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Effect of feed supplementation with *Origanum vulgare* L. essential oil on sea bass (*Dicentrarchus labrax*): A preliminary framework on metabolic status and growth performances

Francesca Rita Dinardo, Michele Deflorio, Elisabetta Casalino *, Giuseppe Crescenzo, Gerardo Centoducati

Department of Veterinary Medicine, University of Bari Aldo Moro, Casamassina km 3, 70010 Valenzano, Italy

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ABSTRACT

This study provided a preliminary framework for the effects of *Origanum vulgare* L. essential oil (EO) on sea bass (*Dicentrarchus labrax*) health status over a 60-day feeding trial. Fish were fed twice a day until apparent satiety with three different diets: a control diet (CD), and two experimental diets supplemented with 100 (D100) and 200 (D200) ppm of oregano EO. No mortality was observed in each treatment. Feeding on D100 diet resulted in high growth performances and better food conversion and protein efficiency ratios. Additionally, the supplementation of 100 ppm EO diet also improved ($P < 0.05$) hepatosomatic and viscerosomatic indices, compared both to control and D200 diets. EO feeding positively affected ($P < 0.05$) several serum biochemical indices (amylase activity and total proteins, glucose, triglycerides, and cholesterol levels). Focusing on the antioxidant potential of blood, D100 led to the highest ($P < 0.05$) ferric reducing antioxidant power values and the lowest ($P < 0.05$) thiobarbituric acid-reactive substances levels in blood.

1. Introduction

During the last decades, fish products have attracted considerable attention as a source of valuable proteins, vitamins, minerals, and polyunsaturated fatty acids, as well for cultural and gastronomic reasons, leading to a dramatic increase in fish consumption (FAO, 2018; Jennings et al., 2016). Limited natural resources are incompatible with growing market trends, so aquaculture is gaining interest to ensure fish supply (Mendes and Gonçalves, 2008). The success of breeding treatments is crucial to preserve the healthiness of fish, and consequently to guarantee high yields (Long et al., 2019). However, farming conditions, such as high density of cultured fish, confinement and harvest may directly affect the water quality and animal health, resulting in low immune competence and high susceptibility to disease (Tort, 2011). For this reason, alternatives biocontrol measures are needed to develop profitable aquaculture productions (Diler et al., 2017). Feed additives are non-nutritive ingredients that are included in feed formulations to improve health status of farmed aquatic specimens (Harikrishnan et al., 2011). Plant derivates used as feed additives may act as immunostimulants or antimicrobial agents, and represent valuable alternatives to antibiotics and other chemical drugs (Galindo-Villegas and Hosokawa, 2004). Several studies have reported the use of plant essential oils (EO) or their purified constituents (*e.g.*, thymol, carvacrol, resveratrol, curcumin, hydroxytyrosol) in the diets of large and small animals (Dalle Zotte et al., 2016; Frankič et al., 2009). Due to its heterogeneous composition, the biological effects of EO may be the result of synergistic effects among different molecules (Sutili et al., 2018). The EO constituents seem to exert positive effects on the reproductive and growth performances, as well on reduction of morbidity and mortality rates (Abd El-Hack et al., 2016). Thus, EO may provide a safe and ecological alternative to synthetic antibiotics (Peś et al., 2016; Saccol et al., 2013; Zheng et al., 2009). EO may also modulate gut bacterial composition and positively affect the intestinal functions of farmed aquatic animals (Sutili et al., 2018). Finally, EO as feed additives may exert preservatives effects on fish fillets, counteracting lipid oxidation and bacterial spoilage, as well their use may represent an effective strategy to fortify fish fillets with bioactive compounds having health-promoting effect for humans (Erkan et al., 2011; Kykkidou et al.,

* Corresponding author.
E-mail addresses: francesca.dinardo@uniba.it (F.R. Dinardo), michele.deflorio@uniba.it (M. Deflorio), elisabetta.casalino@uniba.it (E. Casalino), giuseppe.crescenzo@uniba.it (G. Crescenzo), gerardo.centoducati@uniba.it (G. Centoducati).

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2009; Tironi et al., 2010).

Oregano (*Origanum vulgare* L., 1753) is an aromatic plant of the *Lamiaceae* family growing in several Mediterranean regions. Oregano is worldwide used as flavoring agent or in pharmacological formulations (Chishti et al., 2013). Several *in vitro* and *in vivo* studies reported the positive effects of oregano and its bioactive components on human health (*e.g.*, to counteract chronic degenerative and infectious diseases) (Dundar et al., 2008; Oflaz et al., 2002; Pezzani et al., 2017). The major constituent of oregano EO, carvacrol, has been recognized as a safe food additive, therefore carvacrol-rich oregano oils have been applied in farmed animal diets as growth- and health-promoter (Baser, 2008; Burt, 2004). Previous studies have linked the effects of oregano EO to the metabolic performances and healthiness of farmed fish (Diler et al., 2017; Zhang et al., 2020; Zheng et al., 2009).

Sea bass (*Dicentrarchus labrax* L., 1758) is one of the most cultured and traded fish in Mediterranean area due to its high growth performance, low farming costs, appreciated taste and nutritional quality (Vandeputte et al., 2019). There are few studies about diet supplementation with oregano EO in the management of sea bass, mainly concerning the anesthetic effect and the immunostimulation against pathogens (Bodur et al., 2018; Khalil et al., 2018; Volpatti et al., 2014). Therefore, the present work aimed to fill the knowledge base gaps by investigating the potential effects of different levels oregano EO supplementation on *Dicentrarchus labrax*, focusing on some blood biochemical parameters, oxidative stress biomarkers and growth performances.

2. Materials and methods

2.1. Fish and culture conditions

Experimental samples of juveniles sea bass were obtained from the commercial fish farm "Ittica Caldoli'' (San Nazario, Lesina, FG, Italy). Fish were acclimatized to laboratory conditions for 15 days and then randomly distributed in nine cylindrical fiberglass tanks (2 m^3 water capacity), totalizing 25 fish per tank (average body weight of 80.83 ± 2.11 g/fish and average stock density of 1.00 kg/m^3). The tanks were linked to a closed recirculating system, with a water flow of 7200 L/h (eight total volume recirculate per day), equipped with chilling and heating devices and with mechanical, biological and UV filter. Once per week the 10 % of total water was changed with reconstituted sea water. Data about temperature, dissolved oxygen, pH and salinity of the water were collected by means of a tester HI-9829 (Hanna Instruments, Padova, Italy) and total ammonia, nitrite and nitrate were measured with colorimetric kit (Testlab Marin, JBL), on weekly basis throughout the experimental period (including acclimatization and feeding trials). During the experimental period, lasted from June to September 2019, water temperature and salinity were maintained close to 25 ± 2 °C and at 30‰ ± 2, respectively. Dissolved oxygen concentration was 7.4 ± 0.5 mg/L, pH was 7.7 ± 0.1, and light was in natural photoperiod conditions. Total ammonia (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) concentrations were kept below 0.05 mg/L, 0.20 mg/L, and 2.0 mg/L, respectively.

2.2. Experimental diets

The commercial feed "Basic 3" was provided by Veronesi Mangimi A.I.A. S.p.A (Verona, Italy) and its nutritional composition is reported in Table 1. Oregano EO was purchased from Farmalabor S.r.l. (Canosa di Puglia, Italy) and its chemical composition is reported in Table 2. The procedure of oregano EO addition to the diet was according to the protocol described by Dairiki et al. (2013) and de Souza Silva et al. (2019). Briefly, oregano EO was dissolved in grain alcohol to prepare EO suspensions at different concentrations. Commercial pellets were ground and the resultant powder was mixed with the EO suspensions to reach the final concentrations of 100 (D100) and 200 (D200) ppm of EO

Table 1
Nutritional composition of commercial feed[a].

Ingredients	Composition (%)
Crude protein	42.00
Crude fat	18.00
Crude fiber	3.20
Ash	9.00
Total carbohydrates	18.80
Mineral supplement	1.40
Vitamin C (mg/Kg)	160.00
Vitamin E (mg/Kg)	160.00
Gross energy (mj/Kg)	18.44

[a] The feed was supplied by Veronesi Mangimi A.I.A. S.p.A-Italy.

Table 2
Chemical constituents of *Origanum vulgare* L. essential oil[a].

Compounds	Concentrations
Hydrocarbons	15 %
Citral	0.5 %
Geraniol	0.2 %
Limonene	0.3 %
Linalool	0.3 %
Eugenol	0.1 %
Arsenic	<1 mg/kg
Lead	<1 mg/kg
Mercury	<1 mg/kg
Cadmium	<1 mg/kg
Total heavy metals	<10 mg/kg
Carvacrol	60–80%
Beta-caryophyllene	2.5–8.0 %

[a] The essential oil of *Origanum vulgare* L. was supplied by Farmalabor S.R.L.-Italy.

(Arciuli et al., 2017). In the control diet (CD), the same amount of pure grain alcohol (100 mL per kg) was added to the feed, without EO supplementation. The mixtures were homogenized, pelleted and left to dry at 25 °C for 24 h before being packed in plastic bags and stored at −18 °C. The required amount of feed (ca. 600 g) was stored at 4 °C just one day before administration. Each diet was tested in triplicate (3 tanks per treatment). The fish were fed twice a day for 60 days, and each ration was dispensed until apparent satiety of fish.

Management of the animals and sampling have been carried out to minimize stress and health risks. All procedures involving animals were carried out in compliance with the Italian guidelines for animal care (DL 26/14) and the European Communities Council Directive (2010/63/UE), and approved by the General Directorate of Animal Health and Veterinary Drugs of Ministry of Health, with authorization n° 444/2019-PR on the 12nd of June 2019.

2.3. Sample collection and analysis

After 30 and 60 days of feeding, fifteen fish from each treatment (5 fish per tank) were sampled and anesthetized with fish clove oil at a dose of 30 mg/L (Mylonas et al., 2005). Blood was drawn from the caudal vein, collected in plastic tubes and allowed to clot at room temperature. Then, serum was separated by centrifugation at 3000 rpm for 5 min and stored at −80 °C until further analysis.

Viscera and liver weights were recorded at the end of experimental period. After blood collection, fish were soaked in ice-slurry to achieve death by hypothermia, and viscera were quickly removed to be weighed at once (Blessing et al., 2010).

At the end of the feeding trial, the fish were sampled, weighed and measured to determine the performance parameters.

2.4. Determination of fish perfomance and feed utilizations indices

Growth performances were determined according to Li et al. (2019)

as follow: Weight gain (WG) = 100 * (final body weight – initial body weight)/initial body weight; Specific growth rate (SGR) = 100 * [Ln (final body weight) – Ln (initial body weight)]/days of feeding trial.

Feed utilizations of fish were calculated according to Yılmaz et al. (2012) as follows: Feed conversion rate (FCR) = feed intake/WG; Protein efficiency ratio (PER) = WG/protein intake.

2.5. Determination of biometric indices

Total fish length, total fish weight, liver and viscera weight were recorded in order to calculate hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (CF). HIS was determined according to Busacker et al. (1990) as follow: HSI = 100 * (liver weight /total fish weight). VSI was estimated according to Ricker (1979) as follow: VSI = 100 * (viscera weight/total fish weight). CF was estimated according to Fu et al. (1998): CF = 100 * [total fish weight/ (total fish length)^3].

2.6. Serum biochemical indices

Proteins concentrations were determined by the Bradford assay procedure using bovine serum albumin as standard (Bradford, 1976) and expressed as g/dL. Glucose, Cholesterol, and Triglycerides were determined through commercial colorimetric kits (FAR S.r.l., Pescantina (VR), Italy), and concentrations were expressed as mg/dL. Amylase (EC 3.2.1.1) and Lipase (EC 3.1.1.3) activities were determined through commercial colorimetric kits according to the manufacturer instructions (Sigma-Aldrich, St. Louis, MO, USA). Enzymes activities were reported as U/L. Each sample analysis was performed in triplicate.

2.7. Oxidative stress indices

The thiobarbituric acid-reactive substances (TBARS) assay was performed in serum to quantify peroxidative damage to lipids occurred with free radical generation (Buege and Aust, 1978). The amount of malonaldehyde (MDA) produced, a marker for lipid peroxidation, was determined spectrophotometrically at 535 nm. TBARS levels were expressed as nmol MDA/mg protein. Ferric reducing antioxidant power (FRAP) assay was used to measure total antioxidant potential according to the method described by Benzie and Strain (1996) with a slight modification. 3 mL of freshly prepared FRAP reagent (1 mL of a 10 mM TPTZ solution in 40 mM HCl plus 1 mL of 20 mM $FeCl_3$ in 10 mL H_2O solution and 10 mL of 300 mM acetate buffer, pH 3.6) was incubated at 37 °C for 40 min after mixing with 100 µL of serum sample. The absorbance of the reaction mixture was recorded at 593 nm and the antioxidant power was expressed as µmol Trolox Equivalents (TE)/mL. Each sample analysis was performed in triplicate.

2.8. Statistical analysis

Treatments were performed in triplicate. Data were reported as means ± standard deviations. One-way analysis of variance (ANOVA) were performed to detect significant differences in growth performance, feed utilization and biometric indices among feeding treatments. Data for blood parameters and oxidative stress biomarkers were submitted to a one-way ANOVA to show significant differences within the same sampling day or within the same diet. Data were analyzed using Statistica 13.0 (Statsoft Inc., Tulsa, USA). Tukey post hoc tests was used to compare means, with significance level of 5%.

3. Results

3.1. Growth perfomance and feed utilization indices

After 60 days, fish fed on diet containing 100 ppm (D100) oregano EO showed a significantly higher final body weight compared both to control (CD) and 200 ppm EO diet (D200) (Table 3). The highest weight gain (WG) and specific growth rate (SGR) values were recorded in D100 (Table 3). Regarding feed utilization indices, feed conversion ratio (FCR) was significantly improved in D100. D100 and D200 showed the highest and the lowest protein efficiency ratio (PER) respectively (Table 3).

3.2. Biometric measurements

Significant variations were observed in hepatosomatic (HSI) and viscerosomatic (VSI) indices among the diet treatments. Fish fed on diet formulated by adding 100 ppm EO showed the highest ($P < 0.05$) values of HSI and VSI indices (Table 4). In contrast, D200 diet led to the lowest ($P < 0.05$) value of VSI (Table 4). Regarding the condition factor (CF), no significant differences were found among feeding treatments ($P > 0.05$) (Table 4).

3.3. Serum constituents and digestive enzyme activities

Overall, the total protein concentration increased during the feeding trials (Table 5). The highest ($P < 0.05$) levels were detected in D100 both at 30 and 60 days (2.69 ± 0.23 and 3.33 ± 0.45 g/dL, respectively). No significant differences were observed for the protein levels between CD and D200 (2.40 – 2.34 g/dL at 30 days and 2.85–3.07 g/dL at 60 days, respectively) (Table 5).

The glucose content in CD increased ($P < 0.05$) after 30 days, and then returned to the initial value after 60 days (117.65 ± 0.95 mg/dL) (Table 5). Conversely, in fish fed on 100 ppm, the glucose concentration remained stable after 30 days (124.22 ± 1.04 mg/dL) and then significantly ($P < 0.05$) decreased after 60 days (90.62 ± 0.89 mg/dL), resulting in levels significantly ($P < 0.05$) lower than CD and D200 (Table 5). No significant changes ($P > 0.05$) were observed with D200 for the glucose content during the feeding trials (113.47 ± 0.64 and 109.91 ± 0.78 mg/dL at 30 and 60 days, respectively). Overall, comparing feeding treatments, the lowest ($P < 0.05$) glucose values were recorded with D100 (Table 5).

The cholesterol content in fish fed on control diet remained stable (336.21−332.44 mg/dL), whereas it constantly went down ($P < 0.05$) throughout the feeding trials with both D100 and D200 diets (217.76 ± 0.46 and 215.75 ± 0.30 mg/dL at 60 days, respectively) (Table 5). The triglycerides level increased ($P < 0.05$) after 30 days (1192.39 ± 9.02 mg/dL), and then slightly decreased ($P < 0.05$) after 60 days (986.57 ± 7.19 mg/dL) with the CD (Table 5). A similar trend was observed with D200, whereas the triglycerides level constantly went down ($P < 0.05$) throughout the feeding trials in fish fed on 100 ppm (976.73 ± 7.38 and 653.73 ± 4.26 mg/dL at 30 and 60 days, respectively) (Table 5).

Regarding amylase activity, in fish fed on 100 and 200 ppm EO a significant decrease was found after 30 days (1.68−1.57 U/L, respectively) and 60 days (1.71–1.83 U/L, respectively) with respect to the initial activity level (2.26 ± 0.01 U/L) (Table 5). No significant variations were observed with the CD (Table 5). Lipase activity did not change ($P > 0.05$) during the feeding trials (38.60–39.37 U/L).

Table 3
Growth performance and feed utilization indices of sea bass (mean ± standard deviations) fed on control diet (CD) or experimental diets (D100 and D200) for 60 days.

	CD	D100	D200
Initial body weight (g)	80.83 ± 2.11	80.83 ± 2.11	80.83 ± 2.11
Final body weight (g)	240.41 ± 4.95^b	290.72 ± 5.71^a	199.64 ± 6.17^c
Weight gain (%)	197.43 ± 1.28^b	259.67 ± 1.58^a	146.99 ± 1.13^c
Specific growth rate (%/d)	1.82 ± 0.04^b	2.13 ± 0.07^a	1.51 ± 0.08^c
Feed conversion ratio	1.40 ± 0.03^b	1.09 ± 0.07^c	1.98 ± 0.25^a
Protein efficiency ratio	1.70 ± 0.08^b	2.17 ± 0.11^a	1.20 ± 0.07^c

Different letters (a–c) in the same row indicate significant differences ($P < 0.05$) for each parameter.

Table 4
Biometric parameters (mean ± standard deviations) of sea bass fed on control diet (CD) or experimental diets (D100 and D200) for 60 days.

	CD	D100	D200
Hepatosomatic index	1.24 ± 0.26^{b}	1.51 ± 0.37^{a}	1.29 ± 0.35^{b}
Viscerosomatic index	8.23 ± 0.43^{b}	9.97 ± 0.61^{a}	7.09 ± 0.52^{c}
Condition Factor	1.04 ± 0.05	1.05 ± 0.07	1.04 ± 0.05

Different letters (a–c) in the same row indicate significant differences ($P < 0.05$) for each parameter.

Table 5
Serum biochemical indices (mean ± standard deviations) of sea bass fed on control diet (CD) or experimental diets (D100 and D200) for 60 days.

	DAYS	CD	D100	D200
Total Protein (g/dL)	0	2.33 ± 0.12^{B}	2.33 ± 0.12^{B}	2.33 ± 0.12^{B}
	30	2.40 ± 0.09^{Bb}	2.69 ± 0.23^{Ba}	2.34 ± 0.21^{Bb}
	60	2.85 ± 0.11^{Ab}	3.33 ± 0.45^{Aa}	3.07 ± 0.28^{Aab}
Glucose (mg/dL)	0	118.00 ± 0.95^{B}	118.00 ± 0.95^{A}	118.00 ± 0.95
	30	151.07 ± 1.46^{Aa}	124.22 ± 1.04^{Ab}	113.47 ± 0.64^{c}
	60	117.65 ± 0.95^{Ba}	90.62 ± 0.89^{Bc}	109.91 ± 0.78^{b}
Cholesterol (mg/dL)	0	336.21 ± 1.06	336.21 ± 1.06^{A}	336.21 ± 1.06^{A}
	30	339.29 ± 1.54^{a}	281.33 ± 0.16^{Bb}	240.14 ± 0.73^{Bc}
	60	332.44 ± 0.48^{a}	217.76 ± 0.46^{Cb}	215.75 ± 0.30^{Cb}
Triglycerides (mg/dL)	0	1074.49 ± 8.81^{B}	1074.49 ± 8.81^{A}	1074.49 ± 8.81^{B}
	30	1192.39 ± 9.02^{Ab}	976.73 ± 7.38^{Ac}	1254.43 ± 9.86^{Aa}
	60	986.57 ± 7.19^{Ba}	653.73 ± 4.26^{Bc}	684.27 ± 4.28^{Cb}
Amylase (U/L)	0	2.26 ± 0.01	2.26 ± 0.01^{A}	2.26 ± 0.01^{A}
	30	2.23 ± 0.03^{a}	1.68 ± 0.03^{Bb}	1.57 ± 0.03^{Cc}
	60	2.22 ± 0.04^{a}	1.71 ± 0.03^{Bc}	1.83 ± 0.02^{Bb}

Different capital letters (A–C) in the same column indicate significant differences ($P < 0.05$) among sampling days within the same diet.
Different lowercase letters (a–c) in the same row indicate significant differences ($P < 0.05$) among diets within the same sampling days.

3.4. Oxidative stress evaluation

The ferric reducing antioxidant power (FRAP) and the levels of thiobarbituric-acid reactive substances (TBARS) levels are shown in Fig. 1. Overall, the FRAP levels decreased during the feeding trials, in particular in fish fed on CD and D200 (Fig. 1A). TBARS concentrations significantly varied ($P < 0.05$) between the diets, with highest levels detected with CD and D200 (Fig. 1B).

4. Discussion

In aquaculture, much attention has been given to feed additives in order to preserve the fish healthiness, and to improve the fillet nutritional quality (Galindo-Villegas and Hosokawa, 2004). High stocking density or any other stressful condition require specific physiological responses to counteract stressors and to cope with additional energetic requirements. Thus, nutritional strategies focused on the use of plant extracts to modulate the immunological and physiological response, to counteract the oxidative stress, or to promote the gastrointestinal tract functions have attracted increased interest in animal production (Sutili et al., 2018). EO modes of action includes different mechanisms, such as antimicrobial, antioxidant, immunomodulatory, hypoglycaemic, and hypocholesterolemic activities, and regulation of enzyme and hormones secretion/function (Acar et al., 2015; Gülec et al., 2013; Rafieepour et al., 2019; Sutili et al., 2018).

European sea bass was selected for the present study as one of the most cultured species in the Mediterranean area with a well-known sensitivity to stress. According to some authors, the use of EO in fish diet may affect its metabolic functions, wellness, and growth performances, although studies are relatively recent and results do not always agree (Abdel-Latif and Khalil, 2014; Sharif Rohani et al., 2011; Soltani et al., 2013). The different findings reported in the literature could be due to the different age of fish used in experiments, the stocking density, and, especially, to the level of adding of oregano EO in the diet, and to the EO composition.

Under the conditions of our study, a positive effect on biometric parameters was observed when sea bass was fed on diet containing oregano EO compared to the control diet. HSI is generally used as indirect measure of energy status since the liver is an important store of energy reserve. A positive correlation was previously found between HSI and the glycogen reserves, which are mobilized by fish when food

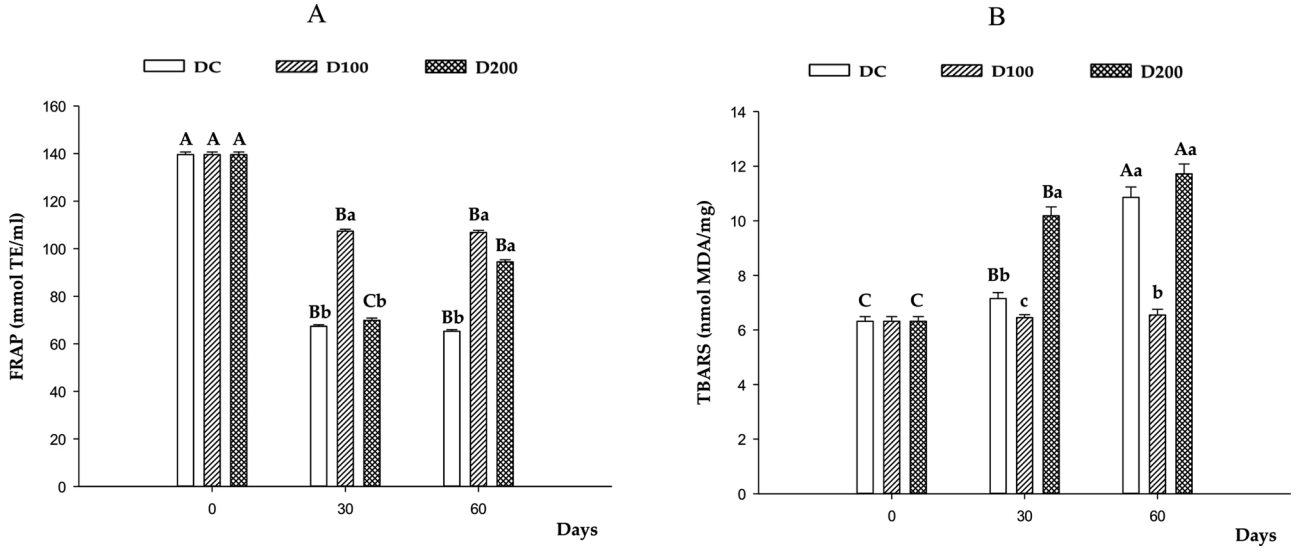

Fig. 1. Level of FRAP (A) and TBARS (B) in sea bass fed on control diet (CD) or experimental diets (D100 and D200) for 60 days. Data are reported as means ± standard deviations. Different capital letters (A-C) indicate significant differences ($P < 0.05$) among sampling days within the same diet. Different lowercase letters (a-c) indicate significant differences ($P < 0.05$) among diets within the same sampling day.

availability is scarce (Chellappa et al., 1995). A reduction in HSI was reported in rainbow trout (*Oncorhynchus mykiss* W., 1972) and sea bream (*Sparus aurata* L., 1758) held at high stocking density (Leatherland and Cho, 1985; Montero et al., 1999). Our results showed higher HIS and lower glucose level in serum in fish fed on 100 ppm oregano EO with respect to CD. Yılmaz et al. (2016) also reported a decrease of glucose levels in sea bass after feeding with thyme (*Thymus vulgaris* L.), rosemary (*Rosmarinus officinalis* L.), and fenugreek (*Trigonella foenum--graceum* L.) seed powders. Several authors positively assessed the reduction of glucose levels due to diet supplementation with plant extracts, since serum glucose has been suggested as a nonspecific stress marker in fish (Acar et al., 2015; Morgan and Iwama, 1997; Yılmaz et al., 2016). The decrease of serum glucose concentrations might be due to the to the stress-mitigating effect of oregano EO (Acar et al., 2015; Gülec et al., 2013). We may not exclude the hypoglycaemic effect of carvacrol, the major component of oregano EO we used. The role of carvacrol in blood glucose regulation has been demonstrated through *in vitro* and *in vivo* studies on mice and rats (Bayramoglu et al., 2014; Ezhumalai et al., 2015; Habtemariam, 2018). Similar results have been observed in rainbow trout fed on diet supplemented with carvacrol (Yılmaz et al., 2015), although the mechanism by which carvacrol reduces blood glucose in aquaculture species is not known. Molecules others than carvacrol have been shown to exert hypoglycemic effect in seabass, enhancing insulin action, facilitating the insulin/insulin receptor interaction, thus improving glucose-uptake by the tissues (Martins et al., 2020). EO treatment also led to a decrease of serum amylase activity, likely as consequence of the hypoglycaemic activity of carvacrol (Ghorani et al., 2018; Stojanović et al., 2019). Even though in the interest of researchers, there is a lack of knowledges about the mechanism underlying amylase activity, secretion and biosynthesis in fish (Krogdahl et al., 2005). The reduced activity of serum amylase as a consequence of EO supplementation likely indicates a shift in the carbohydrate metabolism attributable to lower energy needs. On the contrary, high levels of amylase activity occur in fish to cope with high metabolic needs under stressful condition or impaired pancreatic function (Inyang et al., 2016; Nwamba et al., 2006).

Carvacrol is expected also to underlie the decrease of triglyceride and cholesterol levels observed in serum in fish fed on EO, accordingly to results reported on diabetic mice fed on carvacrol for 35 days (Ezhumalai et al., 2015). Our results for triglyceride and cholesterol showed similarity with the results of Yılmaz et al. (2015) that found reduced concentrations in sea bass fed with thyme, rosemary, and fenugreek seed powders. Citrus EO was also shown to decrease the triglyceride and cholesterol levels in tilapia (*Oreochromis mossambicus*) (Acar et al., 2015). Usually, farmed seabass has higher overall lipid levels than its wild counterpart (Orban et al., 2003). Lipogenesis enzymes in seabass is depending by the serum cholesterol levels, and the regulation of cholesterol *de novo* biosynthesis is mainly controlled by the rate-limiting enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) (Castro et al., 2015; Dias et al., 1998, 2005). EO components have been reported to inhibit HMGCR, thus deactivating cholesterogenic processes (Lee et al., 2004).

Plant derivates used as feed additives may promote several non-specific immune defense mechanisms and, to a lesser extent, some specific defense responses, increasing the resistance of fish species to infectious agents. Serum proteins level is an important nonspecific immune response of fish (Dorucu et al., 2009). Under the condition of our study we observed an increase of serum proteins concentration in fish fed on experimental diet enriched with 100 ppm EO. Similarly, Pourmoghim et al. (2015) found that adding a dietary extract of *O. vulgare* to the diet stimulated non-specific immunity in rainbow trout. Supporting the role of serum protein in humoral defense system, a recent study reported an increase level of such constituents combined with a stronger resistance to *Tenacibaculum maritimum* diseases in sea bass fed on 1% *O. vulgare* oil extract for 28 days (Khalil et al., 2018). Thus, we may state that the inclusion of oregano EO in the diet may enhance the non-specific immune response and resistance to stressful condition of sea bass.

Blood antioxidant power is important to counteract the oxidative stress and to maintain redox homeostasis (Domínguez et al., 2018). Compared to the control diet, the higher levels of FRAP and the concomitant lower levels of TBARS highlighted a better oxidative balance in fishes fed on 100 ppm oregano EO due to the ability of EO to withstand oxidative stress and to counteract the lipid peroxidation. We attributed the beneficial effects of oregano EO to carvacrol as it acts as reducing agent, free radical scavenger as well as metal chelator (Barillari et al., 2005; Hanafi et al., 2010; Maqsood et al., 2013).

The growth performance seemed to be differently affected by oregano EO depending on the level of feed supplementation. Compared to the control diet, a positive effect on growth parameters (*e.g.*, final body weight, weight gain, and specific growth rate) was observed when sea bass was fed diet containing 100 ppm EO, while the same parameters got worse in 200 ppm EO diet. Some reports suggested that peculiar aromatic flavour of oregano EO increase feed palatability, resulting in high voluntary feed intake and weight gain (Abdel-Latif and Khalil, 2014). On the other hand, the intense smell due to high level of EO could reduce palatability of the ration, leading to poor growth performances. Our growth performances results were supported by previous reports (De Moraes França Ferreira et al., 2014; Diler et al., 2017; Zheng et al., 2009) on rainbow trout, yellowtail tetra (*Astyanax altiparanae*), and channel catfish (*Ictalurus punctatus*). Feed parameters were in line with growth performances. Fish fed on 100 ppm EO diet showed better FCR and PER, compared both to control and 200 ppm EO diet. The positive effect of D100 on FCR may be due to the inherent functional properties of EO (*e.g.*, regulation of digestive enzyme and hormones secretion, immune stimulation, synergistic interactions with the gastro-intestinal microbiota, and antibacterial and antioxidant activities) which may result in increased digestibility, nutrients absorption and protein conversion (El-Dakar et al., 2008; Giannenas et al., 2018; Rafieepour et al., 2019). Accordingly, Gonçalves et al. (2019) reported that supplementation of diets with a mixture of essential oils, including oregano oil, improved feed utilization indices in sea bass. An improvement of feed parameters was observed also in sea bass fed on diet added with thyme (Yılmaz et al., 2012), whereas no effects were recorded by Cararo et al. (2017) on silver catfish juveniles fed on oregano EO.

5. Conclusions

Summarizing, the preliminary framework we constructed clearly shows that feed supplementation with oregano EO may differently affect several biomarkers of sea bass wellness depending on the level of feed supplementation. In particular, low doses of oregano EO hold a promising potential as health promoter for sea bass. Several biometric indices, blood parameters, and oxidative stress biomarkers benefited from the administration of oregano EO. One mechanism underlying the metabolic effects of oregano EO might be imputable to the biological properties of its main constituent, carvacrol.

CRediT authorship contribution statement

Francesca Rita Dinardo: Validation, Formal analysis, Investigation, Data curation. **Michele Deflorio:** Investigation. **Elisabetta Casalino:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision. **Giuseppe Crescenzo:** Supervision, Funding acquisition. **Gerardo Centoducati:** Conceptualization, Resources, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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