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Chemical Composition Of Root Parts Of Ferula Elaeochytris Korov. And Its Food-Borne Effects On Growth Of Goldfish (*Carassius auratus L.*)

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Abstract
The Ferula genus that belongs to Apiaceae has been used in traditional medicine in the Middle East, Central Asia and Mediterranean since its properties such as aphrodisiac, antibacterial, antiviral, antifungal and as well as its positive effects on dyspepsia, arthritis, and diabetes. The present study examined the dietary effects of root parts of Ferula elaeochytris on growth of Goldfish (Carassius auratus), indicating its chemical composition. For this purpose, fish fed with experimental diets containing five different levels of F. elaeochytris (control, 0.5‰, 1 ‰, 0.5 %, 1%,) a period of 60 days. Relatively growth rate (RGR), feed conversion ratio (FCR), and specific growth rate %/day (SGR) were calculated. 180 of C. auratus individuals in total were used for five treatments in triplicates. The root parts of F. elaeochytris were extracted by different methods to determine total protein and essential oil compositions. The WG, FCR, and SGR values of C. auratus have shown significant changes in the treatments with the supplementation of F. elaeochytris root to feed ($P<0.05$), comparing to the control. The highest WG and SGR values were found in 0.5 % of F. elaeochytris root treatment as 81.99±1.53% and 0.99±0.01 respectively. These values in the control were 58.92±2.56 and 0.79±0.07, respectively. Also, the lowest FCR value, 1.33±0.09, was found in 0.5 % of F. elaeochytris root treatment. These results indicated profitable effects of dietary containing 0.5 % of F. elaeochytris root on growth parameters of C. auratus.

Keywords: Ferula elaeochytris, Chemical composition, C. auratus, Growth parameters

INTRODUCTION

Nowadays, aquaculture production is increasingly demanded due to facing to decrease or extinction risk for natural fish stocks. The aquaculture industry, as an important branch of food production in the world, has an around 10% rate of growth. All over the world, aquaculture total production reached 55 million tons through fish farming facilities in 2004 while 101 million tons in 2012 [1]. On the other hand, this kind of production reached 80.000 tons in 2000, exceed 240.000 tons in 2015 in Turkey [2]. The growing aquaculture industry has caused increase in its requirements. Synthetic chemicals, such as antibiotics and chemotherapeutics which have ability to provoke growth and struggle for diseases have been used widely. However, overuses of these chemicals have some harmful effects on not just fish health but also other animals, and even human. For instance, usage of antibiotics can induce antibiotic resistance of fish pathogens to these antibiotics over time. Therefore, plant extracts and essential oils are considered as alternatives of these chemicals in aquaculture.

Previous studies have shown that some plant extracts affect fish growth performance positively. For instance, Dügenci et al., 2003 [3] suggested that extracts of mistletoe (*Viscum album*) and nettle (*Urtica dioica*) at a ratio of ‰1 in feed of rainbow trout (*Oncorhynchus mykiss*) significantly increased specific growth rate (SGR) when compared with the control. Çağıltay et al., 2011[4] added bay leaf (*Laurus nobilis*) to rainbow trout feed, and observed a decrease in food conversion ratio (FCR) and an increase in SGR. Bahabadi et al., 2014 [5] reported that ‰1, 5 and 10 of dietary administration of yarrow extract caused increase in weight gain SGR, and condition factors, decrease in FCR in rainbow trout after 15 and 30 days of feeding periods.

Ferula (Apiaceae) is a genus of about 185 species of flowering plants. This genus distributes from the eastern Mediterranean, the Middle East, and the Central Asia. The *genus* Ferula contains not only tannins, starch, essential oils saponins, terpenes, and resins but also phytoestrogens which could have effects on some reproductive hormones such as derivatives and analogues of progesterone, testosterone and estrogen [6], [7].

There is limited knowledge about the effects of *Ferula* species on fish growth performance. Therefore, in the present study, the aim was to investigate the effects of *Ferula elaeochytris* Korov. root powder on growth performance of goldfish (*Carassius auratus*).

MATERIALS AND METHODS

Experimental fish and conduction of experiment

180 goldfish with an average weight of 11.09 ± 0.80 grams were used in the experiment. This study was approved by the local ethical committee of Çanakkale University. Fish were distributed among 15 experimental glass aquariums as 12 fish/aquarium, triplicate for each treatment. In order to provide adaptation of the fish to the experimental environment, they were fed with commercial feed for 15 days. Experiments were initiated by individual weighing of fish. In the 60-day experiment, fish were fed by hand at 2% of their body weight twice a day.

Experimental Feeds

Experimental feeds were prepared at Çanakkale Onsekiz Mart University, Faculty of Marine Science and Technology, Fish Feeding and Feed Laboratory. Fish meal, soy meal, wheat meal, corn starch, fish oil, vitamin, mineral mixtures used in experimental feeds were obtained from a fish feed factory, while *Ferula elaeochytris* root powder was obtained from a commercial firm. Formulations with similar protein

(35%) and oil (7%) content were prepared following the nutritional analyses such as moisture, protein, oil and ash on feedstuffs. For the study, *F. elaeochytris* root powder was added to the control feeds corresponding to ‰ 0.5, **‰** 1, %0.5 and %1. All raw materials were sieved prior to feed preparation, then passed through feed mill. Primarily, dry raw materials and then liquid raw materials were homogenized in laboratory type feed mixer. Thereafter, pellets obtained by passing through a laboratory type pelletizing machine were dried to a humidity of 10% in an air-circulated environment. The prepared feeds were stored in locked polyethylene bags at -20˚C until the beginning of feeding experiments. Ingredients and chemical composition analyses of the prepared feeds are given in Table 1.

Calculation of Growth Performance Indices and *F. elaeochytris* extraction

Growth performance and feed utilization were calculated using following equations;

FCR (Feed conversion ratio) = feed consumed / weight gain

RGR (Relatively growth rate %) = [(final wet weight - initial wet weight) / initial wet weight] × 100

SGR (Specific growth rate %/day) = [(ln final wet weight – ln initial wet weight) / days] × 100

F. elaeochytris root powder extracted with ethyl acetate. The essantial oils were analyzed by a Shimadzu GC-MS QP 2010 Ultra system. A library search was carried out using the Wiley GC-MS Library. Another extraction was prepared by a Tris buffer, pH 7.5 for total protein concentration which was determined by Bradford protein assay [8].

Statistical analyses

Values of all measured variables are expressed as least square means and standard deviation. Statistical significance was determined by one-way analysis of variance (ANOVA), followed by Tukey pairwise comparisons. Statistical significance was established at $P < 0.05$.

Table 1. Dietary ingredients and chemical analyses of the diets used in the study.

Control	‰ 0.5	‰ 1	% 0.5	% 1
Dietary ingredient (%)				
Fish meal	23.00	23.00	23.00	23.00	23.00
Soybean meal	37.00	37.00	37.00	37.00	37.00
Wheat meal	12.00	12.00	12.00	12.00	12.00
Fish oil	5.00	5.00	5.00	5.00	5.00
Mineral/ vitamin premix[1,2]	4.00	4.00	4.00	4.00	4.00
Starch	19.00	18.50	18.50	18.50	17.00
Ferula elaeochytris	0	0.05	0.10	0.50	1.00
Total	100	100	100	100	100
Chemical analyses (Dry matter. %)					
Protein	35.17	35.59	35.22	35.18	35.47
Lipid	7.21	7.32	7.19	7.40	7.15
Ash	5.63	5.74	5.68	5.36	5.19

[1]Vitamin Mix: Vit. A, 18000 IU; Vit. D3, 2500 IU; Vit. E, 250 mg/kg; Vit. K3, 12 mg/kg; Vit. B1, 25 mg; Vit. B2, 50 mg; Vit. B3, 270 mg; Vit. B6, 20 mg; Vit. B12, 0.06 mg; Vit. C, 200 mg; Folic acid, 10 mg; Calcium d-pantothenate, 50 mg; Biotin, 1 mg; Inositol, 120 mg; Choline chloride, 2000 mg.

[2]Mineral Mix: Fe, 75.3 mg; Cu, 12.2 mg; Mn, 206 mg; Zn, 85 mg; I, 3 mg; Se, 0.350 mg; Co, 1 mg

RESULTS AND DISCUSSION

The species of Ferula *genus* have some applications in both traditional medicine and industry. They could be used as anti-microbial, anti-oxidant, anti-carcinogenic, antispasmodic, molluscicidal and antithelminthic (particularly for intestinal parasites), and also for stomach pain, coronary disease, lowers cholesterol and wound-healing remedy. Moreover, these species are advertised as a natural therapeutic for sexual function enhancement [9], [10]. These characteristics of Ferula *genus* are encouraged usage of them as feed additives. For this reason, *F. elaeochytris* root powder, which is a commercially available product, has been added to feed of Goldfish (*Carassius auratus*) to reveal the effect of it.

In this study, the total protein concentration of *F. elaeochytris* root powder was found as 20.5±1.8 mg/g dry wet. According to the essential oil analyses, the main components of *F. elaeochytris* root powder were determined as α–pinene, nonane, ß–pinene, naphthalene, hexenal, octanal, and ß–caryophyllene. Başer et al., 2000 [1] reported that nonane, α–pinene, ß-caryophyllene, and germacrene B were composed of more than 50% of total essential oils of *F. elaeochytris*. There are contents differences not just among the Ferula species, but also a species from different localization. For instances, essential oil compositions of *F. orientalis* from different localities had different oil components, and also different quantities of essential oils [12].

Fish have an advantage over other farm animals in terms of effectiveness of growth performance. Many of farm animals are homeotherms, and they spend their energy obtained from feed for maintaining thermal homeostasis, but fish are poikilotherms [13]. This kid of loss of energy is not subjected in fish farming. Feeding in fish farming directly affects growth performance, and it is easily modifiable. Therefore, determination of feed ratio and feed strategy depending on species has a critical role on fish feeding. The direct effects of feed and its ingredients on growth performance are needed to be calculated. For this reason, some calculations can be used such as feed conversion ratio (FCR) meaning to the number of units of feed a fish produced one unit of product, specific growth rate (SGR) meaning to estimation of production of fish after a certain period, relative growth rate (RGR) meaning to growth rate relative to size. Growth in fish is correlated with proper environmental factors, proper ingredients of feed for relevant fish species, and digestibility of feed [14]. Therefore, most of the attention has been paid to several herb extracts and they were tested for their growth promoting activity in aquatic animals [15], [16].

The use of chemicals to promote growth and resistance against bacterial disease in fish culture result in many problems like resistance to antibiotics while the utilization of chemicals can be harmful to fish and consumer health as well as the environment [17]. Natural products constitute a viable substitute to antibiotics in aquaculture. The most important of the possibilities natural products pose is the use of herb extracts. They are obtained from many plant materials such as flowers, buds, seeds, leaves, fruits [18]. Accordingly, this study determined the effect of *F. elaeochytris* root powder on the growth performance of goldfish as a growth promoter.

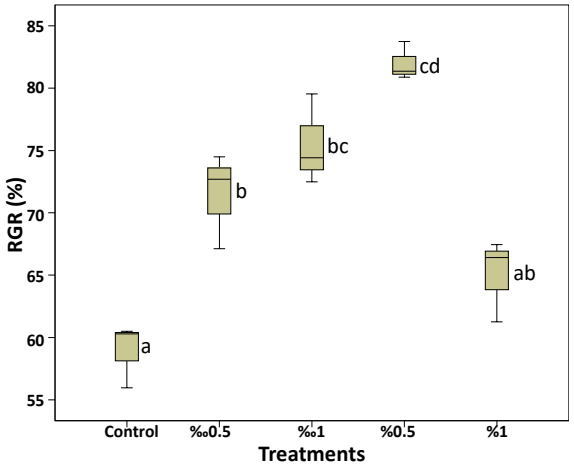

Figure 1. Relative growth rate (RGR) of goldfish (*Carassius auratus*) in the control and the treatments (*Ferula elaeochytris* in the feed at a rate of ‰ 0.5, ‰ 1, % 0.5, and % 1) at the end of the experiment. The same superscript letters did not differ from each other ($P \leq 0.05$). The box plot represents the median and 25/75th percentile.

During the feeding experiment in the current study the average water temperature was 20±0.2°C, the dissolved oxygen was 8.5±0.2 mg L^{-1} and the pH was 8.10±0.2. No mortality was recorded during the experiment. The relative growth rate (RGR%), feed conversion rate (FCR) and specific growth rate (SGR) values of goldfish have shown in Figure 1, Figure 2 and Figure 3, respectively. Significant differences obtained in the treatments with the supplementation of *F. elaeochytris* root to feed ($P<0.05$), comparing to the control. The highest RGR and SGR values were found in 0.5 % of *F. elaeochytris* root treatment as 81.99±1.53% and 0.99±0.01 respectively. These values in the control were 58.92±2.56 and 0.79±0.07, respectively. Also, the lowest FCR value was found as 1.33±0.09 in 0.5 % of *F. elaeochytris* root treatment.

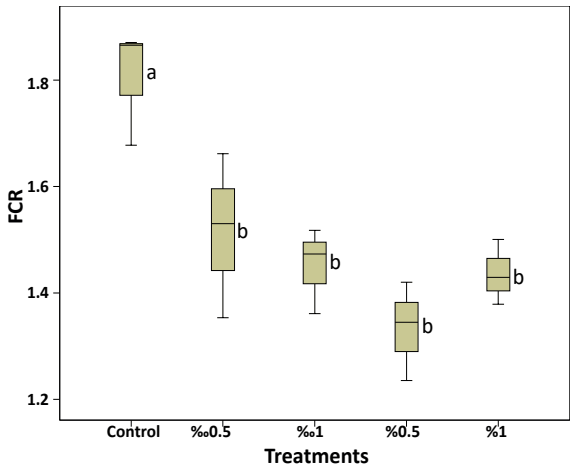

Figure 2. Feed conversion ratio (FCR) of goldfish (*Carassius auratus*) in the control and the treatments (Ferula elaeochytris in the feed at a rate of ‰ 0.5, ‰ 1, % 0.5, and % 1) at the end of the experiment. The same superscript letters did not differ from each other ($P \leq 0.05$). The box plot represents the median and 25/75th percentile.

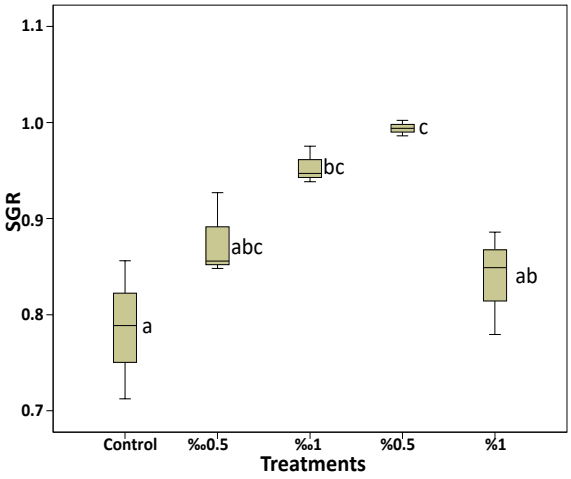

Figure 3. SGR (Specific growth rate) of goldfish (*Carassius auratus*) in the control and the treatments (*Ferula elaeochytris* in the feed at a rate of ‰ 0.5, ‰ 1, % 0.5, and % 1) at the end of the experiment. The same superscript letters did not differ from each other ($P \leq 0.05$). The box plot represents the median and 25/75th percentile.

Yılmaz et al., 2006 [19] reproted that *F. coskunii* 1.5, 3, and 4.5 g/kg in diet, no significant differences in growth performances but body composition in *Cyprinus carpio* fry. They showed that *F. coskunii* suplamentation to the feed caused an increased in protein and lipid content of body composition. The obtained different growth performance results between their study and the present study may be caused by the difference of Ferula species or fish species. Moreover, they used fry fish while mature fish were used in the current study. Positive effects of herbal extracts on growth performance of different fish have been reported by other authors [20], [21] and [22]. For instance, dietary supplementation of *Rehmannia glutinosa* increased the growth rates of *C. carpio*. The positive effects of *F. elaeochytris* root powder on growth performance could explain with the *F. elaeochytris* root powder components resulting in better intestinal health and improved digestion and absorption. However, the reduction in growth performance of goldfish in this study in 1% *F.elaochytris* group can be explain in dose dependent manner. In earlier studies, growth performance in dose-dependent manner following use of some herbal feed additives in fish species were reported by Acar et al., 2015 [23] and and Baba et al., 2017 [24] . The selection of optimum dose is highly significant for the effective use of herbal supplements as feed additives in fish diets,

CONCLUSION

This study provided the effects of *F.elaochytris* as a feed additive on fish growth. The fish fed with the diet containing 0.5% *F. elaochytris* root powder had no negative effects on goldfish survival also, significantly improved the growth performance of fish. This natural feed additive may represent an alternative to chemicals in goldfish aquaculture practices as an growth promoter. Further research on health status of goldfish should be conducted to better understand the effects of *F.elaochytris* root powder in fish culture.

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