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## Effects of Figs and Rosemary Extracts on Rainbow Trout (*Oncorhynchus mykiss*) on Growth Performance and Blood Parameters

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### Abstract

In this study the effects of dietary fig and rosemary extract supplementation on growth performance, feed utilization, biometric indexes and some blood parameters in rainbow trout (*Oncorhynchus mykiss*) were investigated. Two experimental diets were supplemented with medicinal herb extracts at 0,5,1,2 g/kg. The fish were fed 60 days of trial diet. The addition of fig extract and rosemary extract did not have a positive effect on development and feed intake. In addition, the addition of fig extract and rosemary extract did not cause a change in the number of red blood cell count, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration ( $P>0.05$ ). However, the addition of fig extract and rosemary extract to the feed increased the spleen somatic index, while decreasing viscerosomatic index and hepatosomatic index ( $P<0.05$ ).

**Keywords:** *Oncorhynchus mykiss*, fig extract, rosemary extract, growth performance, feed utilization, blood parameters

### İncir ve Biberiye Ekstraktlarının Gökkuşluğu Alabalığı (*Oncorhynchus mykiss*)'nda Gelişim Performansı ve Kan Parametreleri Üzerine Etkileri

#### Özet

Bu çalışmada incir ve biberiye ekstraktının Gökkuşluğu alabalığı'nda (*Oncorhynchus mykiss*) büyüme performansı, yem değerlendirme ve kan parametreleri üzerine etkileri araştırılmıştır. Bu amaçla iki bitki ekstraktı deneme yemlerine 0,5-1 ve 2g/kg oranlarında ilave edilmiştir. Balıklar 60 gün boyunca deneme yemleriyle beslenmiştir. Araştırmamız sonucunda, yeme incir ekstraktı ve biberiye ekstraktı ilavesinin gelişim ve yem alımı üzerinde olumlu etkisi bulunmamıştır. İlave olarak, yeme incir ekstraktı ve biberiye ekstraktı ilavesi kırmızı kan hücre sayısı, hematokrit miktarı, ortalama eritrosit hacmi, eritrosit başına düşen hemoglobin miktarı ve eritrosit başına düşen ortalama hemoglobin konsantrasyonu seviyelerinde bir değişikliğe sebep olmamıştır. Bununla birlikte, yemlere incir ekstraktı ve biberiye ekstraktının ilavesi viscerosomatik indeks ve hepatosomatik indeksi azaltırken, dalak somatik indeksini artırmıştır.

**Anahtar kelimeler:** *Oncorhynchus mykiss*, incir ekstraktı, biberiye ekstraktı, büyüme performansı, yem dönüşümü, kan parametreleri

## INTRODUCTION

The amount of fisheries production has been increasing in our country and in the world. The obtained amount of fisheries through cultivation has reached about 73 million tons in the world while it has reached 240 thousand tons in Turkey (Fao, 2016). This situation leads to more intensive use of the resources and increases the risk of stress and makes fish suffer from diseases. Antibiotics and a variety of synthetic chemicals used for the prevention of diseases and stress cause undesired chemical usages in terms of environment and consumers as well as economic losses (Yıldırım and Okumus, 2004). In addition, the problems arising from the different stages of production of fish and fish exposed to such applications directly affect the production and business economy. Sudden light changes, pathogenic microorganisms in adaptation periods and species-specific developmental stages, manipulations, water quality and stocking density are some of the examples (Can, 2006; Altun et al., 2007). Furthermore, fish are influenced by various stress factors such as stocking density, water

pollution, inadequate food, handling and transportation. These conditions adversely affect fish health and increase the risk of disease. Various chemicals (antibiotics, hormones, chemotherapeutics and vitamins) in order to reduce or prevent such effects have been used for many years in aquaculture industry (Citarasu, 2010). Moreover, synthetic substances are intended for the treatment of diseases, coloration, strengthening the immune system, prevention of stress, improvement of the feed intake and to promote the growth. However, damages caused by the chemicals to the environment, fish and human as a result of fish consumption are undesirable (Harikrishnan et al., 2011). Therefore, the additives used for combating the diseases to increase production through aquaculture, to grow fish faster, to make them more resistant to the ambient conditions and the drugs used in various stages in today's cultivation are being replaced by organic products. Today, activities related to alternative sources have been increased in all areas to prevent everlasting destruction of irreversible damages caused by the use of chemicals. These kinds of researches have been conducted and progressed in the field of agriculture and animal husbandry. As an alternative to the use of chemicals; many substances such as marine algae, probiotics, bacterial compounds, vegetable feed additives and enzymes have been used in many studies (Sakai, 1999; Nikoskelainen et al., 2003; Bonaldo et al., 2007; Torrecillas et al., 2007; Firouzbaksh et al., 2011). Rosemary and *Ficus carica* are known to have antimicrobial and antioxidant effects (Ryu and Jung, 1999).

In the limited number of researches carried out with the aim of determining the opportunity of using plants and the active agents they contain in the cultivation, it has been reported that plant extracts added to the feed and water enhance the feed consumption, feed conversion, and growth and carcass quality (Simsek et al., 2005; Immanuel et al., 2009; Oskoi et al., 2012). Several studies have reported that oral administration of fig extract in *Paralichthys olivaceus* (Cho, 2011), and *Paralichthys olivaceus* (Lee et al., 2015) and rosemary extract in African catfish (Turan and Yiğitaslan, 2016) improved growth performance.

The aim of this study was to investigate the effects of fig and rosemary extracts on growth performance and blood parameters of rainbow trout (*Oncorhynchus mykiss*).

## MATERIALS and METHODS

### Fish and Experimental Protocol

This study was approved by the Local Ethical Committee of the Adnan Menderes University (Protocol Number: 2018/017). The experiment was set up in commercial trout farm (Cansuyu) in Denizli. Feed analyzes were made in the Tarbiyomer unit of Adnan Menderes University, blood analysis was done in a special medical laboratory. In experiment, 21 polyester tanks (3×0.8×0.6 m) and 840 rainbow trout (*O. mykiss*) (initial mean weight  $12.47 \pm 0.15$ ) were used. The experiment was conducted in the flow-through system. Each trial group was carried out with triplicate. Fig extract (Talya herbal product) and rosemary extract (Talya herbal product) were added to a commercial trout feed (BioAqua, Turkey, pellet size: 2 mm, Table 1). The herbs extract were added to the feed at 0,5 g/kg (Groups F1, R1)-1 g/kg (Groups F2, R2)-2 g/kg (Groups F3, R3). The addition of extracts to the feed was carried out with alcohol via spraying method. With the aim of protecting the activities of the components in plant extracts, feeds were prepared on a weekly basis and stored in glass flacons at +4°C. Additionally, a control group was fed a diet without herbal extract supplementation (Sonmez et al., 2015). Fish were fed 2 times a day in the ratio of % 2 of their body weight during adaptation and experiment period (60 days). Water temperature, dissolved oxygen, and pH were measured at 10.5°C, 8.5 mg·L<sup>-1</sup>, 9.6, respectively.

Proximate analyses of commercial trout extruder feed were performed using standard methods (AOAC, 1998). Moisture was detected after drying at 105°C until a constant weight was achieved. Crude protein was analyzed by the Kjeldahl method, and crude ash by incineration at 525°C in a muffle furnace for 12 h. Crude fat was analyzed by methanol/chloroform extraction (Folch et al., 1957). (Table 1)

**Table 1.** Commercial trout extruder feed (pellet size: 2 mm) ingredients

Parameters	Values
<i>Proximate analyses</i>	
Crude Protein (%)	49
Crude Lipid (%)	19
Crude Cellulose (%)	3
Crude Ash (%)	13
<i>Macro elements (%)</i>	
Calcium %	1-2
Total Phosphore %	1.5
Sodium	0.2/1

Ingredients: Fish meal, fish oil, soybean and by products, wheat and by products, yeast and by products, amino acids, vitamins and minerals.

### Growth Performance and Biometric indices

Growth performance and feed utilization were calculated according to the formulae given below:

WG (g) = final weight (FW) (g) – initial weight (IW) (g)

SGR = (%/d) [(ln final weight (g) - ln initial weight (g)) / days] x 100

FCR = feed intake (g) / weight gain (g)

CF = body weight(g)/standard length<sup>3</sup> x 100

Biometric indices were calculated from the following formulae:

Visceral fat index (VFI) = {wet weight of visceral fat (g)/[wet body weight (g) – wet weight of visceral fat (g)]} × 100

Hepatosomatic index (HSI) = {wet weight of liver (g)/[wet body weight (g) – wet weight of liver (g)]} × 100

Viscerosomatic index (VSI) = {wet weight of viscera and associated fat (g)/ [wet body weight (g) – wet weight of viscera and associated fat (g)]} × 100

Bilesomatic index (BSI) = {wet weight of bile (g)/[wet body weight (g) – wet weight of bile (g)]} × 100

Spleen somatic index (SSI) = {wet weight of spleen (g)/[wet body weight (g) – wet weight of spleen (g)]} × 100

### Blood Collection

Blood samples of five fish/groups were collected randomly from the caudal vein using a vacutainer fitted 5 mL on day 60. For blood sampling, fish were anaesthetized with MS222 (Sigma Aldrich, Steinheim, Germany) (Smith et al., 2007). They were well wiped and cleaned in order to avoid mucus mixing into the blood, and blood was taken from the fish through the caudal vein by a 2.5-mL plastic syringe without harming the fish. Then, 200 µL of blood was transferred to ethylenediaminetetraacetic acid (EDTA) tubes (BD, Oxford, UK) for hematological analysis. The other 600 µL of blood was harvested in plastic biochemistry tubes (Vacutest Kima s.r.l., Piove di Sacco, Italy). After the blood was coagulated, the tubes were centrifuged at 4000 × g for 10 min for serum separation, which was stored below –20°C (Bricknell et al., 1999).

### Hematological Analysis

Red blood cells (RBC, 10<sup>6</sup> mm<sup>3</sup>), hematocrit (Hct, %) and hemoglobin (Hb, g/dL) were determined by using the method by Blaxhall and Daisley (Blaxhall and Daisley, 1973). RBC was counted with a Thoma hemocytometer using Dacie's diluting fluid. Hct was determined using a capillary hematocrit tube. Hb concentration was determined by spectrophotometry (540 nm) using the cyanomethaemoglobin method. The hematological indices of mean cell haemoglobin concentration (MCHC: g·dL<sup>-1</sup>), mean cell haemoglobin (MCH: pg) and mean cell volume (MCV: fL) were calculated using the total RBC count, Hb concentration and Ht (Lee et al., 1998). Mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the following formula (Bain et al., 2006).

$$\begin{aligned} \text{MCV } (\mu\text{m}^3) &= [(\text{Hct}, \%) \times 10] / (\text{RBC}, \times 10^6 \text{ per mm}^3) \\ \text{MCH (pg)} &= [(\text{Hb}, \text{g/dL}) \times 10] / (\text{RBC}, \times 10^6 \text{ per mm}^3), \text{ and} \\ \text{MCHC } (\%) &= [(\text{Hb}, \text{g/dL} \times 100) / (\text{Hct}, \%) \end{aligned}$$

### Statistical Analysis

The variation analyses were carried out with Duncan multiple comparison tests, and the differences between groups were carried out via the use of SPSS 21 statistics program in order to evaluate the relationships between the data of blood, proximate composition and growth parameters obtained from the test group.

### RESULTS

There was not any significant differences were detected related to weight increase, feed conversation ratio and specific growth rate between the experimental group and the control group. (Table 2,  $P > 0.05$ ). Growth data are presented in Table 2.

**Table 2.** Growth performance and feed utilization in *Oncorhynchus mykiss* that were fed diets containing different levels of fig and rosemary extract (0 (Control), 0,5, 1, or 2 g/kg of feed; for 60 day)

	Control	F1	F2	F3	R1	R2	R3
<b>Initial fish weight (g)</b>	12.45±0.13	12.40±0.076	12.42±0.02	12.41±0.10	12.49±0.05	12.52±0.07	12.56±0.13
<b>Final fish weight (g)</b>	25.38±0.20	25.42±0.18	25.82±0.26	25.87±0.16	25.38±0.30	25.31±0.14	25.34±0.61
<b>Weight gain (%)</b>	516.80±13.07	520.80±9.35	536.00±11.20	538.40±4.45	515.86±13.31	511.33±8.22	511.06±28.58
<b>FCR</b>	0.89±0.007	0.89±0.007	0.88±0.010	0.88±0.002	0.90±0.010	0.90±0.004	0.90±0.020
<b>SGR (%/d)</b>	0.64±0.01	0.64±0.01	0.65±0.01	0.66±0.00	0.63±0.01	0.63±0.00	0.63±0.02

Values are mean ±SE (n=6).

There was not any significant differences were detected related to crude protein, crude lipid, crude ash and crude moisture between the experimental group and the control group. (Table 3,  $P > 0.05$ ). The whole-body proximate compositions of fish presented in Table 3.

**Table 3.** Whole-body proximate composition (%) of *Oncorhynchus mykiss* fed diets with different levels of fig and rosemary extract for 60 day

Composition (%)	Control	F1	F2	F3	R1	R2	R3
<b>Crude Protein</b>	15.40±0.62	14.05±0.66	15.77±1.71	14.58±0.86	15.08±0.54	14.86±0.92	14.36±0.48
<b>Crude Lipid</b>	4.29±0.16	4.26±0.19	4.49±0.74	4.63±0.20	4.54±0.51	4.26±0.48	4.08±0.21
<b>Crude Ash</b>	2.82±0.87	3.39±1.12	2.86±0.73	2.32±0.24	2.91±0.31	2.77±0.37	2.77±0.09
<b>Moisture</b>	71.12±1.04	72.90±1.06	72.01±1.78	71.44±0.69	70.38±1.80	71.69±2.34	72.18±0.84

Values are mean ±SE (n=6).

**Table 4.** Viscerosomatic index (VSI), Hepatosomatic index (HSI), Spleen somatic index (SSI) and Visceral fat index (VFI) of *Oncorhynchus mykiss* fed different diets containing fig and rosemary extract extract for 60 day

	Control	F1	F2	F3	R1	R2	R3
<b>VSI</b>	15.93±0.57 <sup>a</sup>	13.75±0.53 <sup>b</sup>	13.74±1.10 <sup>b</sup>	14.15±0.26 <sup>b</sup>	13.86±0.94 <sup>b</sup>	14.17±0.56 <sup>b</sup>	14.24±0.79 <sup>b</sup>
<b>HSI</b>	1.48±0.01 <sup>a</sup>	1.13±0.28 <sup>b</sup>	1.10±0.05 <sup>b</sup>	1.11±0.06 <sup>b</sup>	1.16±0.10 <sup>b</sup>	1.18±0.03 <sup>b</sup>	1.20±0.03 <sup>b</sup>
<b>SSI</b>	0.13±0.01 <sup>b</sup>	0.20±0.01 <sup>a</sup>	0.21±0.02 <sup>a</sup>	0.20±0.01 <sup>a</sup>	0.20±0.02 <sup>a</sup>	0.19±0.03 <sup>a</sup>	0.18±0.01 <sup>a</sup>
<b>VFI</b>	2.85±0.80	2.35±0.16	2.43±0.31	2.53±0.23	2.37±0.17	2.51±0.03	2.37±0.07

Values are mean ±SE (n=6). Different letters in the same line indicate significant differences among groups (P<0.05).

At the end of 60 days, VSI, and HSI values were found lower in the group fed with fig and rosemary extracts compared to the control group (P<0.05) (Table 4). SSI values were found higher in the groups fed with fig and rosemary extracts compared to the control group (P<0.05) (Table 4). On the other hand, VFI index not affected by application of these plant extracts.

**Table 5.** Hematological parameters in rainbow trout that were fed diets containing different levels of fig and rosemary extract for 60 day.

Blood Parameter	Control	F1	F2	F3	R1	R2	R3
<b>Hb(g·dL<sup>-1</sup>)</b>	6.26±0.46	6.86±0.96	6.67±0.73	6.59±1.49	6.68±1.18	6.75±1.50	6.86±1.65
<b>Hct (%)</b>	35.40±2.70	37.80±0.83	37.00±1.58	36.60±1.81	36.40±0.89	36.60±1.14	37.00±2.82
<b>RBC(10<sup>6</sup> mm<sup>3</sup>)</b>	3.54±0.13	3.77±0.13	3.69±0.16	3.65±0.16	3.59±0.23	3.62±0.20	3.69±0.27
<b>MCV(μm<sup>3</sup>)</b>	99.78±5.62	100.14±1.26	100.03±1.09	100.21±2.24	101.63±6.68	101.21±3.28	100.26±0.57
<b>MCH(pg)</b>	17.68±1.40	18.20±2.81	18.10±2.50	18.11±4.30	18.61±3.20	18.60±3.73	18.48±3.58
<b>MCHC(g·dL<sup>-1</sup>)</b>	17.81±2.19	18.17±2.69	18.09±2.46	18.05±4.13	18.38±3.37	18.43±4.00	18.42±3.50

Values are mean ±SE (n=6).

The effects of extracts on rainbow trout hematological variables are presented in Table 5. The RBC count, Hb concentration, Hct, MCV, MCH, and MCHC in the treatment groups did not very significant from the values observed for the control group (p>0,05).

## DISCUSSION

The addition of fig extract to rainbow trout feeds did not positively affect growth according to the results of this study. Similarly, Cho (2011) stated that fig extract did not show positive effect on growth in rainbow trout. Lee et al. (2015) also reported no change in the survival rate, weight increase and specific growth rate of *Paralichthys olivaceus* (6.5 g) that was fed with a diet containing % 2,5 fig (*Ficus carica*).

The addition of rosemary extract to rainbow trout feeds did not positively affect on growth in the present study. Similarly, Kivrak and Didinen (2017) stated that rosemary oil did not positively affected on rainbow trout growth. In contrast, Turan and Yiğitaslan (2016) reported rosemary extract has positive effect on growth in African catfish with no apparent effects on health status. This difference between the findings might be due to the use different fish species in different experiments.

The use of medical plants in fish newly started in this field. It was reported that fig extract, onion extract, and indian fig have no effect on the condition factor and HSI (Cho, 2011). For example, in a study carried out with rats, it was reported that rosemary and thyme were more effective than many medicinal plants (including cummin) in decreasing α-glucosidase activity of intestine and it was deduced that especially rosemary could be used to prevent obesity and diabetes (Koga et al., 2006). In addition, rosemary showed antidiabetogenic feature in rabbits. (Bakirel et al., 2008). Rosemary's effect of reducing on α glucosidase enzyme was associated with its prevention of fat absorption. For

this reason, it should not be ignored that rosemary can prevent fat absorption in fishes. However, in the present study, visceral fat index (VFI) relatively decreased by use of fig and rosemary extracts in rainbow trout ( $p>0.05$ ).

In this study, use of fig and rosemary extracts in feed did not significantly affect on the red blood cell count, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in rainbow trout. Similarly, Kivrak and Didinen (2017) stated that the use of rosemary oil in the feed of rainbow trout does not change the number of erythrocytes and hemoglobin concentration.

In conclusion, the addition of rosemary and fig extracts to the feed did not affect on the growth and hematological parameters in rainbow trout. However, use of these plant extracts in rainbow trout feeds increased spleen somatic index and decreased viscerosomatic index and hepatosomatic index. In addition, visceral fat index relatively decreased in rainbow trout. Therefore, these plant extracts should be further studied to explore reducing effects of fish body lipid in aquaculture.

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