How the Use of Orange (*Citrus sinensis*) Peel Essential oil Affected the Growth Performance of Rainbow Trout (*Oncorhynchus mykiss*)?

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Abstract

The most important portion of the aquaculture production with 40-60% of the operating expenses is the feed cost. The decline in natural fish stocks and the resulting increase in the cost of fish meal have led to feed producers and researchers to search for new and/or alternative raw materials for fish feed production. Orange peel essential oil (EO) contains 94.72% D-limonene. Four essential oil supplemented diets (0%, 0.5‰, 1‰ and 3‰) prepared for rainbow trout juvenile. Four groups fish were stocked 100 L aquarium (55 fish per aquarium) with free flow water system for triplicate study in 90 days feeding trial. At the end of the 90-days experimental feeding period; feed conversion ratio is importantly decreased, relative growth rate, specific growth rate and protein efficiency ratio are importantly increased at the 1‰ rate orange peel essential oil additives group ($p < 0.05$).

Keywords: *citrus sinensis*, essential oil, rainbow trout

INTRODUCTION

Aquaculture production is increasing due to decrease of natural stocks. The amount of world aquaculture production is 101 million tons, of which approximately 50% is fish production. The decline in natural fish stocks and the resulting increase in the cost of fish meal have led to feed producers and researchers to search for new and/or alternative raw materials for fish feed production. It is expected that these raw materials, which are considered as alternatives at the beginning, be provide many positive factors in aquaculture as well as being an alternative nowadays. In these studies, plants and microorganisms or extracts derived from its; such as essential oils, active substances, polysaccharides and oligosaccharides are used (Gültepe *et al.*, 2011, 2014, 2015a, 2017; Acar *et al.*, 2015). Also, the use of antibiotics is restricted and/or prohibited, especially for the resistance of

fish pathogens, the effects of aquatic microbiota, ecological balance and human health. Researchers are started the search for natural products, such as; non-specific immune system stimulating and/or supporting products, enzymes, organic acids, probiotics-prebiotics and plant extracts, instead of chemicals for breeding animals. Essential oils are on the GRAS list of foods published by US FDA (Gültepe *et al.*, 2015b). There are two different hypotheses for mechanism of essential oil. The first of these, improving of nutrient utilization through the increasing of enzyme volume and activity at the result of stimulation of endogenous enzymes. The second is the regulation of the intestinal microbial flora and the protection of the animal health (Zhang *et al.*, 2005; Gültepe, and Kesbiç, 2016).

The amount of world orange production is approximately 69 million tons; this is 57% of all

Table 1. Formulation and chemical composition of the diets

	<i>Ingredients (g kg⁻¹)</i>			
	Control	0.5‰	1‰	3‰
Fish meal	48.00	48.00	48.00	48.00
Soy meal	25.00	25.00	25.00	25.00
Wheat meal	9.20	9.20	9.20	9.20
Fish oil	10.00	10.00	10.00	10.00
Vit-Min. Mix*	4.00	4.00	4.00	4.00
Corn starch	3.80	3.75	3.70	3.50
OEO	0.00	0.05	0.10	0.30
	<i>Chemical composition (g kg⁻¹, DM)</i>			
	Control	0.5‰	1‰	3‰
Protein	45.20	45.20	45.20	45.20
Lipid	15.10	15.10	15.10	15.10
Ash	8.53	8.53	8.53	8.53
Carbohydrate	27.20	27.20	27.20	27.20

*Vit-Min. (Vitamin-Mineral) per kg diet: 18000 IU retinyl acetate, 2500 IU cholecalciferol, 250 mg DL- α -tocopheryl acetate, 12 mg menadione sodium bisulphate, 0.06 mg cyanocobalamin, 200 mg ascorbyl polyphosphate, 1 mg D-biotin, 2000 mg choline chloride, 10 mg folic acid, 50 mg pantothenic acid, 50 mg pyridoxine, 50 mg riboflavin, 25 mg thiamin, 120 mg inositol, 270 mg nicotinic acid, 75.3 mg Fe, 12.2 mg Cu, 206 mg Mn, 85 mg Zn, 3 mg I, 0.350 mg Se, 1 mg Co.

citrus production. Approximately, 31% of the world orange production is processing (FAO, 2016). Orange gives 55-60% peel waste at the processing stage. It means that there is approximately 10-15 million tons' waste. Orange peel essential oil have a lot of advantages for usage of animal feedstuff, because of highly D-limonene content (84-96%), sustainable feedstuff, inexpensive raw material, recycling of waste products, and direct integration into animal feed production. Thus, one of the aim of this study is evaluation of these wastes in aquaculture. Second aim isto assess the effects of orange peel essential oil on the growth performance of rainbow trout juveniles.

MATERIALS AND METHODS

Fish, culture conditions and experimental diets

The experiment was performed on 660 healthy rainbow trout (*Oncorhynchus mykiss*) with the mean weight 4.48 ± 0.03 g. At the four weeks adaptation period, the fish were fed a commercial diet containing 45% protein and 15% fat. After the adaptation, fish were randomly divided into 12 aquarium of 100 L with 55 fish per aquarium and equipped with the free-flow system. During the experiment, water properties (mean $\pm$ SE) were measured daily with the following values obtained: temperature 18.9 ± 0.6 °C, pH 7.7 ± 0.3 , dissolved oxygen 7.32 ± 0.28 mg·L⁻¹, and conductivity 559.3 ± 44.5 μ S·L⁻¹. Four experimental diets were formulated with similar protein, fat and ash content. for the fish. Three of them contained orange essential oil (OEO) at

0.5‰, 1‰ and 3‰ concentrations (Acar *et al.*, 2015; Gültepe *et al.*, 2015b; Gültepe, and Kesbiç, 2016; Gültepe *et al.*, 2017); the control group was without supplementation with OEO. The feed was produced with a standard pelleting machine. The formulation and chemical composition of the four experimental diets are presented in Table 1. A pellet size of 2 mm was used for the diets. Pellets were air-dried at 40°C and stored at -20°C until use. Fish were ad libitum fed by hand to apparent visual satiety twice a day at morning and midafternoon for 90 days. The experiment was performed in accordance with the guidelines for fish research from the animal ethics committees at Kastamonu University, Turkey.

Extraction and analysis of essential oils

The orange peel used in this study was obtained from Turkey. The OEO was obtained from fresh peel by hydro-distillation, using a Clevenger system with 150 g dry plant material and 1500 mL water. The gas chromatography-mass spectrophotometry (GC-MS) analysis of the obtained essential oil was conducted by using anAgilent 7890A GC System / 5975C Inert Mass Selective Detector (Acar *et al.*, 2015). The analysis results of the active ingredient components of the OEO were given in Table 2.

Growth performance detection

At the end of 90 days feeding trial, fish in each aquarium were individually weighed and

Table 2. Active ingredient components of the OEO

Active Substance	%	RT ^a (min)
E-2-hexanal	0.015	2.750
1-hexanol	0.043	3.216
α-pinene	0.761	4.902
3-hexanol	0.045	5.216
4-methyl-2-pentanol	0.051	5.509
β-phellandrene	0.323	5.989
Octanal	0.190	6.608
β-pinene	2.397	6.723
3-carene	0.461	7.374
<i>D</i> -limonene	94.742	8.221
β-ocimene	0.037	9.015
γ-terpinene	0.096	9.343
1-octanol	0.165	9.888
(4)-4-carene	0.123	10.726
p-menth-1-en-4-ol	0.225	14.952
Decanal	0.173	16.852
Cis-citral	0.153	20.216

^aRT (min): Reaction time (minute).

then growth performance calculated according to follows.

$$WG (\%) = \frac{(FW - IW)}{IW} \times 100$$

$$SGR = \frac{\ln(FW) - \ln(IW)}{IW} \times 100$$

$$FCR = \frac{\text{Feed Consumption}}{(FW - IW)}$$

$$DFI = \frac{\text{Initial Feed Consumption}}{\text{Days}}$$

$$DPI = \frac{\text{Daily Feed Consumption} \times \text{Protein of Feed}}{100 \times n}$$

$$DLI = \frac{\text{Daily Feed Consumption} \times \text{Lipid of Feed}}{100 \times n}$$

$$PER = \frac{\text{Weight Gain}}{\text{Protein Consumption}}$$

Abbreviations: IW: Initial weight (g), FW: Final weight (g), FI: Feed intake (g), WG: Weight gain, SGR: Specific growth rate, FCR: Feed conversion ratio, DFI: Daily feed intake, DPI: Daily protein intake, DLI: Daily lipid intake, PER: Protein efficiency ratio, n=165 per group.

Proximate analysis in feed ingredients and diets were determined by standard methods (AOAC, 2009).

Statistical analysis

Statistical parameters were examined by ANOVA. Levels of significance were determined using Tukeys HSD test, with critical limits being set at $P < 0.05$. Values are expressed as means $\pm$ standard deviation (SD) for each measured variable (Gültepe *et al.*, 2015a).

RESULTS AND DISCUSSIONS

Table 3 shows the growth performance of fish fed with the experimental diets. Survival at the end of the experiment was 100% in all experimental groups. Citrus essential oil is a complex mixture, which are included terpenic hydrocarbons, alcohols, ketones, aldehydes, oxygenated compounds and other compounds. The limonene is a monoterpene and also main component of lime and other citrus oils. It has been determined that the orange peel essential oil content contains approximately 95% pure D-limonene. The chemicals used in extraction and chromatography systems can also be found in analysis results, even at low rates. Given this situation, this study can be considered as a study made with almost pure D-limonene. Several researchers reported that OEO have antimicrobial and antifungal effects (Moreira *et al.*, 2005; Sharma, and Tripathi, 2006; Dambolena *et al.*, 2008; Chee *et al.*, 2009). Besides, the beneficial effects of essential oils obtained from orange peel on growth and immune system

Table 3. Growth parameters of experimental groups

Parameters	Control	0.5‰	1‰	3‰
<i>IW</i>	4.46±0.02	4.49±0.06	4.51±0.01	4.47±0.03
<i>FW</i>	16.83±0.55 ^a	19.71±0.38 ^b	21.18±0.09 ^c	18.81±0.33 ^b
<i>FI</i>	745.13±2.74	746.40±4.60	744.17±7.75	748.35±1.95
<i>WG</i>	276.98±11.05 ^a	338.91±12.15 ^b	369.66±3.15 ^c	321.30±6.15 ^b
<i>SGR</i>	1.47±0.03 ^a	1.64±0.03 ^b	1.72±0.01 ^c	1.60±0.02 ^b
<i>FCR</i>	1.46±0.06 ^c	1.16±0.03 ^b	1.06±0.02 ^a	1.25±0.03 ^b
<i>DFI</i>	0.164±0	0.165±0.001	0.164±0.002	0.165±0
<i>DPI</i>	0.074±0	0.075±0	0.074±0	0.075±0
<i>DLI</i>	0.025±0	0.025±0	0.025±0	0.025±0
<i>PER</i>	1.52±0.07 ^a	1.92±0.04 ^b	2.10±0.03 ^c	1.80±0.06 ^b

The values are expressed mean ± SD (n=55 for the growth parameters to each group). Values with different superscript letters are significantly different ($P<0.05$).

Figure 1. Time-dependent WG in groups

were determined for tilapia (Acar *et al.*, 2015). FW of Control, 0.5‰, 1‰ and 3‰ groups ranged 16.83±0.55 g, 19.71±0.38 g, 21.18±0.09 g, and 18.81±0.33 g, respectively. There were significant effects of OEO added diets both within OEO supplemented groups (0.5‰, 1‰ and 3‰) and between Control group ($P<0.05$). FW of the group fed with 1‰ OEO added feed was higher than the others groups. WG (%) was weighed 276.98±11.05, 338.91±12.15, 369.66±3.15 and 321.30±6.15 for the control, 0.5‰, 1‰ and 3‰ OEO fed groups, respectively. SGR was calculated for the control, 0.5‰, 1‰ and 3‰ OEO fed groups, 1.47±0.03, 1.64±0.03, 1.72±0.01 and 1.60±0.02, respectively. PER were found 1.52±0.07, 1.92±0.04, 2.10±0.03 and 1.80±0.06 for the control, 0.5‰, 1‰ and 3‰ OEO fed groups, respectively. Zheng *et al.*, (2009) reported that oregano essential oil addition to fed improved growth performance of channel catfish. The most of the herbal additives and essential

oils have a positively effects on the fish growth performance due to directly or indirectly effect on the microbiota (Gültepe *et al.*, 2014; Immanuel *et al.*, 2009; Sheikhzadehet *et al.*, 2009; 2011). According to results, WG, SGR and PER values of 1‰ OEO fed group were significantly increase than the all groups ($P<0.05$). Effect of feeding with OEO added was started from the second week onwards on the WG (Figure 1). FCR value of 1‰ OEO fed group was significantly decrease than other groups ($P<0.05$). FCR values of Control, 0.05‰, 1‰ and 3‰ OEO groups were found that 1.46±0.06, 1.16±0.03, 1.06±0.02 and 1.25±0.03, respectively. Similarly, Acar *et al.*, (2015) reported that 1‰ OEO addition of tilapia fed was significantly affected WG, FCR and SGR values of tilapia. There is not enough study the effects of OEO on animal growth parameters. Therefore, this study has an important place in the field.

CONCLUSION

At the end of the experiment; a statistically significant difference was found in terms of FW, WG, SGR, FCR and PER values of groups ($P<0.05$). The best growth performance was found in the group fed with 1‰ OEO.

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