Effects of Diet Supplementing Fennel (\textit{Foeniculum vulgare}) Essential Oil on Haematological Parameters of Rainbow trout (\textit{Oncorhynchus mykiss})

S. Mahdavi, and S. Yeganeh

\textbf{Abstract}—This study investigated effects of dietary fennel essential oil on some haematological parameters of rainbow trout (\textit{Oncorhynchus Mykiss}). For this purpose fish with an average initial weight 30±2.3 gr were divided in three treatments (Control and two groups include 200 and 400 mg/kg fennel essential oil) with three replicates for 4 weeks and fed three times a day at a ratio of 2/5% body weight. At the end of the experiment the haematological parameters such as white blood cells (WBC), red blood cells (RBC), hematocrit (Hct), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were evaluated. Results showed that the highest level of WBC was in diet containing 400 mg/kg fennel essential oil and highest level of RBC was in control group (p<0.05). There were no significant differences in hematocrit percentage, hemoglobin concentration, MCH and MCHC among treatments (p>0.05). Higher MCV obtained in 200 mg/kg fennel essential oil. On the basis of these results using dietary supplementing fennel essential oil can improve fishs immune system.

\textbf{Keywords}—Fennel, Rainbow trout, haematology, hematocrit.

\section{I. INTRODUCTION}

Nowadays to reduce or avoid the dependence of aquaculture on antibiotics, some products of natural plant origin have been considered as an effective alternative way to control bacterial and viral infections [1].

Various herbal products with antimicrobial, antistress immunostimulant and growth promoter properties affect on fish health [2].

Furthermore using herbal compositions promotes haematological and biochemical performance in fish and shrimps [3]. Recently, using plant derivatives found special place because of low expense, not having destructive effect on environment and low side effect in comparison with chemical drugs [4].

Fennel (\textit{Foeniculum vulgare}) is a perennial, herbaceous and aromatic plant from Apiaceae family which originated from Mediterranean and Southern Europe. Because of its various applications (Pharmaceutical, cosmetics and food industry), now in most areas of the world such as Southern and Central Europe, Asia (Indian, Japan, China) and many African countries as well as Brazil and Argentina fennel is widely cultivates in farm lands [5]. Its natural distribution in Iran is in Gorgan, Mazandaran, Azerbaijian, Tabriz, Gilan and Balochestan [6].

22 compounds have been identified in fennel essential oil. But its main components include t-anethole (48/66-75/5%), Limonene (8/84-25/55%), Fenchone (7/85-10/93%), Estragole (2/38-3/47%), α pinen, myrcene and vitamin A [7].

Rainbow trout (\textit{Oncorhynchus Mykiss}) from \textit{Salmonidae} family is the first most commonly cultivated coldwater fish in aquaculture industry of Iran. And is one of the most valuable fish in world [8]. It is locally from North America [9]. Because this species is adaptable with most climates and rearing conditions, now it is culturing in most areas of the world [10].

Several researches have been conducted to study the effect of herbal products on different species of fish. Alishahi et al. [11] in 2010 study the effect of (\textit{Silybum marianum}) extract on common carp. A research done by Asadi et al.[12] in 2012 about the effects of Watercress (\textit{Nasturtium nasturtium}) extract on selected immunological parameters of rainbow trout (\textit{Oncorhynchus mykiss}). Tangestani et al. [13] study the effect of garlic essential oil on juvenile Beluga. Haghighi and Rohani [14] in 2013 study the effects of powdered ginger (\textit{Zingiber officinale}) on the haematological and immunological parameters of rainbow trout (\textit{Oncorhynchus mykiss}). this research was conducted to evaluate the effect of fennel essential oil on some haematological parameters of rainbow trout.

\section{II. MATERIALS AND METHODS}

\textbf{A. Fish and Experimental Condition}

72 fish weight about 30±2/3 were randomly divided in three treatments (Control and two groups include 200 and 400 mg/kg fennel essential oil) with three replicates in 150 litre tanks for four weeks. The whole water of the tanks was exchanged with fresh water every day. Physiochemical parameters of water like temperature, oxygen, pH and salinity were measured periodically.

\textbf{B. Experimental Diet}

Essential oil used in this study were purchased from Barij...
Essence (Iran- Kashan) factory. The basal diet used in present study were provided from a reliable company in Iran. Proximate composition of the diet is shown in table I. For preparation of test diet food were mixed with herbal oil ( 10 gr oil per 1 kg food), then were supplemented with 200 and 400 mg fennel essential oil per 1kg fish food. During the experiment period fish were fed with hand at 2.5 % of their body weight 3 times a day.

C. Haematological Indices

After the trial period fish from each treatment were anesthetized with clove powder (100 mg/L) and blood were obtained from the dorsal aorta. The blood were immediately used to determine the number of RBC and WBC according to the method of Hoston [15]. Hct percentage and Hb concentration were determined by Droebkin [16] method. MCV, MCH and MCHC were calculated by the following equations.

\[
MCV(\text{fl}) = \frac{PCV(\%)}{TRBC} \times 10 \tag{1}
\]

\[
MCH(pg) = \frac{Hb(g/dl)}{TRBC} \times 10 \tag{2}
\]

\[
MCHC(\%) = \frac{Hb(g/dl)}{PCV(\%)} \times 100 \tag{3}
\]

D. Statical Analysis

The results were analyzed with one- way analyse of variance (ANOVA) using SPSS software. Duncans multiple range test was used to compare differences between the means at 5% probability. Data are presented as mean ± SD.

<table>
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<tr>
<th>PROXIMATE COMPOSITION OF THE EXPERIMENTAL DIET (%)</th>
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<tr>
<td>Dry matter</td>
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<td>Crude protein</td>
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<td>Crude lipid</td>
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<td>Ash</td>
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III. RESULTS

Mean water temperature during the experiment was about 12.5±0.4°C , dissolved oxygen about 6.7±0.4 mg/L and pH was 7.4±0.3.

Effect of different levels of fennel essential oil on rainbow trout is presented in Table II. As the results show, the highest level of WBC obtain in 400 mg/kg fennel essential oil and was significantly different with control group (p<0.05). The highest level of RBC observed in control group and adding fennel essential oil decreased RBC (p<0.05). No significant differences were observed in Hct percentage and Hb concentration among different treatments (p>0.05). Values in Table II are means of triplicate groups ± SD. Means with the different letters are significantly different (p < 0.05). The highest level of MCV obtained in 200 mg/kg fennel essential oil and the lowest was in control group and this differences was statically significant (p<0.05). Highest levels of MCH and MCHC was observed in 400 mg/kg fennel essential oil but was not statically significant (p>0.05)

IV. DISCUSSION

Recently, plant origin immunostimulants considering their several advantages found special place in aquaculture researches [17], [18] In present study using fennel essential oil promotes blood Leucocytes in Rainbow trout. Blood parameters such as Leucocytes and Erythrocytes are non specific immune indices which fluctuation in their content can be an appropriate indice of stress response [19]. Our results are in agreement with that reported by Alishahi et al. [11] in 2010 who found using Silybum marianum extract in common carp (Cyprinus carpio), increase WBC in days 20,30,40 in comparison to control group (p<0.05) while no significant differences observed in other parameters such as hemoglobin and RBC (P>0.05). Harikrishnan et al. [20] suggest that adding herbal supplements in gold fish (Carassius auratus) diet, increases WBC. The highest content of RBC found in control group and adding fennel essential oil decrease RBC (p<0.05). Tangestani et al. [13] in 2010 reported that adding garlic (Allium sativum) essential oil in juvenile beluga (Huso huso) diet decreases RBC (p<0.05). incontrast Shalaby et al [21] in 2006 demonstrated that RBC in tilapia (Oreochromis niloticus) increases by increasing garlic essential oil in diet. Hemoglobin content increased with increasing dietary fennel essential oil but it was not statistically significant (p>0.05). Hematocrite also did not show a significant difference among treatments (p>0.05). Haghighi and Rohani [14] in 2013 reported that dietary supplemented with (Zingiber officinale) significantly increase hematocrit , hemoglobin and WBC in Rainbow trout in comparison with control group (p>0.05).

in most animals MCV, MCH and MCHC are blood parameters which use as indices to diagnose anemia[22]. Our results showed no significant difference in MCH and MCHC among treatments (p>0.05), but MCV was higher in 200mg/kg fennel essential oil treatment (p<0.05). Harikrishnan et al. [23] in 2011, demonstrated that pomegranate enriched diet increase MCH and MCHC in olive flounder (Paralichthys olivaceus). But there were no significant differences in MCV among treatments. In a research conducted by Fazlolahzadeh et al. [24] in 2011, found that supplementing garlic (Allium sativum) in rainbow trout (Oncorhynchus Mykiss) diet decreases MCV but no significant differences observed in MCH and MCHC.

V. Variations in the results reported by different sources are mainly related to the differences in experimental conditions, fish species and also fish size. This may be also due to differences in active constituents in essential oil and also researchers demonstrated that their percentage can differ in a certain species in different seasons of year [25].
### References


