vitamin C is added to the ratio would make the rainbow trout face an infectious disease (Halver, 2002). Excess dose of vitamin C will be resist rainbow trout injected with *Vibrio anguillarum* (Navarre and Halver, 1989). The same result has been obtained for viral diseases to rainbow trout with excessive amount of ascorbate-2-phosphate (Anggawati-Satyabudhy *et al*., 1989). However, it is stated by Halver (2002) that a dose of 200 mg kg^{-1} of dry ration would be sufficient, in the case of rainbow trout. The required level of ascorbic acid in Yellowtail (*Seriola dumerili*), European sea bass (*Dicentrarchus labrax*) and common carp (*Cyprinus carpio*) is 122 mg kg^{-1} (Masumoto *et al*., 1991), 20 mg kg^{-1} (Merchie *et al*., 1996) and 270 mg kg^{-1} (Gouillou-Coustans *et al*., 1998), respectively.

By implementing hematological techniques, including evaluation of erythrocyte count, hemoglobin concentration, hematocrit value and leukocyte count, valuable knowledge for fishery biologists in fish health assessment has been provided (Blaxhall, 1972; Talas *et al*., 2012). Results of this study emphasize on the previous findings about significant effects of ascorbic acid on RBCs (Clementi *et al*., 1997; Domezain *et al*., 1997). RBC, Hb and Hct of fish in control group were less than other treatments. There were also significant differences between control group and fish fed with 200 mg ascorbic acid kg^{-1} in third, sixth and ninth weeks of sampling. There is no significant difference between control group and

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fish fed by 50 and 100 mg ascorbic acid kg^{-1}, and between fish treated with 200 and 400 mg kg^{-1} ascorbic acid.

MCH and MCHC in fish fed with 200 mg ascorbic acid kg^{-1} were significantly more than control group just in the sixth week. In addition, no significant differences were observed in other times and in MCV. Since MCV and MCHC are a ratio between RBC, Hb and Hct, the results were predictable. The increase of Hb and MCHC indicates that vitamin C can be effective in hemoglobin concentration of fish erythrocyte.

WBC and ratio of different kinds of WBC are other important indexes for showing the state of health and immune system in animals (Shalaby *et al*., 2006; Ates *et al*., 2008; Talas *et al*., 2012). WBC in fish fed by diet supplemented with 200 mg ascorbic acid kg^{-1} was significantly more than the control group. In agreement with Kumari and Sahoo, (2005) also demonstrated that supplementation of 2000 mg ascorbic acid kg^{-1} in catfish diet improves growth performance and the immune system.

Supplementary ascorbic acid in fish feed increases the production of antibody against bacteria and improves phagocytosis and lysosome activity which have important roles to reduce stress (Hardie *et al*., 1991; Henrique *et al*., 1998; Shiau and Hsu, 2002; Talas *et al*., 2014). Lymphocytes are the most common type of WBCs that are used against viral diseases (Groff and Zinkl, 1999). The amount of lymphocyte in aquatic animals like mammalians is affected by stress and hormones (Secombes and Ellis, 2012). It has been proven that vitamin C regulated the lymphocyte B activity (Bergsten *et al*., 1995). Significant increase of lymphocyte level in fish, treated by using 200 mg kg^{-1} ascorbic acid in comparison with the control group, showed that cultured brown trout defense ability against viruses had increased. Incidence of viral diseases is a widespread problem that may threat Brown trout stocks in Caspian Sea. Ascorbic acid can improve fish defense ability by increasing lymphocyte production, especially when it is used for a long time. Increasing trend in lymphocyte production over time shows positive effects of ascorbic acid on immune system improvement, chronic stress reduction, fish adaptation to culture environment and increases body resistance to diseases in Caspian brown trout (Garcia *et al*., 2007; Innocent *et al*., 2011).

In the present study, neutrophil number in fish feed diet supplemented with ascorbic acid was significantly lower than control group. Also with increasing ascorbic acid level and time, more decrease was observed in neutrophil number. Neutrophil has phagocytes property and increases immunity against bacterial diseases. Different factors such as pathogens, stress and fish species could also influence on the number of neutrophil (Stoskopf, 1993; Talas *et al*., 2008; Orun *et al*., 2011). Normal culture

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condition and lack of stress cause reduction in neutrophil number (Davis *et al*., 2008). In the present study, neutrophil percentage decreased over the time which could be related to improvement of fish immune system and their easier adaptation to the culture condition. Enhanced innate immunity by increase of lysosome activity could be another reason for neutrophil reduction over the experiment.

In conclusion, the current research showed that dietary L-ascorbyl-2-polyphosphate influences the complete blood count of Caspian brown trout fingerlings after 9-week feeding trial with the optimum amounts by feeding 200 mg ascorbic acid kg^{-1} diet.

References

Ai, Q.H., Mai, K.S., Zhang, C.X., Xu, W., Duan, Q.Y., Tan, B.P. and Liufu, Z.G., 2004. Effects of dietary vitamin C on growth and immune response of Japanese seabass, *Lateolabrax japonicus*. *Aquaculture*, 242, 489-500.

Ai, Q., Mai, K., Tan, B., Xu, W., Zhang, W., Ma, H. and Liufu, Zh., 2006. Effects of dietary vitamin C on survival, growth, and immunity of large yellow croaker, *Pseudosciaena crocea*. *Aquaculture*, 261, 327-336.

Adunni Adewolu, M. and Aro, O.O., 2009. Growth, feed utilization and hematology of *Clarias gariepinus* (Burchell, 1822) fingerlings fed diets containing different levels of vitamin C. *American Journal of Applied Science Publication*, 6: 1675-1681.

Anggawati-Satyabudhy, A.M., Grant, B.F. and Halver, J.E., 1989. Effects of L-ascorbyl phosphates (AsPP) on growth and immunoresistance of rainbow trout to infectious hematopoietic necrosis (IHN) virus. Proceeding Third International Symposium on Feeding and Nutrition in Fish, Toba, Japan.

Arab, N., Rajabi Islami, H. and Shamsaei Mehrjan, M., 2013. Effect of dietary vitamin C on survival and growth parameters of Caspian brown trout (*Salmo trutta caspius*) fingerlings. *Journal of Fisheries*, 66, 331-346. (In Persian).

Armantrout, N.B., 1980. The freshwater fishes of Iran. Ph.D. Thesis Oregon State University. Oregon, USA, 427 P.

AOAC Association of Official Analytical Chemists, 2000. Official Methods of Analysis, Gaithersburg, Maryland, USA.

Ates, B., Orun, I., Talas, Z.S., Durmaz, G. and Yilmaz, I., 2008. Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) exposed to Pb^{2+} and Cu^{2+}. *Fish Physiology and Biochemistry*, 34, 53-59.

Bergsten, P., Yu-Kehrl, J. and Levine, M., 1995. Ascorbic acid transport and distribution in human B lymphocytes. *Archive of*

[Downloaded from jifro.ir on 2024-03-29]

[DOR: 20.1001.1.15622916.2016.15.3.19.7]

Biochemistry and Physiology, 317, 208-214.

Blaxhall, P.C., 1972. The haematological assessment of the health of the freshwater fish. A review of selected literature. *Journal of Fish Biology*, 4, 593-604.

Blaxhall, P.C. and Daisley, K.W., 1973. Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771-781.

Brander, G.C. and D. M., Pugh. 1977. Veterinary Applied Pharmacology and Therapeutics, Third Edition. The English language Book Society and Bailliere Tindall, London, UK.

Cerezuela, R., Cuesta, A., Meseguer, J. and Ángeles Esteban, M., 2009. Effects of dietary vitamin D_3 administration on innate immune parameters of seabream (*Sparus aurata* L.). *Fish and Shellfish Immunology*, 26, 243-248.

Clementi, M.E., Cataldi, E., Capo, C., Peteru Zzelli, R. and Giardina, B., 1997. Purification and characterization of the hemoglobin components from Adriatic sturgeon, *Acipenser naccarri*. Proceedings of 3rd International symposium of sturgeon, Italy.

Coad, B.W., 2000. Criteria for assessing the conservation status of taxa (as applied to Iranian freshwater fishes). *Biologia*, 55, 539-557.

Davis, A.K., Maney, D.L. and Maerz, J.C., 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology*, 22, 760-772.

Domezain, A., Garcia, O., Gallego, M., Domezain, J. and Sanz, A., 1997. Evolution during growth of the biometry and the blood constants of the sturgeon, *Acipenser naccarii*. Proceedings of 3rd International Symposium of sturgeons, Italy.

Garcia, F., Pilarski, F., Onaka, E.M., de Moraes, F.R. and Martins, M.L., 2007. Hematology of *Piaractus mesopotamicus* fed diets supplemented with vitamins C and E, challenged by *Aeromonas hydrophila*. *Aquaculture*, 271**,** 39-46.

Gouillou-Coustans, M.F., Bergot, P. and Kaushik, S.J., 1998. Dietary ascorbic acid needs of common carp (*Cyprinus carpio*) larvae. *Aquaculture*, 161, 453-461.

Groff, J.M. and Zinkl, J.G., 1999. Hematology and clinical chemistry of Cyprinid fish. Common carp and goldfish. *Veterinary Clinics of North America: Exotic Animal Practice*, 2, 741-746.

Halver, J.E., 2002. The vitamins. In: Halver, J.E. and Hardy, R.W. (Eds), Fish Nutrition, 3rd edition. Academic Press Inc, San Diego, CA, USA.

Hardie, L.J., Fletcher, T.C. and Secombes, C.J., 1991. The effect of dietary vitamin C on the immune response of the Atlantic salmon (*Salmo salar L.*). *Aquaculture*, 95**,** 201-214.

Henrique, M.M.F., Gomes, E.F., Gouilou-Coustans, M.F., Oliva-Teles, A. and Davies, S.J., 1998. Influence of supplementation of

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[DOR: 20.1001.1.15622916.2016.15.3.19.7]

practical diets with vitamin C on growth and response to hypoxic stress of seabream, *Sparus aurata*. *Aquaculture*, 161**,** 415-426.

Houston, A.H., Dobric, N. and Kahurananga, R., 1996. The nature of hematological response in fish. *Fish Physiology and Biochemistry*, 15, 339-347.

Hrubec, T.C., Smith, S.A. and Robertson, J.L., 2001. Age related in hematology and chemistry values of hybrid striped bass chrysops *Morone saxatilis*. *Veterinary and Clinical Pathology*, 30, 8-15.

Ibiyo, L. M. O., J. O. Atteh, J. S. Omotosho, and C. T. Madu, 2007. Vitamin C (ascorbic acid) requirements of *Heterobranchus longifilis* fingerlings. *African Journal Biotechnology*, 6(13): 1559-1567.

Innocent, B.X., Fathima, S.A. and Sivagurunathan, A., 2011. Haematology of *Cirrhinus mrigala* fed with vitamin C supplemented diet and post challenged by *Aphanomyces invadens*. *Journal of Applied Pharmaceutical Science*, 1, 141-144.

Kiabi, B.H., Abdoli, A. and Naderi, M., 1999. Status of fish fauna in south Caspian basin of Iran. *Zoology in the Middle East*, 16, 57-65.

Kori-Siakpere, O., Ake, J.E.G. and Idoge, E., 2005. Hematological characteristics of the African snakehead, *Parachacnna obscura*. *African Journal of Biotechnology*, 4, 527-530.

Kumari, J. and Sahoo, P.K., 2005. High dietary vitamin C affects growth, non-specific immune responses and disease resistance in Asian catfish, *Clarias batrachus*. *Molecular and Cellular Biochemistry*, 280, 25-33.

Magnadóttir, B., 2006. Innate immunity of fish (overview). *Fish and Shellfish Immunology*, 20(**2**), 137-151.

Masumoto, T., Hidetuyo, H. and Shimeno, S., 1991. Ascorbic acid's role in aquaculture nutrition. Proceedings of the Aquaculture Feed Processing and Nutrition Workshop. American Soybean Association, Indonesia.

Merchie, G., Lavens, P., Storch, V., Ubel, U., De Nelis, H., Leenheer, A. and Soregeloos, P., 1996. Influence of dietary vitamin C dosage on turbot (*Scophthalmus maxmus*) and European sea bass (*Dicentrachus labrex*) nursery stages. *Comparative Biochemistry and Physiology*, 11, 336-341.

Mulero, V., Esteban, M.A., Munoz, J. and Meseguer, J., 1998. Dietary intake of levamisole enhances the immune response and disease resistance of the marine teleosts gilthead seabream (*Sparus aurata* L.). *Fish and Shellfish Immunology*, 8, 49-62.

Navarre, O. and Halver, J., 1989. Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. *Aquaculture*, 79, 207-221.

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[DOR: 20.1001.1.15622916.2016.15.3.19.7]

Orun, I., Talas, Z.S., Ozdemir, I. Alkan, A. and Erdogan, K., 2008a. Antioxidative role of selenium on some tissues of (Cd^{2+}, Cr^{3+}) induced rainbow trout. *Ecotoxicology and Environmental Safety*, 71, 71-75.

Orun, I. and Talas, Z.S., 2008b. Antioxidative role of sodium selenite against the toxic effect of heavy metals (Cd^{2+}, Cr^{3+}) on some biochemical and hematological parameters in the blood of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). *Fresenius Environmental Bulletin*, 17(**9**), 1242-1246.

Orun, I., Talas Z.S. and Alkan, A., 2011. Modulating effect of selenium on gills of fish exposed to heavy metals. *Fresenius Environmental Bulletin*, 20, 104-108.

Ramezani, H., 2009. Effects of different protein and energy levels on growth performance of Caspian brown trout, *Salmo trutta caspius* (Kessler, 1877). *Journal of Fisheries and Aquatic Sciences*, 4, 203-209.

Saber, A., Abedian kinari, A.M. and Hayati F., 2005. Effects of dietary protein and energy levels on growth and body composition of Caspian brown trout (*Salmo trutta caspius*). *Journal of Marine science and Technology*, 4, 45-54. (In Persian)

Satheeshkumar, P., Ananthan, G., Senthil Kumar, D. and Jagadeesan, L., 2011. Haematology and biochemical parameters of different feeding behavior of teleost fishes from Vellar estuary, India. *Comparative Clinical Pathology*, 21(**6**), 1187-1191.

Secombes, C.J. and Ellis, A.E., 2012. The immunology of teleosts. In: Roberts, R.J. (ed) Fish Pathology, Fourth Edition. Wiley-Blackwell, Oxford, UK.

Shalaby, A.M., Khattab, Y.A. and Abdel Rahman, A.M., 2006. Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). *Journal of Venomous Animal and Toxins Including Tropical Disease*, 12, 172-201.

Shiau, S.Y. and Hsu, T.S., 1999. Quantification of vitamin C requirement for juvenile hybrid tilapia, *Oreochromis niloticus*×*Oreochromis aureus*, with L-ascorbyl-2 monophosphate-Na and L-ascorbyl-2-monophosphate-Mg. *Aquaculture*, 175, 317-326.

Shiau, S.Y. and Hsu, C.Y., 2002. Vitamin E sparing effect by dietary vitamin C in juvenile hybrid tilapia, *Oreochromis niloticus*× *O. aureus*. *Aquaculture*, 210, 335-342.

Soltani, M., Omidbeigi, R., Rezvani, S., Mehrabi, M.R. and Chitsaz, H., 2001. Study of anaesthetic effects induced by clove flower (*Eugina caryophillata*) on rainbow trout (*Oncorhynchus mykiss*) under various quality condition. *Journal of the Veterinary Medicine*, 56, 85-89. (In Persian)

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[DOR: 20.1001.1.15622916.2016.15.3.19.7]

Sotoudeh, E., Abedian kenari, A. and Habibi Rezaei, M., 2011. Growth response, body composition and fatty acid profile of Caspian brown trout (*Salmo trutta caspius*) juvenile fed diets containing different levels of soybean phosphatidylcholine. *Aquaculture International*, 19, 611-623.

Stoskopf, M.A., 1993. Fish medicine. Saunders Company, USA. 882P.

Svobodova, Z., Pravda, D. and Palakova, J., 1991. Unified methods of hematological examination of fish. Research institute of fish culture and hydrobiology. Vodnany, Czech Republic, 31P.

Talas, Z.S., Orun, I., Ozdemir, I., Erdogan, K., Alkan, A. and Yılmaz, I., 2008. Antioxidative role of selenium against the toxic effect of heavy metals (Cd^{2+}, Cr^{3+}) on liver of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). *Fish Physiology and Biochemistry*, 34, 217-222.

Talas, Z.S., Dundar, S.P., Gulhan, M.F., Orun, I. and Kakoolaki, S., 2012. Effects of propolis on some blood parameters and enzymes in carp exposed to arsenic. *Iranian Journal of Fisheries Sciences*, 11(**2**), 405-414.

Talas, Z.S., Mehmet Fuat Gulhan, M.F., Erdogan, K. and Orun, I., 2014. Researching of antioxidant effects of propolis in carp (*Cyprinus carpio*) exposed to arsenic: biochemical and histopathologic findings. *Diseases of Aquatic Organisms*, 108, 241-249.

Tewary, A. and Patra, B.C., 2008. Use of vitamin C as an immunostimulant. Effect on growth, nutritional quality, and immune response of *Labeo rohita* (Ham.). *Fish Physiology and Biochemistry*, 34, 251-259.

Vázquez, G.R. and Guerrero, G.A., 2007. Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). *Tissue Cell*, 39, 151-160.

Xiaoyun, Z., Mingyun, L., Khalid, A. and Weinmin, W., 2009. Comparative of haematology and serum biochemistry of cultured and wild Dojoloach *Misgurnus anguillicadatus*. *Fish Physiology and Biochemistry*, 33, 435-441.

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