# EFFECT OF DIETARY VITAMIN C ON SURVIVAL AND GROWTH PERFORMANCE OF CASPIAN BROWN TROUT (*SALMO TRUTTA CASPIUS*) FINGERLINGS

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## EFEKAT VITAMINA C IZ HRANE NA PREŽIVLJAVANJE I RAST MLAĐI KASPIJSKE POTOČNE PASTRMKE (*SALMO TRUTTA CASPIUS*)

*Apstrakt*

Ovo istraživanje je sprovedeno da bi se utvrdio uticaj vitamina C na rast, preživljavanje i hepatosomatski indeks (HSI) kaspijske potočne pastrmke (*Salmo trutta caspius*). Korišćeno je pet različitih vrsta hrane koje sadrže 0, 50, 100, 200 i 400 mg $kg^{-1}$ vitamina C u obliku L-Askorbil-2-polifosfata. 600 jedinki pastrmske mlađi (9,6±0,6 g) nasumično je podeljeno u pet tretmana sa po tri ponavljanja, od kojih je svaki sadržavao 40 riba. Hranjenje je vršeno devet nedelja a pokazatelji rasta, uključujući prirast (WG), specifičnu stopu rasta (SGR), factor kondicije (CF), stopu efikasnosti proteina (PER), stopu konverzije hrane (FCR), i HSI izračunati sun a kraju eksperimenta. Rezultati su pokazali da je stopa preživljavanja riba u svim tretmanima bila100 %, a tokom eksperimenta kod mlađi kaspijske pastrmke nisu zabeleženi znaci nedostatka askorbinske kiseline. Značajne razlike između tretmana ($p<0.05$) utvrđene su u pogledu finalne težine (FW), prirasta (WG), specifične stope rasta (SGR), stope efikasnosti proteina (PER), stope konverzije hrane (FCR), faktora kondicije (CF) i hepatosomatskog indeksa (HIS). Rezultati su pokazali das u ribe koje su hranom dobijale 200 mg $kg^{-1}$ askorbinske kiseline pokazale imale najbolje rezultate, iako nisu zapažene značajne razlike između tretmana sa 200 i 400 mg askorbinske kiseline. Dakle, za najbolji rast mlađi kaspijske potočne pastrmke optimalna količina vitamina C za svaki kilogram hrane je 200 mg askorbinske kiseline.

*Keywords*: Vitamin C, *Salmo trutta caspius,* preživljavanje, rast.
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## INTRODUCTION

Vitamin C (Ascorbic acid) is an essential vitamin which plays important roles in the physiological activities of animals like fish (Tolbert, 1979; Boyle, 2005). Due to the lack of L-Gulonolactone Oxidase enzyme, most of the teleosts cannot synthesize ascorbic acid and are depended to the diet supplementation (Fracalossi et al., 2001; Ai et al., 2004). Low levels of ascorbic acid will result in skeletal abnormality, impaired collagen formation, internal bleeding, reduced growth rate, and feeding appetite (Gouillou-Coustans et al., 1998; Oikawa et al., 2008). In cultured fish species diets with the lack of ascorbic acid may even leads to increased mortality rate (Ortuno et al., 2001; Lin and Shiau, 2005).

Adequate amount of ascorbic acid in fish diet, especially in life early stages, plays an important role in disease resistance followed by fish immunity and survival. However, ascorbic acid need is variable among different aquatic species and is accompanied by fish size, diet composition, and cultural system (Ortuno et al., 2001; Lim et al., 2002; Ai et al., 2004; Azad et al., 2007; Garcia et al., 2007). Therefore, determination of the vitamin requirements is necessary for normal growth and cultural performance.

The Caspian brown trout (*Salmo trutta caspius,* Kessler, 1877) is one of the salmonid species which is distributed in the south western coasts of the Caspian Sea and migrates to the freshwater rivers for spawning (Berg, 1949; Nikolskii, 1961). In spite of several studies on the nutritional requirement of the Caspian brown trout (Saber et al., 2005; Ramezani, 2009; Sotoudeh et al., 2011), little information is present about the vitamin needs as the most important factor in grows and immunity. Considering the vitamin C importance for reaching to suitable weight, the current research was done on the how different levels of ascorbic acid in fish diet will affect the survival, growth, hepatosomatic index of Caspian brown trout fingerlings.

Several studies have been done to preserve and restore the resources of the Caspian brown trout (Saber et al., 2005; Sayyad Burani et al., 2006; Zamani et al., 2007; Rahbar et al., 2009).

## MATERIALS AND METHODS

### Diets Preparation

Basal diet was prepared according to the Saber et al. (2005) suggestion for normal growth of Caspian brown trout to contain 49.60 % crude protein, 14.96 % lipid and 17.50 % ash. The other experimental foods were made by adding L ascorbyl-2-polyphosphate (LAPP) (25% ascorbic acid equivalent, Tiger, China) to obtain 0, 50, 100, 200 and 400 mg ascorbic acid equivalent $kg^{-1}$ diet, respectively. The precise level of ascorbic acid in each experimental diet was analyzed by a reverse-phase high-performance liquid chromatogram (HPLC, KNAUER pump 1000, German).

### Proximate Food Composition

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 a constant weight. Ash content was determined based on AOAC (1998) method 938.08 by heating an aliquot of the samples in a muffle furnace at 550 °C for 3 h and weighing the remaining 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 according to the Bligh and Dyer (1959)

method using a chloroform-methanol (1:1 by vol.) mixture. All of the measurements were done according to the AOAC (1995) and the result expressed as g/100 g diet.

Ascorbic acid content was also analyzed according to Shiau and Hsu (1999) with some modifications. Approximately 3–5 g of grounded feed 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 6 min at 2739 ×g. After this, 20 µl of supernatants were sieved through a 0.22 µm 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 2.8 with phosphoric acid) and the effluent was monitored by a UV-detector (254 nm wave length).

**Experimental Procedure**

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 food (Behparvar Co., Karaj, Iran). Average weight of fish fingerlings at the beginning of the test was adjusted to 9.6±0.6 g and water was provided from a spring with flow of 6 L $s^{-1}$ during the experiment.

This study was conductedin five raceway ponds (5×1×0.8 meter), and each had an input flow rate of 1 L $s^{-1}$. Each pond was divided to three equal parts by lace fabric with tiny holes and 40 fish fingerlings have been randomly distributed in these 15 testing areas. Mortality was not observed during periods of the adaptation and the experiment. The bioassay was in triplicate performed for nine weeks. The blocks were randomly divided into five treatments including 50, 100, 200 and 400 mg $kg^{-1}$ ascorbic acid along with control treatment. Fish were hand-fed to an apparent satiation at 8, 12 and 16 o'clock in each treatment and the consumed amounts of food were recorded for each pond. It should be noted that food consumption by fingerlings fish was too fast and no food remained in water. The water temperature, dissolved oxygen, pH and electrical conductivity were measured every week. During the experiment period, the temperature ranged from 9.6 to 10.2, the pH ranged from 7.5 to 7.7, electrical conductivity ranged from 248.3 to 252.8 and the dissolved oxygen content was approximately 10.2 mg $l^{-1}$.

Growth parameters were determined during nine weeks. The fish were fasted 24 hours before harvest. The total body weight (0.01 g) and total length (0.1 cm) were determined after anesthesia by 250 ppm clove oil based on Soltani et al. (2001) recommendation.

**Calculations and Statistical Analysis**

The growth parameter including weight gain (WG), specific growth rate (SGR), condition factor (CF), protein efficiency rate (PER), feed conversion rate (FCR), and hepatosomatic index (HSI) were calculated for each treatment based on the formula suggested by Sotoudeh et al. (2011).

All statistical analysis was done using SPSS-16 software package. The means were subjected to the analysis of variances (ANOVA) after examination of normality by Colmogornov-Smirnov test. Where the differences were occurred, the Tukey's HSD test was used to determine the difference. Results are expressed as mean±SE and a P-values less than 0.05 were determined as significant.

## RESULTS

### Proximate composition of experimental diets

The results of the proximate composition for each experimental diet are presented in Table 1. No significant difference was found between the treatments in the amount of moisture, protein, lipid, and ash. Reduction in the amount of ascorbic acid in all experimental diet after food preparation could be related to the heating of diet during the process.

**Table 1.** Proximate composition of experimental diets (%)

| | Moisture | Protein | Lipid | Ash | Ascorbic acid |
|---|---|---|---|---|---|
| Control | 5.23±0.35 | 49.6±0.25 | 15.01±0.57 | 17.40±0.13 | 9.8 |
| 50 mg AA | 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 |

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

### Survival and growth

The survival rate of fish in all treatments was 100% and no symptoms of ascorbic acid deficiency in the Caspian brown trout fingerlings were observed during the 9-week experiment. The results showed that nine weeks of testing 200 mg AA $kg^{-1}$ diet caused a significant increase in the growth compared with the control treatment and 50 mg AA $kg^{-1}$ diet ($p<0.05$), however no significant difference was observed in final weight of fishes with 100, 200 and 400 mg treatment after nine weeks (Figure 1).

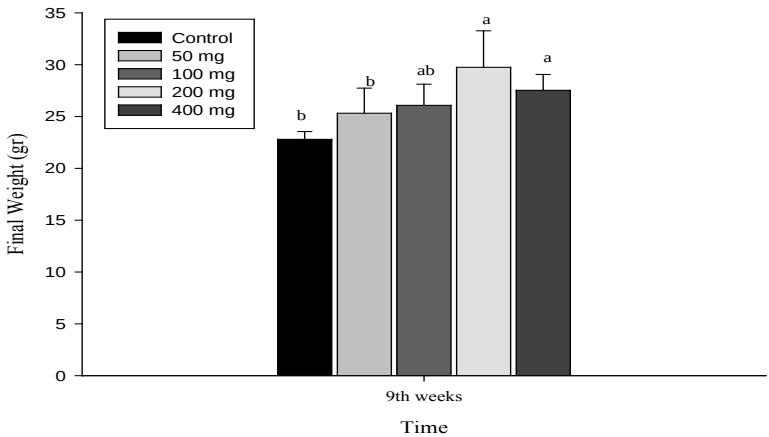


**Figure 1.** Effects of ascorbic acid on Final weight of Caspian brown trout (*Salmo trutta caspius*). Similar letters at the same column show no significant difference between results for each treatment ($p<0.05$).

Weight gain had also shown the same trend. The fingerlings fed by the control diet had the lowest weight gain, while the highest weight gain was found in the 200 mg AA treatment (20.10±0.98 g) after nine weeks. Moreover, the weight gain rate in 100 and 400 AA treatments were lower than 200 mg AA treatment ($p<0.05$), although no significant difference was recorded after the 9-week experiment (Table 2).

Specific Growth Rate (SGR) was also significantly different between treatments after nine weeks experiment ($p<0.05$). The 200 mg AA treatment had the maximum SGR with the rate of 0.77±0.02 % $d^{-1}$, while had no significant differences with 100 and 400 mg AA treatments. The control treatment had also the lowest rate of SGR by 0.59±0.03 % $d^{-1}$.

Specific Growth Rate in treatment of 200 mg ascorbic acid per 1 kg food was higher than in other treatments. However, there were no significant differences between treatments of 100 and 400 mg ascorbic acid per 1 kg food (Table 2).

**Table 2.** Weight gain and SGR of Caspian brown trout (*Salmo trutta caspius*) fed diets with graded levels of ascorbic acid for 9 weeks.

| Target vitamin C supplementation (mg $kg^{-1}$) | Analyzed ascorbic acid level (mg $kg^{-1}$) | Growth response | |
|---|---|---|---|
| | | Weight Gain (g) | SGR (% $d^{-1}$) |
| Control | 9.8 | 13.14±0.10$^{c}$ | 0.59±0.03$^{b}$ |
| 50 mg AA | 43.8 | 15.66±3.74$^{b}$ | 0.64± 0.11$^{b}$ |
| 100 mg AA | 89.4 | 16.42±1.27$^{b}$ | 0.68±0.03$^{ab}$ |
| 200 mg AA | 188.5 | 20.10±0.98$^{a}$ | 0.77±0.02$^{a}$ |
| 400 mg AA | 384.2 | 17.87±1.47$^{b}$ | 0.72±0.03$^{a}$ |

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

The results showed a significant difference the amount of Protein Conversion Ration (PER) between the treatments ($p<0.05$). The lowest and highest PER was observed in control and 200 mg AA treatments with the rate of 1.32±0.12 and 2.02±0.09, respectively. However, no significant difference was found between 200 and 400 mg AA treatments. Besides, the 50 and 100 mg AA treatments showed no significant differences in the amount of PER with control treatment (Table 3). Fish fingerlings fed by control diet had the highest FCR with ration of 1/52±0/02 after the 9-week experiment, although no significant difference showed with 50 and 100 mg AA treatments. The lowest FCR was observed in the 200 and 400 mg AA treatment the ratio of 0.99±0.07 and 1.13±0.16, respectively (Table 3).

An increasing trend of HIS was attained by increase of AA in the feeding diet. The highest HSI of 1.55±0.05% was recorded in 200 mg AA treatment, although had no significant differences with 400 mg AA treatment. No significant difference was observed between 50, 100, and 200 mg AA treatments. There was no significant difference in the value of Condition Factor (CF) between treatments (Table 3).

**Table 3.** Growth response of Caspian brown trout (*Salmo trutta caspius*) fed diets with graded levels of ascorbic acid for 9 weeks

| | PER | FCR | HSI | CF |
|---|---|---|---|---|
| Control | 1.32±0.12$^{b}$ | 1.52±0.02$^{a}$ | 1.30±0.08$^{c}$ | 1.00±0.04$^{a}$ |
| 50 mg AA | 1.57±0.37$^{b}$ | 1.49±0.46$^{a}$ | 1.40±0.12$^{b}$ | 1.00±0.02$^{a}$ |
| 100 mg AA | 1.65±0.12$^{b}$ | 1.23±0.16$^{a}$ | 1.44±0.07$^{b}$ | 1.02±0.05$^{a}$ |
| 200 mg AA | 2.02±0.09$^{a}$ | 0.99±0.07$^{b}$ | 1.55±0.05$^{a}$ | 1.03±0.04$^{a}$ |
| 400 mg AA | 1.80±0.14$^{ab}$ | 1.13±0.16$^{ab}$ | 1.46±0.03$^{ab}$ | 1.02±0.03$^{a}$ |

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

## DISCUSSION

The Caspian brown trout is of the economically valuable species which the recruitment rate affects their population in the southern area of the Caspian Sea. According to an accepted theory, fish with higher growing rate will have higher survival rate by rapidly change in their role from hunt to hunter (Bergenius et al., 2002). It means that specimens with higher weight will have more opportunities for remigrating to the maternal rivers.

This study showed that vitamin C as an essential component of the diet increases the growing rate of Caspian brown trout fingerlings. The lower final weight of the fingerlings in control treatment indicates the direct effect of vitamin C on the growth of Caspian brown trout. Ascorbic acid is an essential coenzyme in Tyrosine amino acid oxidation and phenylalanine (Brander and Pugh, 1977) which can increase weight gain and protein efficiency rate. Accordingly, the higher levels of ascorbic acid lead to the faster growth rate by increasing protein content (Brander and Pugh, 1977; Faramarzi, 2012), although determination of amino acid profile in Caspian brown trout fingerlings muscles is necessary for common scenes. Ascorbic acid can also affect the synthesis of collagen as a structural protein which their synthetize can cause higher final weight gain (Smedsrød et al., 1993;Terova et al., 1998).

No significant difference in growth rate of Caspian brown trout fingerlings in 200 and 400 mg ascorbic acid treatments may be related to the supplement of ascorbic acid needs for biological activities and its saturation in storage tissues such as liver and muscle. However, continuous adding of ascorbic acid into fish feed can result in a lower amount of vitamin C needed to prevent symptoms of ascorbic acid deficiency in the Caspian Brown trout fingerlings, such as deformation of the gill operculum and spine, internal bleeding and reluctance to absorb food, and even high level of mortality (Lin and Shiau, 2005; Xie and Niu, 2006; Ibiyo et al., 2007; Tewary and Patra, 2008). No effects of ascorbic acid deficiency in control treatment could be also related to the previous vitamin C storage in the target tissues of the fingerlings (Ai et al., 2004; Li et al., 2007; Soltani et al., 2008). Therefore, longer period of vitamin C absence may cause deficiency signs of ascorbic acid in the Caspian brown trout fingerlings.

Higher rate of HSI in this study indicates the higher storage of glycogen assisting the Caspian brown trout fingerlings in energy saving, resistance to stressful situations and immunity against pathogens (Moon et al., 1989; Sampaio and Criscuolo, 2006). Confirming the previous studies, this study shows that ascorbic acid can impact on FCR (Ibiyo et al., 2007; Soltani et al., 2008; Adewolu and Aro, 2009). The best 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. Therefore, 200 mg ascorbic acid can commercially be considered as the optimum amount for rearing of Caspian brown trout fingerlings by reduction of food costs and prevention of secondary contaminations.

The results of this study indicate that 200 mg ascorbic acid $kg^{-1}$ diet of the Caspian brown trout fingerlings have significant effect on their SGR compared to the other treatments. In contrast, other salmonid members have been shown no significant differences of SGR with similar or higher levels of ascorbic acid (Dabrowski, 1991; Thompson et al., 1993). The dissimilarity in results could be due to differences in species specific characteristics, water temperature, fish density and other cultural conditions. The initial weight of the specimens, a critical factor affecting SGR, in this study was 9.6±0.6 g which was lower than initial fish weight in similar studies (Dabrowski, 2001; Ai et al., 2004; Handeland et al., 2008). Therefore, the higher SGR of Caspian brown trout fingerlings in this study can be due to their lower initial weight and the potential to use ????? as a supplementary factor in natural metabolisms and weight gain (Ibiyo et al., 2007; Soltani et al., 2008; Adewolu and Aro, 2009). The condition factor in ninth weeks of the experiment had no significant difference between the experimental diets supplemented with or without ascorbic acid. In support of previous studies, condition factor higher than 1 in the present research illustrated that increasing ascorbic acid in the diet will improve the growing condition of Caspian brown trout fingerlings (Cetinkaya and Sen, 2005; Ibiyo et al., 2007; Adewolu and Aro, 2009).

## CONCLUSIONS

Results of the present study show that 200 mg ascorbic acid treatment have the best effect on growth rates of Caspian brown trout fingerlings compared with other experiment treatments. However, appropriate amount of ascorbic acid depends on the other factors such as age, weight, diet composition, physiological conditions, and the maturation stage (Dabrowski, 1986; Wang et al., 2002; Ai et al., 2004; Ibiyo et al., 2007). Further research particularly on the effect of ascorbic acid in non-specific immunity factors for longer periods will help to provide a more precise basis about the ascorbic acid necessity in Caspian brown trout diet.

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