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# Evaluation of *Sargassum fusiforme* and *Ecklonia cava* as Dietary Additives for Olive Flounder (*Paralichthys olivaceus*)

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**Abstract**

A 6-week feeding trial was conducted to evaluate the efficiency of hijiki (*Sargassum fusiforme*) and ecklonia (*Ecklonia cava*) as dietary supplements for juvenile olive flounder. Fish growth performance, hematology, innate immunity and disease resistance were examined. A basal experimental diet was used as a control and supplemented with 6% of either *S. fusiforme* or *E. cava* and another diet was prepared to contain 0.5% oxytetracycline (designated as Con, Hij-6, Eck-6 and OTC-0.5, respectively). The experiment was conducted in triplicate (25 fish per replicate) and the fish (initial body weight, 18.3±0.04 g) were fed one of the test diets at a rate of 3% body weight twice daily. Significantly higher ($P<0.05$) antioxidant capacity and polyphenolic compounds concentration were detected in hijiki and ecklonia supplemented diets. At the end of the feeding trial, fish growth performance and feed utilization were not significantly affected by dietary treatments. Significantly higher ($P<0.05$) hemoglobin level and respiratory burst activity were found in fish fed Hij-6 diet. The fish fed Hij-6 and Eck-6 diets exhibited higher resistance against *Edwardsiella tarda* challenge compared to those fed Con and OTC-0.5 diets. The findings in this study may show that hijiki and ecklonia can be used as environment friendly substitutes for antibiotics in diets for olive flounder.

*Keywords*: Olive flounder, *Sargassum fusiforme*, *Ecklonia cava*, oxytetracycline, *Edwardsiella tard.*

## Introduction

Olive flounder is currently the most important marine aquaculture species in Korea. Its production has been increased by improvements in its culturing techniques. However, disease caused by viruses, bacteria and parasites has been the main constraint to its culture (Jung *et al*., 2005).

*Edwardsiella tarda*, a Gram-negative, motile, flagellated bacterium, is causative agent of edwardsiellosis in commercially important freshwater and marine fish species. *E. tarda* infection occurs in a variety of fish species including Chinook salmon (Amandi *et al*., 1982), Carp (Sae-Oui *et al*., 1984), eel (Minagawa *et al*., 1983), tilapia (Kubota *et al*., 1981) and flounder (Nakatsugawa, 1983). It produces septicemia with extensive skin lesions and affects internal organs which results in extensive mortalities (Plumb, 1999).

In Korea, 27 antibacterial substances including 23 antibiotics and 4 sulfa drugs are used for treatment of fish disease (Park, 2009). The potential hazards of using antibiotics in aquaculture are development of antibiotic-resistant microorganisms, antibiotic residuals in fish products, contamination of surrounding ecological systems and reduced efficiency of antibiotics against the diseases caused by resistant pathogens (McPhearson *et al*., 1991; Hernández Serrano, 2005). The occurrence of antibiotic resistant *E. tarda* has been widely reported all over the world (Aoki *et al*., 1989).

Prevention of fish disease through the stimulation of non-specific immune response by natural compounds is a potential solution for development of sustainable antibiotic-free aquaculture. Seaweeds are rich in bioactive compounds and they produce a great variety of secondary metabolites with broad spectrum biological activities. Numerous compounds with cytostatic, antiviral, anthelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae (Lindequist and Schweder, 2001; Newman *et al*., 2003).

Hijiki (*Sargassum fusiforme*), an edible brown algae, is widely distributed in Korea and Japan. Its positive effects on antioxidant activity (Karawita *et al*., 2004; Siriwardhana *et al*., 2003a; 2003b; 2004; 2005), immune response and disease resistance have

been reported in human (Shan *et al*., 1999), mice (Liu *et al*., 1997; Okai *et al*., 1997; 1998) and fish (Pham *et al*., 2006). The kelp Ecklonia (*Ecklonia cava*), a brown marine algae, is distributed in temperate coastal zone of Korean peninsula and generally forms highly persistent populations in clear waters (Kang *et al*., 2001). *E. cava* has been widely used for production of fucoidan which is well-known as an antitumor, anticoagulant and antithrombin polysaccharide (Takashi *et al*., 1999; 2000). Also, its antioxidant (Kim *et al*., 2004; Heo *et al*., 2003; 2005) and cytoprotective (Kang *et al*., 2005) activities have been reported.

There is little available information on the use of these two marine algae in fish species. Therefore, a feeding trial was carried out to examine their efficiency as candidates for antibiotic replacement in diets for olive flounder.

## Materials and Methods

### Experimental Diets

Formulation and proximate composition of the experimental diets are presented in Table 1. A basal diet was used as a control and three experimental diets were prepared by supplementing 6% *S. fusiforme* or *E. cava* and 0.5% oxytetracycline (designated as Con, Hij-6, Eck-6 and OTC-0.5, respectively). Proximate composition of dietary ingredients is given in Table 2. All the diets were formulated to be isonitrogenous (50% crude protein) and isocaloric (17.2 MJ/kg). The energy content of diets was calculated by using values of 16.7 KJ/g of carbohydrate and protein, and 37.7 KJ/g of fat (Lee and Putnam, 1973). All dry ingredients were thoroughly mixed and after addition of squid liver oil and 30% double distilled water extruded through a meat chopper machine (SMC-12,

**Table 1.** Formulation and proximate composition of the experimental diets (% DM)

| | Diets | | | |
|---|---|---|---|---|
| Ingredients | Con | Hij-6 | Eck-6 | OTC-0.5 |
| White fish meal | 54.0 | 54.0 | 54.0 | 54.0 |
| Soybean meal | 5.0 | 5.0 | 5.0 | 5.0 |
| Corn gluten meal | 5.0 | 4.5 | 4.5 | 5.0 |
| Wheat flour | 9.0 | 6.0 | 6.0 | 9.0 |
| Yeast | 2.0 | 2.0 | 2.0 | 2.0 |
| Starch | 14.0 | 14.0 | 14.0 | 14.0 |
| Hijiki powder[1] | 0.0 | 6.0 | 0.0 | 0.0 |
| Ecklonia powder[2] | 0.0 | 0.0 | 6.0 | 0.0 |
| Oxytetracline[3] | 0.0 | 0.0 | 0.0 | 0.5 |
| Mineral mix[4] | 1.0 | 1.0 | 1.0 | 1.0 |
| Vitamin mix[5] | 1.0 | 1.0 | 1.0 | 1.0 |
| Squid liver oil | 7.0 | 6.5 | 6.5 | 7.0 |
| Cellulose | 2.0 | 0.0 | 0.0 | 1.5 |
| Proximate composition (% DM) | | | | |
| Dry matter | 91.4 | 91.8 | 91.5 | 91.7 |
| Protein | 49.4 | 49.4 | 49.6 | 49.6 |
| Lipid | 10.9 | 10.3 | 10.9 | 10.8 |
| Ash | 10.7 | 13.3 | 11.5 | 11.0 |
| Gross energy, MJ/kg DM | 17.4 | 17.6 | 17.7 | 17.5 |

[1,2] Hijiki and Ecklonia were provided by Professor Y.J. Jeon, Faculty of Applied Science, College of Ocean Science, Jeju National University, Jeju, Korea.

[3] Oxytetracycline hydrochloride (min. 95%), Sigma.

[4] Mineral premix (g/kg of mixture): $MgSO_4.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; $CuCl_2$, 0.2; $AlCl_3$. $6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.

[5] Vitamin premix (g/kg of mixture): L-ascorbic acid monophosphate, 100.0; DL-α tocopheryl acetate, 20.0; thiamin hydrochloride, 4.0; riboflavin, 4.4; pyridoxine hydrochloride, 4.0; niacin, 30.0; D-pantothenic acid hemicalcium salt, 14.5; myo-inositol, 40.0; D-biotin, 0.2; folic acid, 0.48; menadione, 0.2; retinyl acetate, 1.0; cholecalficerol, 0.05; cyanocobalamin, 0.01.

**Table 2.** Proximate composition of dietary ingredients used in the experimental diets (DM%)

| Ingredients | Moisture | Protein | Lipid | NFE[1] | Ash |
|---|---|---|---|---|---|
| White fish meal | 8.72 | 68.33 | 8.56 | 0.32 | 14.07 |
| Soybean meal | 11.68 | 46.91 | 2.52 | 36.44 | 6.54 |
| Corn gluten meal | 9.50 | 61.70 | 1.03 | 26.59 | 1.18 |
| Yeast | 5.49 | 42.15 | 0.49 | 46.25 | 5.62 |
| Hijiki | 8.91 | 17.07 | 0.58 | 57.92 | 15.52 |
| Ecklonia | 5.3 | 14.10 | 0.99 | 79.34 | 0.27 |

[1] Nitrogen Free Extracts = 100 - (Moisture% + CP% + Lipid% + Ash%)

Kuposlice, Busan, Korea) with a 3 mm diameter die. The pellets were crushed into desirable particle sizes subsequently and stored at –20°C until used.

**Experimental Fish and Feeding Trial**

Olive flounder were transported from a private hatchery (Chang-Hae Fisheries Co.) to Marine and Environmental Research Institute (Jeju National University, South Korea). All the fish were fed a commercial diet for two weeks to be acclimated to the experimental facilities and condition. Three hundred fish (initial body weight, 18.3±0.04 g) were randomly distributed into twelve tanks of 100 L capacity (25 fish per tank) in a flow through system and supplied with sand filtered seawater at a flow rate of 3 L/min. Triplicate groups of fish were fed one of the test diets at a feeding rate of 3% body weight twice daily (8:00 and 18:00) for 6 weeks. Fish growth was measured every two weeks and feeding rate was adjusted accordingly. All the fish were fasted for 24 h prior to weighing or sampling. Water temperature varied from 16 to 20°C according to the natural fluctuations in sea water temperature and photoperiod was fixed on a 12 h light:12 h dark cycle. Dissolved oxygen, pH, nitrate and salinity of each tank were measured every two weeks (6.6±0.2 mg/L, 7.9±0.4, 6.3±0.4 mg/L and 33.2±0.7‰, respectively).

**Sample Collection and Analysis**

At the beginning and the end of the feeding trial, all the fish in each tank were bulk-weighed, counted and their total and fork length were measured for calculation of growth parameters including weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), feed intake (FI), condition factor (CF) and survival through the following formulas:

WG = [(final body weight – initial body weight) / initial body weight × 100]

FCR = dry feed fed / wet weight gain

PER= wet weight gain / total protein given

FI = dry feed consumed (g) / body weight (g)

$CF = (\text{body weight} / \text{fork length}^3) \times 100$

At the end of the feeding trial, three fish per tank (nine fish per treatment) were randomly captured, anesthetized in tricaine methanesulfonate (MS-222, Sigma, St.Louis, MO, USA) solution (100 ppm) and blood samples were collected from caudal vein using heparinized syringes for analyses of hematocrit, hemoglobin and respiratory burst activity. The same fish were used for determination of organosomatic indices including hepatosomatic index (HSI = [liver weight / body weight] × 100) and viscerosomatic index (VSI = [viscera weight / body weight] × 100). Also, nine fish per tank (27 fish per treatment) were randomly captured and stored at –20°C for analyses of whole-body proximate composition and liver DPPH radical scavenging activity.

Hematocrit was determined by microhematocrit method (Brown, 1980) and the hemoglobin concentration was determined using an automated blood analyzer (SLIM, SEAC Inc., Florence, Italy). The respiratory burst activity was measured by nitro blue tetrazolium (NBT) assay described by Anderson and Siwicki (1995). Briefly, blood and 0.2% NBT were mixed in equal proportion (1:1) and incubated for 30 min at room temperature. Then 50 µl of the mixture was taken out and dispensed in glass tubes. Finally, one ml of dimethyl formaldehyde (Sigma, USA) was added, centrifuged at 2000×g for 5 min and the optical density of supernatant was measured at 540 nm using spectrophotometer (Genesys 10 UV, Rochester, NY, USA). Dimethyl formaldehyde was used as blank.

Diets and whole-body samples were freeze-dried and finely ground using a grinder. Analyses of moisture and ash contents were performed by the standard procedures (AOAC 1995). Crude protein was measured by using automatic Kjeltec Analyzer Unit 2300 (FossTecator, Höganäs, Sweden) and crude lipid was determined using Soxhlet Extraction System C-SH6 (Korea).

Antioxidant activity of the experimental diets and fish liver were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay described by Brand-Williams (1995) with some modifications. Two gram of samples was homogenized in 20 ml aqueous methanol (80%) at a ratio of 1:4 (whole liver: aqueous methanol) for 1 min using a homogenizer (X-120, Heidolph, Schwabach, Germany). The homogenates were centrifuged (5000 rpm, 4°C, 10 min) and the supernatants were filtered through a 0.45 µm syringe filter (Whatman Inc., Clifton, NJ, USA) prior to the assay. One hundred µL of the filtered extract was pipetted into a 1.5 mL cuvette, then 900 µl of DPPH methanolic solution (100 µm) was added to obtain a final volume of 1 ml. The absorbance of the mixture was measured at 517 nm with 1 min intervals for 10 min using a spectrophotometer (Genesys 10 UV, Rochester, NY, USA). The antioxidant activity of the extract against the DPPH radicals was calculated as percent inhibition:

$$\text{Percent inhibition} = [(A_0 - A_s)/A_0] \times 100$$

where $A_0$ and $A_s$ are the absorbance of the sample at 0 and S min, respectively.

The total polyphenolic compounds concentration of the experimental diets was measured by a colorimetric method described by Skerget *et al*. (2005) with some modifications. Briefly, one gram of

each diet was extracted with 250 ml methanol for 2 h at 40°C. The extracts were cooled and filtered through a 0.45 μm syringe filter (Whatman Inc., Clifton, NJ, USA). Folin-Ciocalteu reagent (0.2 N) (Sigma - Aldrich, Switzerland) of 2.5 ml was added to the filtered solution and incubated for 5 min at room temperature. Then 2 ml of $Na_2CO_3$ solution (75 g/L) was added and the mixture was incubated for 5 min at 50°C. After being cooled, the absorbance of the samples was read at 760 nm using a spectrophotometer (Genesys 10 UV, Rochester, NY, USA). The results were expressed as gram of gallic acid per kg of dry diet.

**Bacterial Challenge**

At the end of the feeding trial, 10 fish from each tank (30 fish/treatment) were randomly captured and subjected to bacterial challenge. *E. tarda* (ATCC 15947, Korea Collection for Type Cultures) was used as pathogenic agent (provided by the Marine Microbiology Laboratory of Jeju National University). The bacterium, originally isolated from diseased olive flounder, was cultured in 10 mL BHI broth (Difco, Detroit, MI, USA) with 1.5% NaCl and incubated with shaking for 24 h at 37 °C. Bacterial growth was measured at an optical density of 700 nm followed by plate counting in BHI-NaCl. The isolated bacteria were identified using the API 20E commercial identification kit (BioMérieux, Marcy l'Etoile, France). The fish were injected intraperitoneally with 1 ml of *E. tarda* suspension containing $3\times10^8$ CFU/ml. The bacterium concentration was determined by plate counting on BHI agar. The pathogenic concentration of the bacterium was determined by a preliminary experiment using the same size of fish. After injection, the fish were stocked in 12 plastic tanks of 65 L capacity and their mortality was monitored and recorded twice daily for 15 days. The cause of death was inspected by the clinical symptoms.

**Statistical Analysis**

Data were subjected to one-way analysis of variance (ANOVA) in SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). The significant differences between group means were compared using Duncan's multiple test at the 5% level of significance ($P<0.05$). The Data are presented as mean ± standard deviations (SD). The percentage data were arcsine transformed before the ANOVA analysis.

## Results

Fish growth performance and feed utilization were not significantly influenced by dietary treatments (Table 3); however fish fed hijiki and ecklonia containing diets showed higher growth rates (over 70% weight gain) compared to the control group (65%). The fish survival rate varied from 84 to 92% without significant differences among dietary treatments.

Whole-body lipid content was significantly ($P<0.05$) increased in fish fed Eck-6 diet compared to the control group and the lowest level was found in

**Table 3.** Growth performance and organosomatic indices of olive flounder (initial body eight, 18.3±0.02) fed the experimental diets for 6 weeks

| Item | Diets | | | |
|---|---|---|---|---|
| | Con | Hij-6 | Eck-6 | OTC-0.5 |
| Final body weight (g) | 30.3±0.4 | 31.5±0.7 | 31.8±1.0 | 31.3±1.2 |
| Weight gain (%) | 65.7±2.2 | 72.1±3.5 | 73.8±6.0 | 70.8±6.3 |
| Feed conversion ratio | 1.62±0.03 | 1.49±0.06 | 1.48±0.21 | 1.32±0.07 |
| Protein efficiency ratio | 1.28±0.03 | 1.39±0.06 | 1.42±0.20 | 1.35±0.06 |
| Feed intake (g/g BW) | 19.46±0.2 | 19.66±0.2 | 19.87±1.2 | 19.93±1.0 |
| Condition factor | 1.02±0.10 | 1.06±0.07 | 1.03±0.03 | 1.14±0.05 |
| Hepatosomatic index | 2.06±0.39 | 2.09±0.27 | 1.77±0.21 | 1.91±0.55 |
| Viscerosomatic index | 3.56±0.32 | 3.41±0.44 | 3.06±0.29 | 3.11±0.10 |
| Survival (%) | 92.0±8.0 | 88.0±10.6 | 84.0±4.0 | 92.0±10.6 |

Values are presented as mean±SD. The lack of superscript letter indicates no significant differences among treatments.

**Table 4.** Whole-body composition of olive flounder fed the experimental diets for 6 weeks

| | Diets | | | |
|---|---|---|---|---|
| | Con | Hij-6 | Eck-6 | OTC-0.5 |
| Moisture (%) | 73.9±1.0 | 73.6±1.0 | 74.4±0.4 | 74.0±1.1 |
| Protein (%) | 18.2±0.7 | 18.1±0.8 | 17.3±0.5 | 18.3±0.6 |
| Lipid (%) | $2.1\pm0.1^b$ | $2.1\pm0.1^b$ | $2.6\pm0.2^c$ | $1.7\pm0.0^a$ |
| Ash (%) | 3.7±0.3 | 3.4±0.5 | 3.5±0.3 | 3.5±0.4 |

Values are presented as mean±SD. Values in the same row having different superscript letters are significantly different ($P<0.05$). The lack of superscript letter indicates no significant differences among treatments.

**Table 5.** Hematological parameters of olive flounder fed the experimental diets for 6 weeks

| | Diets | | | |
|---|---|---|---|---|
| | Con | Hij-6 | Eck-6 | OTC-0.5 |
| Hemoglobin ±g/dL) | 3.97±0.9[a] | 5.79±0.7[b] | 4.24±0.6[ab] | 4.49±1.2[ab] |
| Hematocrit ±%) | 22.3±2.8 | 22.0±1.3 | 21.8±1.8 | 25.2±1.3 |

Values are presented as mean±SD. Values in the same row having different superscript letters are significantly different ±P<0.05). The *lack of superscript letter* indicates *no significant* differences among treatments.

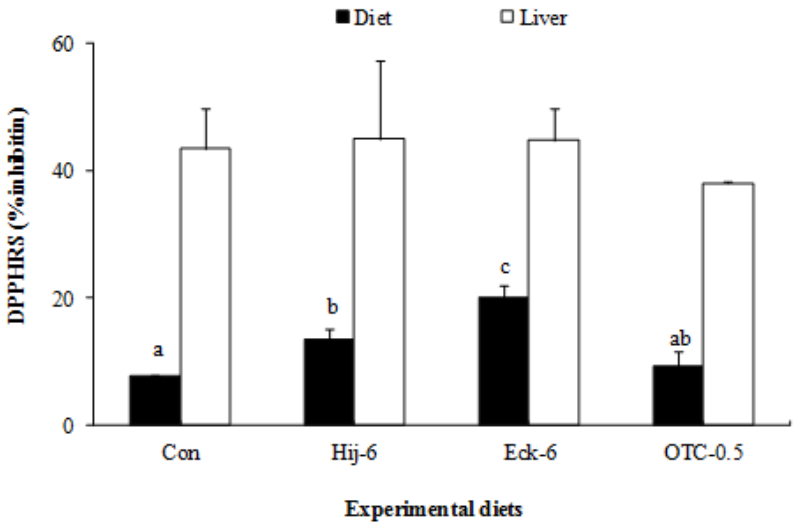


**Figure 1.** Antioxidant capacity of the experimental diets and liver of olive flounder fed the experimental diets for 6 weeks. Values are presented as mean ±SD. Bars with different letters are significantly different (P<0.05)

fish fed OTC-0.5 diet (Table 4). Whole-body protein, moisture and ash contents did not significantly differ among treatments.

The fish fed the Hij-6 diet exhibited significantly higher hemoglobin concentration than those fed the control diet (Table 5). Significantly higher respiratory burst activity was found in fish offered the Hij-6 diet (Figure 2b).

Significantly higher DPPH free radical scavenging activity (Figure 1) and total polyphenolic compounds (Figure 2a) concentration were found in hijiki and ecklonia containing diets. However, this was not result in higher liver DPPH free radical scavenging activity (Figure 1).

During the 15 days of challenge test the first fish mortality was observed on the fourth day after injection where the fish fed the Con and OTC-0.5 diets showed lower disease resistance compared to those fed the hijiki and ecklonia containing diets (Figure 3). However, at the end of the challenge test no significant differences were found among the experimental groups.

## Discussion

Polyphenols are well known as potent natural antioxidants and are widely distributed in plants including marine algae (Bravo, 1998). The antioxidant activity of *S. fusiforme* has been reported by several authors (Siriwardhana *et al*., 2003a, 2003b; 2005; Jang *et al*., 2005). The positive correlation between radical scavenging activity and polyphenolic compounds concentration of *S. fusiforme* has demonstrated that these natural bioactive materials are involved in antioxidant activity of this algae species (Siriwardhana *et al*., 2003a, 2003b, 2005; Karawita *et al*., 2005). Also, recent data from our laboratory indicated free radical scavenging activity of *E. cava* (Kim and Lee, 2008) and the level of activity corresponded to dietary polyphenolic compounds concentration. In the present study, significantly higher DPPH radical scavenging activities were found in Eck-6 and Hij-6 diets and positively correlated with dietary polyphenolic compounds concentration. The differences in free radical scavenging activity of various seaweed extracts is suggested to be due to the variation in phenolic content and/or presence of different types of polyphenolic compounds even in the same algae species (Siriwardhana *et al*., 2003a, 2003b).

The results of growth performance and feed utilization did not show any significant differences among experimental groups. Nevertheless, higher performances were obtained by supplementations of hijiki and ecklonia to the diet. This is in agreement with the results of our previous study where positive effects of hijiki were found on growth performance of olive flounder (Pham *et al*., 2006).

Inclusion of ecklonia in the diet resulted in a higher whole-body lipid content. Similarly, Güroy *et*

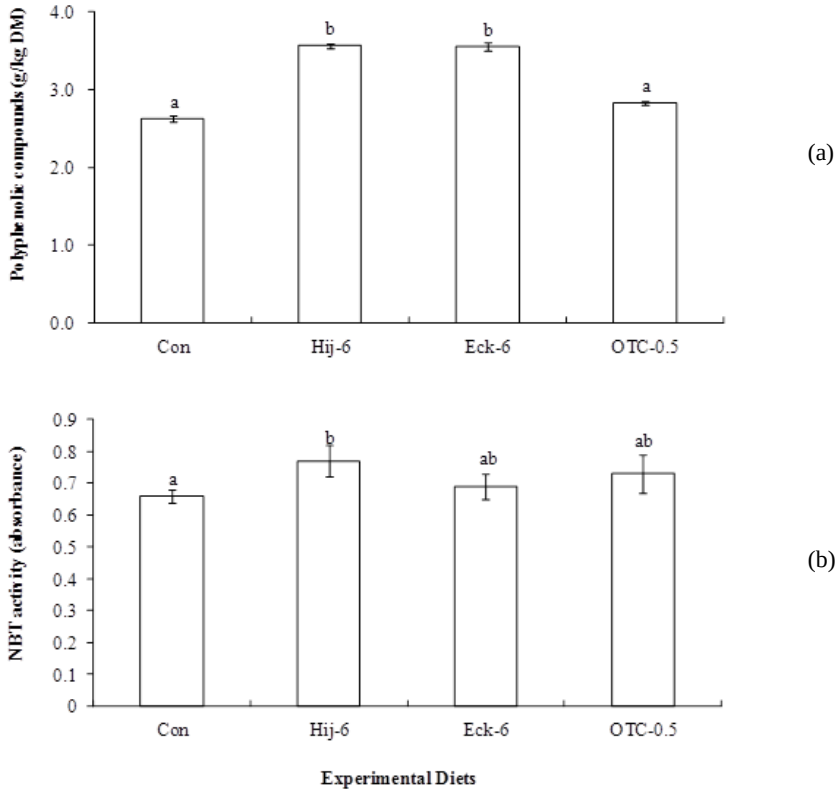


**Figure 2.** Total polyphenolic compounds concentration of the experimental diets (a) and NBT activity (b) of olive flounder fed the experimental diets for 6 weeks. Values are mean of three replicates. Bars with different letters are significantly different (P<0.05).

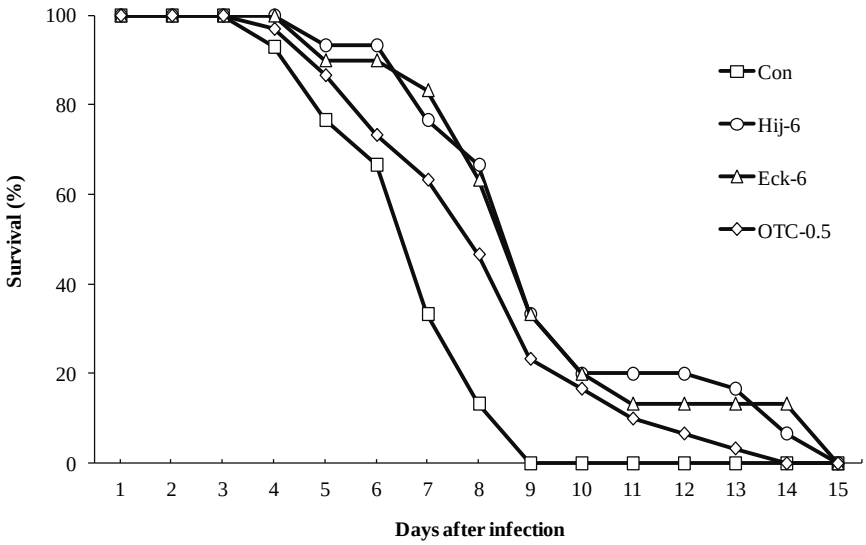


**Figure 3.** Survival rate of olive flounder fed the experimental diets after challenge with *E. tarda.*

*al*. (2007) found a significant increase in whole-body lipid of Nile tilapia fed Cystoseira containing diet. It has been demonstrated that lipid metabolism in fish is affected by dietary supplementation of algae meal (Nakagawa, 2010). However, it seems that the effect of algae meal on fish carcass composition is related to their nutritional value and dietary inclusion level. The other influencing factors are fish species, size, age and experimental protocol (Diler *et al*., 2007; Ergün *et al*., 2008; Dantagnan *et al*., 2009; Güroy *et al*., 2007).

Phagocytes including neutrophils and macrophages are considered as important cellular components of non-specific immune response and play an important role in the host defense against invading pathogens by producing reactive oxygen

intermediates known as respiratory burst activity (Whyte, 2007). It has been demonstrated that this metabolic event can be modulated by various bioactive compounds with immunomodulating activity (Castro *et al*., 2004). There is growing evidence that many edible and inedible types of seaweeds possess biologically active immunemodulators (Okai *et al*., 1998; Shan *et al*., 1999; Castro *et al*., 2004; Leiro *et al*., 2007).

In the present study, significantly higher respiratory burst activity was found in fish fed hijiki containing diet. This finding is in agreement with the results of the study by Pham *et al*. (2006) where the mean number of activated neutrophils, measured by NBT assay, was significantly increased by dietary supplementation of *S. fusiforme*. Similarly, Song *et al*. (2011) found the significant increase of respiratory burst, myeloperoxidase and lysozyme activities in parrot fish fed a *S. fusiforme* containing diet and dietary supplementation of *E. cava* resulted in a higher lysozyme activity. Also, the results of a study by Ahn *et al*. (2008) showed that an enzymatic extract from *E. cava* can enhance the proliferation of splenocytes and increase the number of lymphocytes, monocytes and granulocytes in mice.

Castro *et al*. (2004) examined the effects of eight different seaweed species on respiratory burst activity of turbot (*Psetta maxima* L.) phagocytes *in vitro*. They suggested that polysaccharides are most likely responsible for the immunomodulatory activity of the tested species. In agreement to this, Leiro *et al*. (2007) indicated that several RAW264.7 murine macrophage activities can be efficiently induced by *U. rigida* acidic sulphated polysaccharides, and the sulphate group identified as a key part of this immunostimulating activity. Also, Chen *et al*. (2012) investigated the immunomodulatory activity of polysaccharides from *S. fusiforme* in tumor-bearing mice and their results showed the activation of peritoneal macrophages following polysaccharides administration. Furthermore, the mentioned potential radical scavenging ability of the two tested seaweeds could probably promote the fish defense system by protecting the important parts and functions of the immune system from oxidative damage. It may seem critical in the case of phagocytes that are always threatened by auto-oxidation during oxidative burst activity. On the other hand, the results of recent studies have exhibited the protective effects of polyphenolic compounds on immune system (Aquilano *et al*., 2008; Franova *et al*., 2010).

In this concept, the results of our previous study showed the positive correlation between dietary polyphenols and respiratory burst activity of parrot fish (*Oplegnathus fasciatus*) fed spirulina containing diets (Kim *et al*., 2013). However, further studies are necessary to characterize the exact compounds that are responsible for the immunomodulatory properties of these algal metabolites and to explore the exact underlying mechanisms.

Infectious diseases, caused by wide array of pathogens including bacteria, are one of the most significant factors threatening the on-growing aquaculture industry. Antibiotics have been used widely for controlling fish diseases (Austin and Austin, 2007). Growing concerns about the use of antibiotics in aquaculture has directed attention towards natural compounds with potent antimicrobial and immunostimmulating properties such as various plant-derived compounds (Citarasu, 2010). The beneficial effects of algae-derived bioactive compounds on fish immune function and consequently on their resistance to certain pathogens has been demonstrated in several studies on various fresh water and marine fish species including carp (*Cyprinus ca*rpio) (Fujiki and Yano, 1997; Fujiki *et al*., 1997), turbot (*Scophthalmus maximus* L.) (Skjermo *et al*., 1995), rainbow trout (*Oncorhynchus mykiss*) (Peddie *et al*., 2002), Atlantic salmon (*Salmo salar* L.) (Dalmo and Seljelid, 1995) and grouper (*Epinephelus coioides*) (Cheng *et al*., 2007; Yeh *et al*., 2008). Chiu *et al*. (2008) found that supplementation of sodium alginate, a natural polysaccharide derived from brown algae, to juvenile grouper (*Epinephelus fuscoguttatus*) diets can significantly enhance innate immune response and disease resistance against *Streptococcus* sp. Similar results were found with common carp (*Cyprinus carpio* L.) where the same polysaccharide was observed to enhance the non-specific immune response (Fujiki and Yano, 1997) and disease resistance against *E. tarda* infection (Fujiki *et al*., 1994). The slight enhancement in disease resistance of olive flounder in the present study by dietary administration of the two brown algae may be due to the presence of such bioactive materials including polysaccharides and polyphenols. As suggested earlier by Liang *et al.* (2006), in the present study challenge dosage seemed too high to detect differences among treatments.

According to the results, it can be concluded that dietary inclusion of hijiki and ecklonia can improve non-specific immune response and to some extent the fish disease resistance.

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