



ORIGINAL ARTICLE

## Effect of Dietary inclusion of *Origanum vulgare* extract on non-specific immune responses and Hematological Parameters of Rainbow trout (*Oncorhynchus mykiss*)

Hamid Pourmoghim<sup>1</sup>; Masoud Haghghi<sup>\*2</sup>; Mostafa Sharif Rohani<sup>3</sup>

1. Department of Veterinary Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran

2. \*Coldwater Fishes Research Center, Tonekabon, Iran

3. Iranian Fisheries Research Organization, Tehran, Iran

E-mail: [masoud126@yahoo.com](mailto:masoud126@yahoo.com)

### ABSTRACT

The present study was designed to evaluate the possible effect of dietary *Origanum vulgare* extract as a non-specific immunostimulating agent in rainbow trout (*Oncorhynchus mykiss*). Six hundred (600) average mean weight  $13 \pm 0.05$ g rainbow trout (*Oncorhynchus mykiss*) were randomly allocated into two groups including placebo-treated group (control), and *Origanum vulgare* extract-treated group, each of three replicates. The fishes were hand-fed once a day with diet medicated placebo or *Origanum vulgare* extract (OE) at a rate of 1% of feed weight in the first feeding for 8 weeks. At the end of every two weeks 24 hrs after feeding, fish were bled from caudal vein and blood samples were analyzed for some of hematological and immunological parameters. The results showed that serum total protein, albumin and globulin, respiratory burst activity, phagocytic activity and serum lysozyme activity vary among the two treatment groups which were found to be higher in OE-treated group ( $P < 0.05$ ). It was concluded that supplementation of OE at a rate of 1% registered higher immunological responses. Therefore, dietary inclusion of OE could improve non-specific immune responses in rainbow trout. Future studies to determine optimal herb mixtures and dietary levels should be conducted.

Keywords: Herbal immunostimulant, Iranian medicinal plants, *Origanum vulgare*, Fish

Received 23.11.2014

Revised 30.12.2014

Accepted 11.01.2015

### INTRODUCTION

Major targets in the aquaculture industry are to maintain fish health as well as to improve fish performance. The use of plant extracts in practical diets for fish is a very topical concept in aquaculture industry. One of the most important aspects in rainbow trout farming is the nutrition factor. It can influence the performance as well as the health status of the cultured fish. *Origanum vulgare* is a member of the Labiatae family of plants. It is an aromatic plant with a wide distribution throughout the Mediterranean area and Asia [1]. The essential oil obtained from *O. vulgare* subsp. *hirtum* plant by a steam distillation process comprises more than 20 ingredients, most of which are phenolic antioxidants [2]. Major components are carvacrol and thymol that constitute about 78 to 82% of the total oil [3]. It has been suggested that the essential oil derived from oregano possess *in vitro* antimicrobial [4 and 5] antifungal [6], insecticidal [7] and antioxidant [8] properties. These properties are mainly attributed to carvacrol and thymol. The activity of other constituents such as the two monoterpene hydrocarbons,  $\gamma$ -terpinene and p-cymene, that often constitute about 5 and 7% of the total oil, respectively [3] is uncertain.

### MATERIALS AND METHODS

#### Preparation of *Origanum vulgare* extract

The plant of *Origanum vulgare* was procured from local store and plant species was identified and confirmed by a botanist in Institute of Medicinal Plants. The dried air parts were collected and washed in sterile distilled water. The samples were separately shade-dried for 10 day till weight constancy was achieved. Then, the samples were powdered in an electric blender. The extract was prepared with the

standard method of percolation. To do this, chopped dried air parts of plant in 80% ethanol were percolated for 72 hours. Then, the slurry was filtered with Whattman No. 1 filter paper and centrifuged for 5 min at 5000 rpm. The filtrate obtained from ethanol using a rotary device, the excess solvent was separated from the extract. These crude extract was stored at 4°C until use.

#### **Supplementation of the normal diet with dried *Origanumvulgare* extract**

The formulated fish feed was prepared using the normal fish diet (50% crude protein, 18% crude lipid, 1.9% fiber, 1.3% total phosphorus, 8.3% ashes, and 14.8% nitrogen free extract) with dried *Origanumvulgare* extract or placebo at a ratio 1% of weight food and mixing part by part in a drum mixer. Sufficient water along with the oil ingredients were then added to make a paste of each diet. After it was pelleted and allowed to cool dry. The pellets were air dried and stored in air tight containers until fed.

#### **Fish and experimental conditions**

600 rainbow trout weighing  $13 \pm 0.05$  were used. All experiments were carried out in 1,000 liter round concrete ponds with a continuous water flow of 2.5 liter per second. The fish were kept at an ambient, including uncontrolled water temperature of  $15 \pm 1^\circ\text{C}$ , dissolved oxygen of  $7.2 \pm 0.2 \text{ mg l}^{-1}$  and pH  $8 \pm 0.3$ . After 2 weeks adaptation, fish were randomly allotted in two groups including an experimental group and a control group, in triplicate was maintained in 6 concrete ponds each containing 100 fish. Each group was hand-fed once a day with diet medicated 1% of *Origanumvulgare* extract, or placebo (70% lactose, 10 % starch and 20 % talc) prepared in the laboratory and three times with normal diet at a rate 2% of body weight for 10 weeks.

#### **Bleeding and serum collection**

During bleeding, fish were rapidly netted, tranquillized with 50 mg/l of tricaine methane sulfonate (MS<sub>222</sub>, Sigma chemical Co. St. Louis, MO, USA). Fish were bled from caudal vein using 1ml insulin syringe fitted with 24 gauge needle. To minimize the stress to fish, 1 ml of blood was drawn and the whole bleeding procedure was completed within 1 min. A total number of 15 blood samples were collected from 15 fish in each group (5 samples from each replicate) at the end of every 2 weeks, 24 h after final feeding period. The blood pooling of 5 fish from each replicate divided into 2 halves. Half collected in serological tubes containing a pinch of lithium heparin powder, shaken gently and kept at 4°C to test hematological parameters. Other half collected in tubes without of anticoagulant and allowed to clot at 4°C for 2hrs to test serological parameters. The clot was the spun down at 2000 rpm for 10 min to separate the serum. The serum collected by micropipette and was stored in sterile Eppendorf tubes at -20°C until used for assay.

#### **Hematological assay**

Blood sample was analyzed with routine methods adopted in fish hematology (9). The total red blood cell counts (RBC  $\times 10^6/\mu\text{l}$ ) were determined in a 1:200 dilution of the blood sample in Hayem's solution and total white blood cell counts (WBC  $\times 10^3/\mu\text{l}$ ) in a 1:20 dilution of the blood sample with a Neubauerhemocytometer. The hematocrit (Hct) and leucocrit percentages were determined in duplicate by using micro hematocrit-heparinized capillary tubes of 75 $\mu\text{l}$  volume and a micro hematocrit centrifuge at 15000 rpm for 5 min (10). The percentages of erythrocyte (hematocrit) and leucocyte (leucocrit) volumes were calculated by overlaying the tubes on a sliding scale hematocrit reader.

The hemoglobin (Hb g/dl) concentrations were determined by the cyanomethaemoglobin method [11] using a haemoglobin reagent set (ZiestChem Diagnostics). The all the values of red blood cell indices, the mean values of cell haemoglobin (MCH pg), cell hemoglobin concentration (MCHC %), and cell hemoglobin volume (MCV fl) were calculated according to Wintrobe formulae [12]. The differential leukocytes count was carried out using blood smears stained with Wright-Giemsa. The percentage composition of leukocytes was determined based on their identification characters listed by Ivanava [13].

#### **Biochemical assay**

Serum total protein content was estimated photo metrically by citrate buffer and bromocresol green (BCG) dye binding method [14] using the kit (total protein and albumin kit, Pars Azmun Company, Iran). Albumin was determined BCG binding method. The absorbance of standard and test were measured against blank in a spectrophotometer at 546 nm. Globulin level was calculated by subtracting albumin values from total serum protein. Albumin/globulin (A/G) ratio was calculated by dividing albumin values by globulin values.

#### **Immunological assay**

##### **Separation of leukocytes from the blood**

Leucocytes for assay were separated from each blood sample by density-gradient centrifugation. One milliliter of histopaque 1.119 (Sigma) containing 100 $\mu\text{l}$  of bacto hemagglutination buffer, pH 7.3 (Difco, USA) was dispensed into siliconised tubes. 1 ml of a mixture of 1.077 density histopaque and hemagglutination buffer and 1 ml of blood was carefully layered on the top. The sample preparations were centrifuged at 2500 rpm for 15 min at 4°C. After centrifugation, plasma was collected and stored at -80°C

for future analysis; separated leukocytes were gently removed and dispensed into siliconised tubes, containing phenol red free Hanks Balanced Salt Solution (HBSS, Sigma). Cells were then washed twice in HBSS and adjusted to  $2 \times 10^6$  viable cells/ml.

#### **Respiratory burst activity**

Respiratory burst activity of isolated leukocytes was quantified by reduction of ferricytochrome C [15]. Briefly, 100 $\mu$ l of leukocyte suspension and an equal volume of cytochrome C (2 mg/l in phenol red free HBSS) containing phorbol 12-myristate 13-acetate (PMA, Sigma) at 1 $\mu$ g/ml were placed in triplicate in micro titer plates. In order to test specificity, another 100 $\mu$ l of leukocyte suspensions and solutions of cytochrome c containing PMA and superoxide dismutase (SOD, Sigma) at 300 U/ml were prepared in triplicate in micro titer plates. Samples were then mixed and incubated at room temperature for 15 min. Extinctions were measured at 550 nm against a cytochrome C blank in a multiscan spectrophotometer. Readings were converted to nmoles O<sub>2</sub> by subtracting the O.D. of the PMA/SOD treated supernatant from that treated with PMA given alone for each sample, and converting O.D. to n moles O<sub>2</sub> by multiplying by 15.87. Final results were expressed as nano moles O<sub>2</sub> produced per 10<sup>5</sup> blood leukocytes.

#### **Phagocytosis assay**

Phagocytosis activity of blood leukocytes was determined spectrophotometrically according to Seeley *et al.* (16). This assay involves the measurement of congo red-stained yeast cells which have been phagocytised by cells. To perform the assay, 250 $\mu$ l of the leukocyte solution was mixed with 500 $\mu$ l of the congo red-stained and autoclaved yeast cell suspension (providing a yeast cell: leukocyte ratio of 40:1). The mixtures were incubated at room temperature for 60 min. Following incubation, 1 ml ice-cold HBSS was added and 1 ml of histopaque (1.077) was injected into the bottom of each sample tube. The samples were centrifuged at 2500 rpm for 5 min to separate leukocytes from free yeast cells. Leukocytes were harvested and washed two times in HBSS. The cells then were re-suspended in 1 ml trypsin-EDTA solution (5.0g/l trypsin and 2.0g/l EDTA, Sigma) and incubated at 37°C overnight. The absorbance of the samples was measured at 510 nm using trypsin-EDTA as a blank.

#### **Serum lysozyme assay**

In this study, an assay based on the lysis of *Micrococcus lysodeikticus* was used to determining the lysozyme activity. Serum lysozyme activity was measured spectrophotometrically according to the method Parry *et al.* [17]. Briefly, 0.02% (w/v) lyophilized *Micrococcus lysodeikticus* in 0.05 mM solution phosphate buffer (pH 6.2) was used as substrate. 10 $\mu$ l of fish serum was added to 250 $\mu$ l of bacterial suspension and reduction in absorbance at 490 nm was determined after 0.5 and 4.5 min of incubations at 25°C using a microplate reader. One unit of lysozyme activity was defined as the amount of enzyme causing a decrease in absorbance of 0.001 per min.

#### **Statistical analysis**

All results for each parameter measured were expressed as means  $\pm$  standard errors, and were compared at each time point using Student's *t*-test for independent data. Significant differences between experimental groups were expressed at a significance level of  $P < 0.05$ . All analyses were carried out on 15 fish per group.

## **RESULTS**

### **Hematological analysis**

Dietary *Origanum vulgare* extract incorporated test diets had no significant ( $p < 0.05$ ) effect on red blood cell count (RBC), white blood cell count (WBC), differential leukocytes count (monocyte, lymphocyte and neutrophile), hematocrit (Hct), hemoglobin (Hb), the all the values of red blood cell indices, the mean values of cell hemoglobin (MCH pg), cell hemoglobin concentration (MCHC %), and cell hemoglobin volume (MCV fl) at the end of none of the identical two weeks after feeding in compared to placebo group (Table 1).

### **Biochemical analysis**

*Origanum vulgare* extract had significant ( $p < 0.05$ ) effect in increase of total protein (TP), albumin (AL), and globulin (GL), at the end of the identical every two weeks after feeding in compared to placebo group (Table 2). The maximum level of total protein was recorded on week 2 of exposure duration. Similarly, albumin and globulin contents were significantly higher in *Aloe vera* group as compared to placebo group. However, albumin/globulin ratio was not exhibited significant differences in compared to placebo group at the end of the identical every two weeks after feeding in compared to placebo group ( $p > 0.05$ ; Table 2).

### **Immunological analysis**

#### **Respiratory burst activity**

The respiratory burst activity significantly ( $p < 0.05$ ) enhanced in fish fed with 1% of *Origanum vulgare* extract supplementation feed at the end of the identical every two weeks after feeding in compared to placebo group (Fig. 1).

**Phagocytic activity**

Phagocytic activity of blood leucocytes significantly ( $p<0.05$ ) enhanced in fish treated with 1% of *Origanum vulgare* extract supplementation feed at the end of the identical every two weeks after feeding in compared to placebo group (Fig. 2).

**Lysozyme activity**

Lysozyme activity significantly ( $p<0.05$ ) enhanced in fish fed with 1% of *Origanum vulgare* extract supplementation feed at the end of the identical every two weeks after feeding in compared to placebo group (Fig. 3).

**Table 1** The hematological parameters, WBC, RBC, Hct, Hb, MCH, MCV, MCHC, neutrophil, monocyte, and lymphocyte of rainbow trout fed with 1% placebo (PL) or *Origanum vulgare* extract (OE) in feed for 8 weeks.

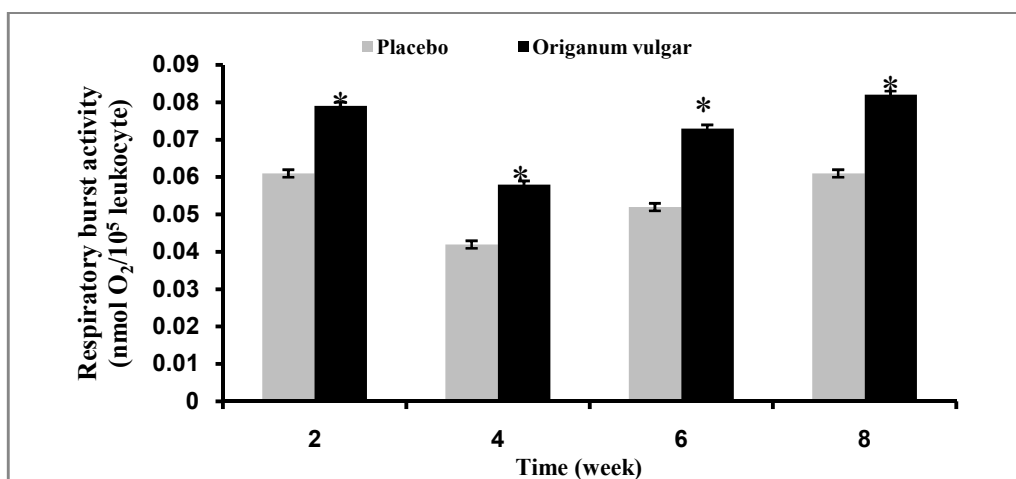
Week	Groups	WBC( $10^3$ /mL)	RBC( $10^6$ /mL)	Hct(%)	Hb(g/dL)	MCH(pg)	MCV(fL)	MCHC(%)	Neut(%)	Mon(%)	Lymp(%)
2	PL	3.90±0.7	0.98±.06	27.33±3.3	9.07±0.6	92.55±2.7	278.8±16	3.31±0.34	7.66±0.1	3.00±0.3	89.0±1.2
	OE	3.92±0.2	1.08±.08	27.77±2.2	9.32±0.9	86.29±4.6	257.1±11	3.35±0.5	8.33±0.1	2.66±0.1	90.0±1.0
4	PL	4.00±0.1	1.69±.05	40.50±4.1	13.50±0.9	79.88±3.1	239.6±13	3.33±0.3	7.66±0.5	2.33±0.2	89.0±2.0
	OE	4.06±0.6	1.71±.04	40.50±5.5	13.56±1.5	79.29±2.9	236.8±9	3.34±0.4	7.66±0.4	2.00±0.5	90.3±0.7
6	PL	4.20±0.5	1.69±.03	36.00±3.7	12.00±1.5	71.00±3.0	213.0±6	3.33±0.3	7.66±0.3	3.33±0.3	89.0±2.1
	OE	4.10±0.2	1.76±.06	37.00±2.0	12.06±0.8	68.52±1.9	210.2±6	3.25±0.2	8.33±0.2	3.00±0.2	88.6±0.9
8	PL	4.95±0.1	1.53±.03	33.00±3.5	10.98±1.8	71.76±3.9	215.6±8	3.32±0.2	8.00±0.1	2.33±0.2	89.6±1.2
	OE	5.31±0.5	1.46±.06	36.66±3.0	12.33±1.3	84.45±4.1	251.0±14	3.36±0.3	7.66±0.5	2.00±0.6	90.0±0.6

Data are expressed as mean±SE (n=15). No significant differences were observed in the *Origanum vulgare* treated groups relative to the placebo group at the end of the identical every two weeks after feeding ( $P>0.05$ ). Neut: neutrophil; Mon: Monocyte; Lymp: Lymphocyte.

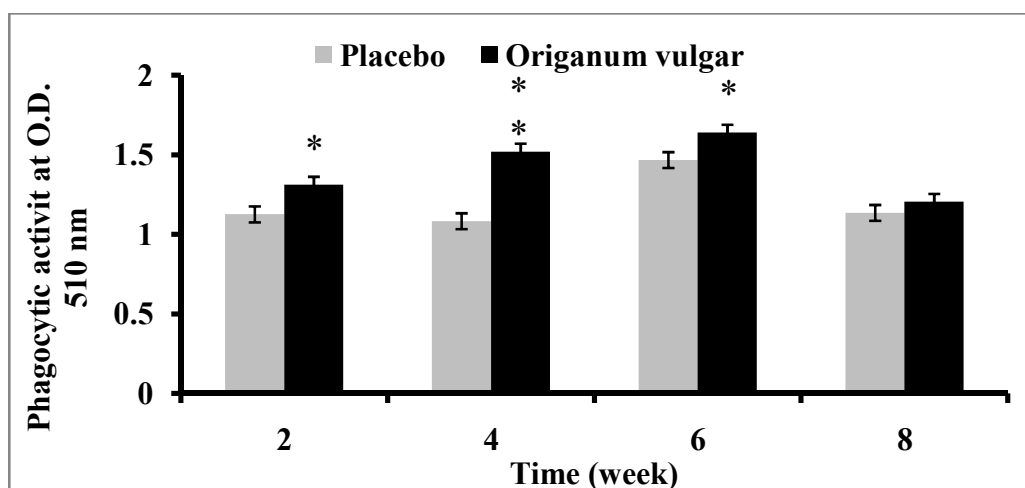
**Table 2** Changes in the serum total protein, albumin, globulin and albumin/globulin ratio of rainbow trout after feeding with 1% placebo, or *Origanum vulgare* extract for 8 weeks.

Week	Groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Albumin/globulin ratio (g/dL)
2	Placebo	4.10±0.01	1.80±0.08	2.20 ±0.08	0.81±0.01
	<i>Origanum</i> extract	4.23±0.01	1.90±0.07	2.33±0.03	0.81±0.02
4	Placebo	4.03±0.01	1.80±0.04	2.23 ±0.08	0.80±0.02
	<i>Origanum</i> extract	4.26±0.02*	1.90±0.05*	2.36±0.04	0.80±0.02
6	Placebo	3.40±0.02	1.53±0.06	1.87 ±0.01	0.81±0.01
	<i>Origanum</i> extract	3.80±0.04*	1.73±0.03*	2.07 ±0.06*	0.83±0.01
8	Placebo	3.93±0.01	1.75±0.06	2.18 ±0.03	0.80±0.01
	<i>Origanum</i> extract	4.19±0.02	1.89±0.05*	2.30 ±0.05*	0.82±0.01

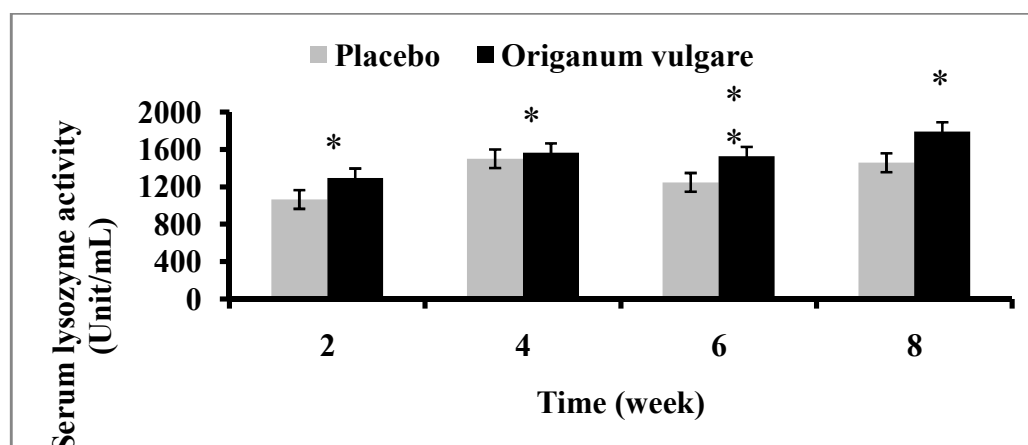
Data are expressed as mean±SE (n=15). \*:  $P<0.05$  compared with the placebo at the end of identical every two weeks.



**Fig.1** Respiratory burst activity of different experimental groups observed on different weeks. Data are expressed as mean±SE. Asterisks indicate significantly different from placebo group in the same week. \* $p<0.05$ ; \*\* $p<0.001$



**Fig.2** Phagocytic activity of different experimental groups observed on different weeks. Data are expressed as mean±SE. Asterisks indicate significantly different from placebo group in the same week. \*p<0.05; \*\*p<0.001



**Fig.3** Serum lysozyme activity of different experimental groups observed on different weeks. Data are expressed as mean±SE. Asterisks indicate significantly different from placebo group in the same week. \*p<0.05; \*\*p<0.001

## DISCUSSION

The present study projects the impact of dried *Origanum vulgare* extract on the hematological and immunological responses in rainbow trout (*Oncorhynchus mykiss*). The hematological parameters in the present investigation such as RBC, WBC, differential leukocytes counts, hemoglobin, and hematocrit, the all of the values of red blood cell indices (MCH, MCHC and MCV) were no significant differences at the end of none of the identical every two weeks after feeding when compared to control group. These observations are in agreement with the obtained results of other researchers, who reported that rainbow trout treated with dietary *Aloe vera* supplementation were no significant differences in RBC and Hct [18], or RBC and Hb [19] among the groups.

In the present study, the dietary *Origanum vulgare* extract supplementation enhanced total plasma protein, albumin and globulin values in comparison with control group. Similar results were reported in rainbow trout fed with garlic [20], ginger [21], lipopolysaccharide [22], *Laurus nobilis* [23], and *Coggyriacoggyria* [24]. Serum proteins are various humoral elements of the non-specific immune system, measurable total protein, albumin and globulin levels suggest that high concentrations are likely to be a result of the enhancement of the non-specific immune response of fish. So, this study revealed that *Origanum vulgare* extracts incorporated diets helped to increase the humoral elements in the serum. Respiratory burst activity is considered as an important indicator of non-specific defense in fish, which is a measure of the increase of oxidation level in phagocytes stimulated by foreign agents [25]. An enhancement of respiratory burst activity has been identified in the present study, that it is in agreement many of studies with dietary immunostimulants [23 and 26]. Respiratory burst and phagocytosis response

by phagocytes in blood present a major antibacterial defense mechanism in fish [27]. Phagocytosis is one of the most important processes in fish. The main cells involved in phagocytosis in fish are neutrophils and macrophages. These cells remove bacteria mainly by the production of reactive oxygen species (ROS) during a respiratory burst. In addition, neutrophils possess myeloperoxidase in their cytoplasmic granules, which in the presence of halide and hydrogen peroxidase kills bacteria by halogenations of the bacterial cell wall. Moreover, these cells have lysozymes and other hydrolytic enzymes in their lysosomes [28]. Similarly, macrophages can produce nitric oxide in mammals and can be as potent as antibacterial agents, peroxy nitrates and hydroxyl groups. Phagocytic activity of leucocytes in rainbow trout was enhanced by dietary dose of powdered ginger rhizome [29, 30] Also, in this study an increasing trend in lysozyme activity has been shown which is in agreement with several reports indicating the role of herbal immunostimulants in enhancing lysozyme activity [31, 32 and 33]. Lysozyme is a humoral component of the non-specific defense mechanism which has the ability to prevent the growth of bacteria by splitting  $\beta$ -1,4 glycosidic bonds in the peptidoglycan of bacterial cell walls, resulting in bacteriolysis. In conclusion, supplementation of OE in aquaculture diets would be used to enhance non-specific immune system in fish. Therefore, further studies are necessary for effective use of *Origanum vulgare* extract with optimal dose, suitable duration, and method of administration.

#### ACKNOWLEDGEMENT

The authors would like to thank Islamic Azad University and the Iranian Fisheries Research Organization (IFRO) for the financial support.

#### REFERENCES

1. Vokou, S., Kokkini, S., and Bessiere, J.M. (1993). Geographic variation of Greek oregano (*Origanum vulgare* subsp. *hirtum*) essential oils. *Biochem Syst Ecol*, 21: 287-295.
2. Vekiari, S.A., Oreopoulou, V., Tzia, C. and Thomopoulos, C.D. (1993). Oregano flavonoids as lipid antioxidants. *J Am Oil Chem Soc*, 70: 483-487.
3. Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T. and Arsenakis, M. (1998). Antifungal activities of *Origanum vulgare* sp. *Hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. *J Agric Food Chem*, 46: 1739-1745.
4. Sivropoulou, A., Paranikolaou, E., Nikolaou, C., Kokkini, S., Lanaras, T. and Arsenakis, M. (1996). Antimicrobial and cytotoxic activities of *Origanum* essential oils. *J Agric Food Chem*, 44: 1202-1205.
5. Lambert, R.J.W., Skandamis, P.N., Coote, P.J. and Nychas, G.J.E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J Appl Microbiol*, 91: 453-462.
6. Kocić-Tanackov, S.D., Dimić, G.R., Tanackov, I.J., Pejin, D.J., Mojović, L.V. and Pejin, J.D. (2012). Antifungal activity of Oregano (*Origanum vulgare* L.) extract on the growth of *Fusarium* and *Penicillium* species isolated from food. *Hem Ind*, 66 (1): 33-41.
7. Karpouhtsis, I., Pardali, E., Feggou, E., Kokkini, S., Scouras, Z.G. and Mavragani-Tsipidou, P. (1998). Insecticidal and genotoxic activities of oregano essential oils. *J Agric Food Chem*, 46: 1111-1115.
8. Botsoglou, N.A., Florou-Paneri, P., Christaki, E., Fletouris, D.J. and Spais, A.B. (2002). Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *Br Poult Sci*, 43(2): 223-230.
9. Blaxhall, P.C. and Daisley, K.W. (1973). Routine haematological methods for use with fish blood. *J Fish Biol*, 5: 771-781.
10. Goldenfarb, B.P., Bowyer, F.P., Hall, T. and Brosious, E. (1971). Reproducibility in the hematology laboratory: the microhematocrit determination. *Am J Clin Pathol*, 56: 35-39.
11. Valery, H., Gowenlock, A.H. and Bell, M. (1991). *Practical clinical biochemistry*. 5<sup>th</sup> ed. CBS Publication and distributors, Delhi, India; p.479-480.
12. Wintrobe, M.M. (1933). Variation in size and haemoglobin concentration of erythrocyte in the blood of various vertebrates. *Folia Haemato*, 51: 32-49.
13. Ivanova, N.T. (1983). Atlas of Fish Blood Cells. *LPP Moscow, Russia* (In Russian).
14. Shaziya, B.I. and Goyal, P.K. (2012). Anthelmintic effect of natural plant (*Carica papaya*) extract against the gastrointestinal nematode, *Ancylostoma caninum* in mice, *ISCA J Biological Sci*, 1(1): 2-6.
15. Secombes, C.J. (1990). Isolation of salmonid macrophages and analysis of their killing activity. In: Stolen, J.S., Anderson, D.P., Roberston, B.S., van Muiswinkel, W.B. editors. *Techniques in fish immunology*. SOS Publications, Fair Haven, p. 137-154.
16. Seeley, K.R., Gillespie, P.D. and Weeks, B.A. (1990). A simple technique for the rapid spectrophotometric determination of phagocytosis by fish macrophages. *Mar Environ Res*, 30: 37-41.
17. Parry, R.M., Chandan, R.C. and Shahani, K.M. (1965). A rapid and sensitive assay of muramidase. *Proc Soc Exp Biol Med*, 119: 384-386.
18. Alishahi, M., Ranjbar, M.M., Ghorbanpour, M., Mesbah, M. and Razijalali, M. (2010). Effects of dietary *Aloe vera* on some specific and nonspecific immunity in the common carp (*Cyprinus carpio*). *Int J Vet Res*, 4: 189-195.

19. Farahi, A., Kasiri, M., Sudagar, M., SoleimaniRaei, M. and Zorriehzahra, S.M.J. (2012). Effect of dietary supplementation of *Melissa Officinalis* and *Aloe Vera* on hematological traits, lipid oxidation of carcass and performance in rainbow trout (*Oncorhynchus mykiss*). *Online J Animal Feed Res*, 1: 1-5.
20. Nya, E.J. (2009). Studies on dietary supplements for the control of *Aeromonashydrophila* infection in rainbow trout (*Oncorhynchus mykiss*, Walbaum). Doctor of Philosophy thesis. School of Life Sciences, Heriot Watt University, Edinburgh, UK.
21. Nya, E.J. and Austin, B. (2009a). Use of garlic, *Aliumsativum*, to control *Aeromonashydrophila* infection in rainbow trout, *Oncorhynchus mykiss*(Walbaum). *J Fish Dis*, 32: 963-970.
22. Nya, E.J. and Austin, B. (2009b). Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *JFish Dis*,32: 971-977.
23. Bilen, S. and Bulut, M. (2010). Effects of Laurel (*Laurusnobilis*) on the non-specific immune responses of rainbow trout (*Oncorhynchus mykiss*, Walbaum). *J Anim Vet Adv*, 9(8): 1275-1279.
24. Bilen, S., Bulut, M. and Bilen, A.M. (2011). Immunostimulant effects of *Coggyriacoggyria* on rainbow trout (*Oncorhynchusmykiss*). *Fish Shellfish Immunol*,30: 451-455.
25. Liaghat, M., Akhlaghi, M., Hosseini, A., Nematollahi, A. and Hosseini, S.M. (2011). Humoral and non-specific immune responses in rainbow trout (*Oncorhynchusmykiss*) naturally exposed to and immunized with *Streptococcus iniae*. *Int J Vet Res*, 5(4): 218-224.
26. Yin, G., Adro, K.D., Thompson, A., Adams, A., Jeney, Z. and Jeney, G. (2009). Chinese herbs (*Astragalus radix* and *Gonodermalucidum*) enhance immune response of carp, *Cyprinus carpio* and protection against *Aeromonashydrophila*. *Fish Shellfish Immunol*, 26: 140-145.
27. Harikrishnan, R., Balasundaram, C. and Heo, M.S. (2010). Herbal supplementation diets on hematology and innate immunity in goldfish against *Aeromonashydrophila*. *Fish Shellfish Immunol*,28: 354-361.
28. Uribe, C., Folch, H., Enriquez, R. and Moran, G. (2011). Innate and adaptive immunity in teleost fish: a review. *Vet Medicina*, 10: 486-503.
29. Dügenci, K.S., Arda, N. and Candan, A. (2003). Some medicinal plants as immunostimulant for fish. *J Ethnopharmacol*, 88: 99-106.
30. Haghghi, M. and Sharifrohani, M. (2013). The effects of powdered ginger (*Zingiberofficinale*) on the haematological and immunological parameters of rainbow trout *Oncorhynchusmykiss*. *Asian J Marine Sci*, 1: 7-11.
31. Rao, Y.V., Das, B.K., Pradhan, J., and Chakrabarti, R. (2006). Effect of *Achyranthesaspera* on the immunity and survival of *Labeorohitainfected* with *Aeromonashydrophila*. *Fish Shellfish Immunol*. 20: 263-273.
32. Choi, S.H., Park, K.H., Yoon, T.J., Kim, J.B., Jang, Y.S. and Choe, C.H. (2008). Dietary Korean mistletoe enhances cellular non-specific immune responses and survival of Japanese eel (*Anguilla japonica*). *Fish Shellfish Immunol*,24: 67-7.
33. Olfati A., MoghaddamGH.,Alijani S., &Rafat, SA. 2012. Effects Of Visual Stimuli Or Change Of The Stimulus Ewe On Libido And Semen Characteristics Of Cross-Bred Rams. *Slovak J Anim Sci*. 45 (3): 76-82.

#### CITATION OF THIS ARTICLE

Hamid P; Masoud H, Mostafa S R. Effect of Dietary inclusion of *Origanum vulgare* extract on non-specific immune responses and Hematological Parameters of Rainbow trout (*Oncorhynchus mykiss*). *Bull. Env.Pharmacol. Life Sci*, Vol 4 [3] February 2015: 33-39