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RESEARCH ARTICLE

The Effects of Different Levels of Ascorbic Acid on Growth Performance and Meat Composition of Brook Trout *(Salvelinus fontinalis)*

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Summary

The effects of different levels of vitamin C added in the diet of brook trout (34.65±0.45 g) for 10 weeks on growth, Condition Factor (CF), Feed Conversion Ratio (FCR) and meat composition were investigated. Fish samples were fed on nutrition added with 0, 100, 500 and 1.000 mg/kg of L-ascorbyl-2-monophosphate (AMP). Experiment was performed using a 3x4 completely randomized factorial design. According to the results, weights at the end of experiment demonstrated differences between the treatments. The highest value was obtained as 98.34±0.10 g with the nutrition added with 500 mg/kg of vitamin C, while the lowest value was observed as 92.40±0.19 g in the control group. Differences between different levels of vitamin C were found statistically significant (P<0.01). Differences between treatment groups regarding feed conversion ratio were also found statistically significant (P<0.01). The best feed conversion ratio was determined as 1.42±0.11 on average in the group fed on nutrition with 500 mg/kg vitamin C addition, and the lowest was as 1.68±0.22 in the control group. According to the results of the analysis on the characteristics of meat composition, condition factor was found statistically insignificant (P>0.05), while hepatosomatic and viscerosomatic indices were statistically significant (P<0.05).

Keywords: *Vitamin C, Brook trout (Salvelinus fontinalis), Growth performance, Meat composition*

Farklı Seviyelerdeki Askorbik Asidin Kaynak Alabalığı *(Salvelinus fontinalis)*'nın Büyüme Performansı ve Et Kompozisyonu Üzerine Etkileri

Özet

Kaynak alabalığının (34.65±0.45 g) 10 haftalık yemleme periyodunda yemlerine katılan farklı seviyelerdeki C vitamininin büyüme, Kondisyon Faktörü (KF), Yem Değerlendirme Değeri (YDD) ve et verim özellikleri üzerine etkileri incelenmiştir. Balıklar, yemlerine 0, 100, 500, 1.000 mg/kg miktarlarında L-ascorbyl-2-monophosphate (AMP) ilave edilerek yemlenmiştir. Deneme 3x4 faktöriyel düzenleme tam şansa bağlı plana göre yürütülmüştür. Elde edilen sonuçlara göre; deneme sonu ağırlıkları muameleler arasında farklılık göstermiştir. En yüksek değer 98.34±0.10 g ile 500 mg/kg seviyeli C vitamini katkılı yemle beslenenlerde, en düşük değerler ise, 92.40±0.19 g'la kontrol grubundan elde edilmiştir. C vitamini seviyeleri arasındaki farklılıklar istatistiki olarak önemli bulunmuştur (P<0.01). Yem değerlendirme değeri bakımından deneme grupları arasındaki fark da istatistiki olarak çok önemli bulunmuştur (P<0.01). En iyi yem değerlendirme oranı ortalama 1.42±0.11 ile 500 mg/kg C vitamini katkılı yemle beslenenlerde, en düşük oran ise, 1.68±0.22 olarak kontrol grubunda görülmüştür. Yaşama oranı yönünden gruplardan elde edilen sonuçlar arasındaki farklılık, C vitamini seviyeleri bakımından önemsiz bulunmuştur (P>0.05). Balık etinin verim özellikleri analizlerinden elde edilen sonuçların C vitamini seviyeleri bakımından; kondüsyon faktörü önemsiz (P>0.05), hepatosomatik indeks ve viscerosomatik indeks değerleri ise istatistiki olarak önemli bulunmuştur (P<0.05).

Anahtar sözcükler: *C vitamini, Kaynak alabalığı (Salvelinus fontinalis), Büyüme performansı, Et kompozisyonu*

INTRODUCTION

Ascorbic acid has significant functions in biochemical reactions of both plant and animal cells. Vitamin C, mainly functioning as a reducing agent in metabolism, has significant roles in physiological events like growth,

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reproduction, disease and stress resistance, antioxidation and the regulation of lipid metabolism [1,2]. This vitamin is reported to have significant functions such as the protection of enzymes and hormones from oxidation, providing stable growth rate, RNA synthesis, collagen synthesis and hydrogen transportation [3-5].

Collagen, an important component of skeleton formation, is required for normal growth. Ascorbic acid has a significant role in collagen formation [3,6]. Sato et al.[7] reported that collagen synthesis ability was weakened in the rainbow trout consuming less than 50 ppm ascorbic acid, while the collagen storage was found rather high in the fish consuming over 100 ppm ascorbic acid.

Many animal species have the ability of ascorbic acid synthesis; however, other high-level animals do not have such mechanisms. For this reason, they have to take necessary levels of vitamin C in diet. Ascorbic acid is a water soluble vitamin that can be synthesized from glucuronic acid, but can't be synthesized by the farmed aquatic species [3,5,7-9].

Nutrient raw materials do not contain adequate levels of ascorbic acid to meet the needs of fish. One of the main reasons for inadequacy of adding vitamin C in the fish commercial rations is that ascorbic acid is sensitive to heat, light and moisture, and could easily be oxidized into dehydroascorbic acid (an inactive form) in the production process. Ascorbic acid should be added in rations later due to the wastes in the preparation and storage period of nutrition [3,6,10,11].

Many researchers reported that ascorbic acid was an indispensable compound for trout and other salmonid species, and therefore should be present in diet [11-15]. Halver [3] reported that ascorbic acid loss was observed during preparation and storage of food, and its requirement increased with stress and disease; therefore, 400-500 mg/kg of ascorbic acid should be added to the diet.

Deficiency symptoms in fish due to the insufficient intake of ascorbic acid were studied by several researchers. Insufficient levels of ascorbic acid in diet could result in reduced hydroxylize activity in collagen. This abnormal collagen stimulates tissue deformation like cartilage scoliosis and lordosis. Collagen synthesis ability decline in the rainbow trout taking less than 50 ppm ascorbic acid. Amount of stored collagen was found higher in the trout taking over 100 ppm ascorbic acid [7]. Principal symptoms of vitamin C deficiency in trout fry was found as low growth speed, low feed conversion ratio, anemia, vertebral deformations (scoliosis, lordosis) and the lesions on gill and operculum [2,11,16].

Recently, some enterprises have started to raise brook trout *(Salvelinus fontinalis)* as alternative product to increase product variety or for hobby purpose in addition to rainbow trout to attract the attention of consumers and market. Different appearance (color, body form, etc.) and meat composition (crispy and less fatty) of this species which grows slower compared to rainbow trout make its production more attractive [17,18].

There have been many studies on rainbow trout. However, the number of studies on brook trout is limited, and especially the nutritional studies are insufficient. In this study, the effects of 0, 100, 500 and 1.000 mg/kg of ascorbic acid in diet on growth, feed conversion ratio, condition factor and meat composition of brook trout *(Salvelinus fontinalis)* are investigated.

MATERIAL and METHODS

This study was carried out in the Fisheries Research Center of Faculty of Fisheries, Rize University. A total of 1.080 brook trout juveniles were selected from 50.000 brook trout juveniles raised in Güneysu Dört Mevsim Trout Farm, and selected samples were randomly separated into tanks, each containing 90 fish. The juveniles had 34.65±0.45 g mean initial weight. Tanks were rectangular with a 1x1 m square base and 70 cm height (1x1x0.7 m), and contained about half ton water. The fish were adapted to the feed for a week before the experiment. Study was organized as 3x4 considering the simple experiment plan [19]. Investigation was carried out in twelve different tanks as three-repeated four units (one control and three different vitamin groups). Commercial extruded trout diets were hand fed according to fish sizes (No: 2, 3, 4) as nutrition material *(Table 1)*. Levels (0, 100, 500, 1.000 mg/kg) of ascorbic acid in diet were determined considering the results of similar studies [3,6].

Table 1. *Fish feed specifications*
Tablo 1. *Balık yeminin özellikleri*

Basic Nutrition Materials (%)	Extruded Pellet No. 2	Extruded Pellet No. 3, 4	Additives	
Dry matter (min)	90	90	Vitamin A IU/g	12.5
Raw protein (min)	47	44	Vitamin D_3 IU/g	2.5
Raw fat (min)	20	20	Vitamin E mg/kg	200
Raw ash (max)	10	10	Ethoxyquin mg/kg	5
Raw cellulose (max)	2	2	BHT mg/kg	150
			Cu mg/kg	96

Ascorbic acid doses (0, 100, 500, 1.000 mg/kg) were measured using a sensitive scale (±0.1 mg). All the nutrition compositions excluding control group were spread on aluminum folio and sprayed with vitamin C dissolved in pure water. Nutrition compositions were stirred for the absorbance of vitamin C. Commercial diets containing vitamin C were calculated from the existing tables which are based on living fish percentage weight and water temperature [3,20]. We estimate that average water temperature over the experimental period was 11°C. There is a ±1°C variation due to seasonal changes, but in general daytime water temperature was fairly constant over the experimental period. The fish in the all groups were fed 1% of their body weight twice per day (at 09:00 and 17:00 h) for 70 days. Fish were weighted with ±0.1 mg sensitive scale a every 14 days during experiment. After fish were weighted, feed amounts that would be given to each group were calculated [21].

At the end of the experiment 4 juveniles randomly chosen from each of the total 12 units were weighed on a digital scale with 0.001g precision, their weights were determined, their total length was measured with 0.1 cm error, and their values of head, fin, carcass weight, hepatosomatic index and viscerosomatic index were measured respectively in order to determine meat composition as described by Moccia et al.[22].

Experiment lasted for 10 weeks. Data obtained in the experiment were subjected to variance analysis. "Statistica for Windows" and one-way analysis of variance (ANOVA) [23] were used in the statistical analysis of data, the mean and standard deviation (SD±) were calculated for all parameters in each group and the mean values were compared with Duncan Test [24,25]. Results obtained in the study were evaluated using the following formulas.

Specific Growth Rate (SGR,% day^{-1}) = 100 x ([ln W_2 - ln W_1]/ feeding days), where W_2 is the final weight (g) of the fish, W_1 is the initial weight (g) [26].

Feed Conversion Ratio (FCR) = F/(A_2 + D - A_1) [20]

F = food amount given in a period (g)

A_1 = weight at the beginning of period (g)

A_2 = weight at the end of period (g)

D = weight of dead fish in period (g)

Condition Factor (%CF) = (W/L^3) x 100 (W = weight (g), L = total height (cm) [27]

Hepatosomatic Index (%HSI) = liver weight (g)/body weight (g) x 100

Viscerosomatic Index (%VSI) = weight of internal organs (g)/ body weight (g) x 100 [28]

RESULTS

Data obtained in this study was taken in 14 days periods during experiment. Average weight of fish increased from 34.65±0.45 g to 92.40±0.19, 96.53±0.15, 98.34±0.10 and 94.71±0.21 g, respectively, at the end of 70 days study *(Table 2)*. Analysis of variance was applied to all data, and according to the results, data was tested at 0.01 and 0.05 confidence levels, then the Duncan's multiple comparison tests was applied to the significant factors. Consequently, changes in specific growth rate, feed conversion ratio were determined in 14 day periods.

Fig.1 shows specific growth rate (SGR) versus time (in periods). In this study, the highest specific growth rate was observed in the first period in 500 mg/kg of vitamin C, and the lowest was found in the fourth and fifth periods in control group. 500 mg/kg group has the highest and control group has the lowest SGR throughout the experiment.

Fig. 2 shows feed conversion ratio (FCR) versus time (in periods). As can be seen in *Fig. 2*, control group had the highest feed conversion ratio (1.86) in the fifth period. 500 mg/kg group has the best (lowest) and control group has the worst (highest) FCR throughout the experiment.

Feed conversion ratios were calculated at the end of this study. Accordingly, it was determined as 1.68±0.22, 1.54±0.19, 1.42±0.11 and 1.47±0.18, respectively. The best FCR is obtained in 500 mg/kg group and worst FCR is obtained in the control group. Results obtained in the study and the multiple comparison tests are given in *Table 3*. As can be seen in this Table, vitamin C addition in the diets had very significant effects on specific growth rates (P<0.01) and feed conversion ratio; however, it was not significant on condition factor (P>0.05). Its effects on hepatosomatic and viscerosomatic indices were found statistically significant (P<0.05).

At the end of study, analysis of body composition was

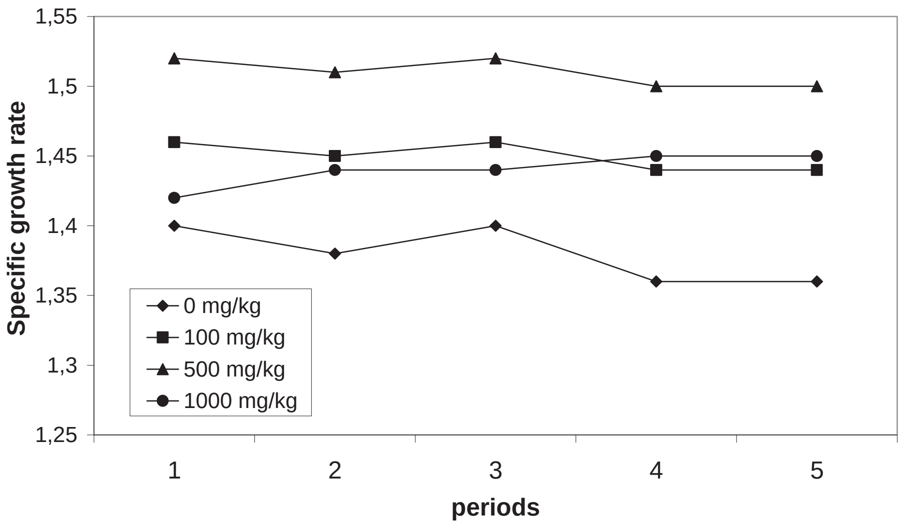

Fig 1. Specific growth rates of brook trout *(S. fontinalis)* in study groups fed with different levels of vitamin C

Şekil 1. Farklı seviyelerde C vitamini ile beslenen kaynak alabalıklarında *(S. fontinalis)* araştırma gruplarına ait spesifik büyüme oranları

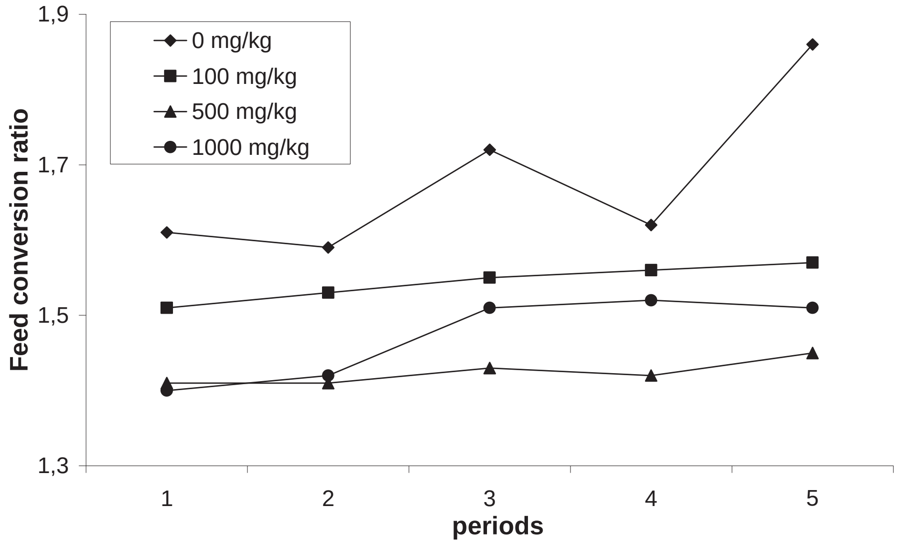

Fig 2. Feed conversion ratio degrees of brook trout *(S. fontinalis)* in study groups fed with different levels of vitamin C

Şekil 2. Farklı seviyelerde C vitamini ile beslenen kaynak alabalıklarında *(S. fontinalis)* araştırma gruplarına ait yem değerlendirme değerleri

Table 2. *Some data obtained from the study*

Tablo 2. *Araştırmadan elde edilen veriler*

Groups	Ascorbic Acid Doses (mg/kg feed)			
	0 X±Sx	100 X±Sx	500 X±Sx	1.000 X±Sx
Initial fish number	270	270	270	270
Fish number at the end of the experiment	257	265	267	267
Mean Individual initial weight (g)	35.05±0.12	35.00±0.25	34.10±0.21	34.45±0.17
Mean Individual final weight (g)	92.40±0.19	96.53±0.15	98.34±0.10	94.71±0.20
Mean weight increase (g)	57.35 ±0.30	61.53±0.40	64.24±0.31	60.26±0.36

X±Sx= Mean value±Standard deviation of mean value

Table 3. *Results of Duncan's test for certain characteristics of brook trout (S. fontinalis) in study groups fed with different levels of vitamin C*

Tablo 3. *Farklı seviyelerde C vitamini ile beslenen kaynak alabalıklarında (S. fontinalis) araştırma gruplarına ait Duncan testi sonuçları*

Parameters	Ascorbic Acid Doses (mg/kg feed)			
	0	100	500	1000
WG(g)±SE	57.35 ±0.30 ^{a}	61.53±0.40^{b}	64.24±0.31^{c}	60.26±0.36^{b}
SGR(%)±SE	1.38±0.05^{ab}	1.45±0.08^{a}	1.51±0.11^{c}	1.44±0.04^{a}
FCR±SE	1.68±0.22^{b}	1.54±0.19^{a}	1.42±0.11^{c}	1.47±0.18^{bc}
CF(%)±SE	1.09±0.04^{a}	1.12±0.32^{a}	1.15±0.10^{a}	1.14±0.07^{a}
HSI(%)±SE	1.46±0.01^{a}	1.51±0.05^{ab}	1.63±0.11^{b}	1.62±0.27^{b}
VSI(%)±SE	10.1±0.05^{c}	9.41±0.21^{b}	9.37±0.05^{a}	9.45±0.06^{b}

WG: *mean weight gain (g),* ***SGR:*** *mean specific growth rate (%),* ***FCR:*** *mean feed conversion ratio,* ***CF:*** *condition factor (%),* ***HSI:*** *hepatosomatic index (%),* ***VSI:*** *viscerosomatic index (%),* ***SE:*** *standard error. Superscripts means that entries within the same row by vitamin C levels which do not share a common superscript letter are significantly different (P<0.05)*

carried out by taking four fish from each group. Protein contents of carcass obtained from groups were 19.27±0.21%, 19.38±0.07%, 19.96±0.11% and 19.53±0.04%, and fat contents were 9.88±0.03%, 8.97±0.12%, 8.02±0.17% and 9.11±0.09%. Results of body proximate analysis and Duncan's multiple comparison tests were given in *Table 4*. Accordingly, vitamin C addition in the diets had statistically insignificant effects on protein and fat content of fish carcass.

Table 4. *Results of body proximate analysis of brook trout (S. fontinalis) in study groups fed with different levels of vitamin C*

Tablo 4. *Farklı seviyelerde C vitamini ile beslenen kaynak alabalıklarında (S. fontinalis) araştırma gruplarına ait vücut proximate analiz sonuçları*

Parameters	Ascorbic Acid Doses (mg/kg food)			
	0	100	500	1000
Dry matter (%)	18.25±0.09	22.17±0.05	24.93±0.23	21.66±0.12
Protein (%)	19.27±0.21[a]	19.38±0.07[a]	19.96±0.11[a]	19.53±0.04[a]
Fat (%)	9.88±0.03[a]	8.97±0.12[ab]	8.02±0.17[b]	9.11±0.09[ab]
Water (%)	81.75±0.09	77.83±0.05	75.07±0.23	78.34±0.12
Ash (%)	1.06±0.18	1.41±0.12	1.58±0.20	1.33±0.08

Different letters (a, b) represents statistical differences within the same row by vitamin C levels (P<0.05)

DISCUSSION

Results obtained in the study were given in *Table 2* and *Table 3*. As can be seen in *Table 3*, different levels (0, 100, 500, 1.000 mg/kg) of vitamin C in diets had positive effects on fish growth. In the group of 500 mg/kg vitamin C, absolute growth 6.89 g higher than control group and percentage growth was 20% better. Difference between groups was found statistically very significant in the analysis of variance (P<0.01).

Altan [29] reported similar results for rainbow trout (*Oncorhynchus mykiss*) in his study investigating the effects of different vitamin C levels in juvenile diets and found the lowest absolute growth as 5.90±0.64 g in control group and the highest as 7.02±0.12 g in the group of 2500 mg/kg vitamin C addition.

In our study 500 mg/kg group gave better result than 1.000 mg/kg group. So there must be an optimal dose below 1.000 mg/kg. Giving more dose than this optimum value does not do much good to the fish. Various doses are used in studies in literature ranging from 50-100 mg/kg to thousands of mg/kg. The optimal dose may depend on age of fish and species of fish and size of fish. Albert and Tacon [30] determined optimal vitamin levels around 50-500 mg/kg for trout in their studies considering the size and age of species. In this study we studied only four levels of vitamin-C doses and the results of 500 mg/kg are better than both 100 mg/kg and 1.000 mg/kg doses. So the optimal value is between these limits for brook trout. Results of our study are similar to findings of Albert and Tacon.

One of the most important factors in aquaculture is feed conversion ratio (FCR) which is a measure of mass of feed that must be given to produce one unit of fish mass. Emre and Kürüm [31], and Çelikkale [20] stated that feed conversion ratio should be between 1 and 3 for trout. Feed conversion ratio is desired to be as low as possible since the feed cost normally accounts for more than 50% of the total expenses of intensive trout farming. Therefore, it should not be more than 2 for profitable farming [32]. Any minor change in FCR can reduce farming costs significantly. Their difference 1.68-1.42=0.26 is rather significant for cost reduction in farming. This means that if 500 mg/kg dose is given, we save cost of 0.26 kg of feed per one kg of fish mass produced. The results show that vitamin-C had a positive effect on FCR.

There are other reports of positive effects of vitamin C on FCR in the literature. For instance, Altan [29] stated in his study investigating the effects of different levels of vitamin C in the diets of juvenile rainbow trout that the best feed conversion ratio was observed as 1.09±0.06 in the group containing vitamin C, and the worst was as 1.26±0.10 in the control group. Althought the fish in Altan's study and ours are not the same, they are from the same family and the findings of this study are compatible with their findings. We expect that these results are valid at least for all fish from trout family.

Another important factor in aquaculture is Specific Growth Rate (SGR). SGR is the percent growth of fish in one day. If SGR stayed constant over the fish life we would observe an exponential growth. However like many other living things the growth rates decrease by age for fish. So the SGR depends on age. In this study we found SGR around 1.4-1.5%. Since the experimental period is relatively short the SGR remained roughly constant over the experimental period. For juvenile rainbow trout this number is roughly around 2.5-3%. Brook trout is a slower growing species than rainbow trout. We found in this study that the control group had a roughly 1.4% SGR and 500 mg/kg group had a 1.5% SGR *(Table 3, Fig. 1)*. Other two vitamin groups had approximately 1.45% SGR. Therefore optimal dose of vitamin-C is between 100 and 1.000 mg/kg and giving this optimal dose increases SGR by a 0.1% at least.

Due to the low sale price of fatty fish, certain measures should be taken to prevent excessive fat formation in fish tissues [28,33]. Ascorbic acid is related to two hydroxylases of carnitine biosynthesis and acts as an active carrier in lipid transportation into mitochondria for lipids to be oxidized through B-oxidation [1,6]. This fact suggests that ascorbic acid could have significant effects on fat percentage of carcass. Results of our study indicate that ascorbic acid reduces the fat percentage a little (about 1-2%) but the effect is minimal. Also an effect of ascorbic acid on protein percentage of carcass is little or no effect. We conclude that ascorbic acid does not affect fat and protein percentages of carcass significantly. This study findings are compatible with the results of the previous studies on the subject and demonstrate that vitamin C addition in fish diets contributes to growth of fish using less amounts of food in a shorter time.

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