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Effect of dietary celery (*Apium graveolens*) on the growth performance, immune responses, and bacterial resistance against *Vibrio anguillarum* of European seabass (*Dicentrarchus labrax*)

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Abstract In this study, we evaluated to reveal the effects of aqueous methanolic extract of celery (*Apium graveolens*) on the growth performance, immune responses, and resistance against *Vibrio anguillarum* in European seabass (*Dicentrarchus labrax*). For this purpose, twenty fish (initial mean weight of 4.80 ± 0.06 g) were placed into twelve tanks (400 L) in triplicate and fish were fed with control (C) and three different levels (0.01, 0.05, and 0.1 g/kg) of *A. graveolens* (AG) extract-containing diets (AG0.01, AG0.05, and AG0.1) for 30 days. Blood and tissue (kidney, spleen, and intestine) samples were taken from the fish every 10 days during the study to determine the immune responses of the fish. Respiratory burst activity (RBA) was significantly decreased in the AG0.1 group compared to all other groups on the 10th day of the study ($P < 0.05$). Significance was noticed in the RBA of fish in all AG groups compared to the C group ($P < 0.05$) on the 30th day of the experiment Lysozyme activity (LYS) was raised on the 10th day of the study in all celery groups compared to the C group ($P < 0.05$). No differences in the myeloperoxidase activity (MPO) were observed among the experimental groups ($P > 0.05$). The final mean weight (FMW) was not affected in any experimental groups ($P > 0.05$). However, in the AG0.05 group, the specific growth rate (SGR) increased, and the feed conversion ratio (FCR) decreased compared to other groups ($P < 0.05$). *IL-1β* in the kidney was highly elevated in the AG0.01 group on the 20th day of the study ($P < 0.05$). Similar results were observed on *IL-6*, *IL-8*, and *TNF-α* expression in the kidney ($P < 0.05$). Anti-inflammatory responses (*IL-10* and *TGF-β*) also increased in all experimental groups and tissues compared to the C group ($P < 0.05$). *COX-2* was upregulated on the 20th day of the study in all tissues ($P < 0.05$). At the end of the feeding trial, the survival rate of the AG0.1 group in fish infected with *Vibrio anguillarum* infection was higher than the C group. Dietary celery extract did not affect growth performance directly but increased innate immune responses and a high survival rate. Overall, compared to the control group, the growth, immunity, and resistance of European seabass fed with a diet containing

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0.05 g/kg celery aqueous methanolic extract has been improved, and this could be used as an immunostimulant feed additive.

Keywords Plant extract · Fish diseases · Vibriosis · Natural immunostimulant · Gene expression

Introduction

Aquaculture is a crucial primary source of increasing the supply of fish (Reverter et al. 2014). As the global population increases, the aquaculture industry has become progressively essential to meet the demand for animal protein for human consumption. It is estimated that by 2030, this demand could reach 62% of the total world production (Ahmad et al. 2021). The first marine non-salmonid species commercially cultivated in Europe is the European seabass (*Dicentrarchus labrax*) (FAO 2022). This species is now one of the most significant species extensively farmed in the Mediterranean Sea, practiced by countries like Spain, Greece, France, Croatia, Egypt, and Turkey (Lees and Thomas 2008; Vandeputte et al. 2019; FAO 2022).

Limited natural marine stocks and growing fish demand have allowed for an increase in fish farming production. Increasing stock density in aquaculture or using low-quality feeds has increased the vulnerability of fish to stress and disease development. Vibriosis, photobacteriosis, and mixobacter infections are the most threatening bacterial diseases to marine fish, especially European seabass (Toranzo et al. 2005), while tenacibaculosis and photobacteriosis are attentional for this species in the Mediterranean region (Muniesa et al. 2020). Vibriosis, a fish disease caused by the Gram-negative bacteria *Listonella* (*Vibrio*) *anguillarum*, is the main reason for typical hemorrhagic septicemia in warm and cold-water fish species and fish affected by vibriosis show hemorrhages in fins, exophthalmia, and corneal opacity (Toranzo et al. 2005).

Antimicrobial agents or antibiotics are predominantly used in aquaculture to treat and prevent these disease outbreaks (Rodgers and Furones 2009; Cabello et al. 2013; Capkin et al. 2015). These agents have some adverse effects on animals, the aquatic environment, and human health and high costs (Uney et al. 2021). Antimicrobial residue accumulation in tissues can occur because of heedlessness of the purpose or route of administration before they can thoroughly be metabolized or secreted from the body (Woodward 1991; Tollefson and Miller 2000; Okocha et al. 2018). Various human health problems may develop from consuming these products (Lee et al. 2001; Cañada-Cañada et al. 2009). Also, antimicrobial resistance in bacterial pathogens after using antibiotics is a serious problem with excessive lethality and mobility. Treating multidrug-resistant patterns in bacteria is challenging and even incurable with conventional antibiotics. It requires the invention of novel treatment alternatives and substitutes for antimicrobial therapies to minimize and control bacterial diseases in fish (Frieri et al. 2017).

Due to the increasing occurrence of resistant pathogens from utilizing antimicrobials which are chemicals in origin, researchers have focused their attention on exploring and using plant-based compounds as an alternative to chemical treatments in aquaculture (Takaoka et al. 2011; Kumar and Bossier 2018; Afzali and Wong 2019; Karga et al. 2020). Plant extracts used in aquaculture support growth and reproduction, increase their appetite, and strengthen the immune system of aquatic organisms (Sönmez et al. 2019; Bilen et al. 2021; Terzi et al. 2021). Also, plant extracts could lessen treatment costs and be more eco-friendly since they tend to be more decomposable than artificial compounds (Reverter et al. 2014; Bilen et al. 2021). Several plant-based immune-enhancing feed additives, such as seaweeds, date palms, cinnamon, etc., are used in European seabass (*D. labrax*) diets to strengthen the immune system and provide resistance against diseases. It has been reported that these improve the health status of fish (Guardiola et al. 2016; Peixoto et al. 2016, 2019; Lobo et al. 2018; Habiba et al. 2021).

Celery (*Apium graveolens*), a popular marshland plant grown globally, is one potential source of plant-based antimicrobials. Seed essential oil extracted from *A. graveolens* showed antimicrobial activities against bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, and *Salmonella* spp., as well as against mold and yeast (Hassanen et al. 2015). the celery-supplemented diet given to common carp (*Cyprinus carpio*), exhibited growth-promoting and immunostimulatory effects and enhancing digestive enzyme activity (Mohamed et al. 2018). Also, the hepatoprotective and antidiabetic effects of celery were found on

striped catfish (*Pangasius sutchi*), black sharkminnow (*Labeo chrysophekadion*), and zebrafish (*Danio rerio*) (Shivashri et al. 2013; Sutthi et al. 2020; Pérez Gutiérrez et al. 2021). Therefore, the current study aimed to determine the effects of celery extracts (*A. graveolens*) on growth performance, immune response, and disease resistance against vibriosis caused by *V. anguillarum* in European seabass (*D. labrax*).

Material and method

Ethics statement

The experimental protocol was approved by the local ethics committee of Kastamonu University, Kastamonu, Turkey, under protocol number KUHADYEK 2019/10.

Plant extraction

The rapaceum variety of celery (*Apium graveolens* var. rapaceum) was used in the experiment. Celery was purchased freshly from a local farmer (Kastamonu, Turkey), and the plant leaves were used in the experiment. The leaves have been washed with distilled water, dried, powdered, and stored in airtight bottles. After pulverization, 50 g were weighed, mixed with 40% methanol in brown bottles, and kept for 3 days. After 72 h, the mixture was filtered, and only the liquid part was evaporated by the extraction method in the evaporator until it reached the consistency of honey. The methanol-free extract was lyophilized and kept in a dark condition until used.

Experimental diets

Four isonitrogenous (50% protein) and isolipidic (18% lipid) diets were formulated, and celery aqueous methanolic extract was added to the feed at the rates of 0, 0.01, 0.05, and 0.1 g/kg instead of corn gluten meal (Table 1). The level of dietary celery extract used in this study was determined by considering the previous research on common carp (Mohamed et al. 2018). The experimental diets were prepared while the first large particle, then small particulate raw material laboratory-type mixer (Dirmak iBT-22, Turkey) with stirring, was pulped after addition to water and oil. This paste mixture was passed in a cold extruder (PTM P6 Extruder, La Monferrina, Italy) at a size suitable for the mouth opening of the fish and dried in a drying cabinet at 40 ± 1 °C for approximately 24 h. Dried feeds were stored in a deep freezer (− 25 ± 1 °C) until the start of the experiment.

Table 1 Formulation and proximate composition of the experimental diets (as fed)

	Control	AG0.01	AG0.05	AG0.1
Fish meal[1]	31.30	31.30	31.30	31.30
Celery extract	0	0.01	0.05	0.10
Dehulled soybean meal[2]	9.70	9.70	9.70	9.70
Pea protein concentrate[3]	9.30	9.30	9.30	9.30
Soy protein concentrate[3]	4.00	4.00	4.00	4.00
Wheat gluten[4]	1.00	1.00	1.00	1.00
Corn gluten[4]	8.00	8.00	8.00	8.00
Blood meal, poultry[5]	2.00	2.00	2.00	2.00
Poultry by-products[6]	6.50	6.50	6.50	6.50
Feather meal hydrolyzed[7]	1.00	1.00	1.00	1.00
Wheat milling quality[8]	7.00	6.99	6.95	6.90
Wheat starch[8]	5.00	5.00	5.00	5.00
Fish oil[9]	14.10	14.10	14.10	14.10
Vitamin-mineral premix[10]	0.30	0.30	0.30	0.30
Dicalcium phosphate[10]	0.80	0.80	0.80	0.80
Chemical composition (%)				
Crude protein	49.59	49.62	49.50	49.68
Crude lipid	18.64	18.71	18.68	18.60
Crude ash	7.98	7.88	7.95	7.90

[1]Anchovy fish meal. Sürsan Feed Mill Company, Samsun, Turkey

[2]Kırcı Soya Company, Balıkesir, Turkey

[3]Sürsan Feed Mill Company, Sinop, Turkey

[4]Cargill, İstanbul, Turkey

[5]Skretting Feed Mill Company, Muğla, Turkey

[6]Cargill, İstanbul, Turkey

[7]Sibal Feed Mill Company, Sinop, Turkey

[8]İpek Wheat Company, Nevşehir, Turkey

[9]Anchovy fish oil. Sibal Feed Mill Company, Sinop, Turkey

[10]DSM Nutritional Products, Turkey

Trial design

European seabass (*Dicentrarchus labrax*) was obtained from the hatchery facility of Kılıç Seafood in Muğla, Turkey, and transferred to the Aquaculture Application Units. Seawater drawn from the Marmara Sea by

a pump with a power of 3.5 kW was first stored in the settling tanks, then through the sand filter and ultraviolet filter, and then stored in the main water tank (the temperature at 22.8 ± 0.75 °C, dissolved oxygen at 7.0 ± 0.5 mg/L and pH at 7.9 ± 0.3). The fish were fed a commercial European seabass diet (48% protein and 18% lipid, Sürsan Feed Company, Samsun Türkiye) during the acclimation period. After acclimatization for 2 weeks, 240 fish with 4.80 ± 0.06 g of initial mean weight were divided into 12 tanks with 400 L water capacity, each containing twenty fish in triplicate. The experimental photoperiod was conducted by applying 12 h of daytime and 12 h of the nighttime light regime. Fish fed ad libitum with the control and three different levels of celery methanolic extract included diets (C, AG0.01, AG0.05, and AG0.1) three times a day for 30 days.

Evaluation of growth performance

Fish were weighed every 10 days, and no feed was given the day before weighing. The daily feed intake of fish was recorded during the trial. All fish were individually weighed at the beginning and end of the trial. The growth performance of fish was calculated according to the following formulas:

$$\text{Specific Growth Rate (SGR, \%/day)} = \frac{[\ln(\text{Final body weight, g}) - \ln(\text{Initial body weight, g})]}{\text{Trial days}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

Isolation of organ and collection of blood samples

Fish (3 per treatment) were anesthetized in 10 L of plastic buckets containing clove oil (40 µL/L) to determine immune responses every 10th day of the study. Blood samples were taken from the caudal vein with 1 mL injectors containing heparin. From each fish, head kidney, spleen, and intestine tissues were collected and placed into an Eppendorf tube containing RNA Later (Invitrogen) solution for determining gene expression. For serum analysis, blood samples in the tubes were centrifuged at 5300 rpm for 5 min, and the obtained serum was stored at − 80 °C until analyzed.

Analysis

Biochemical analysis

Proximate analysis (moisture, crude lipid, crude protein, and crude ash) was conducted on all ingredients and test diets and determined by standard Association of Official Analytical Chemists (AOAC 2000) methodology.

Immunological analysis

Respiratory burst activity (RBA) was determined using the NBT reduction method, according to Siwickiet al. (1994). Lysozyme activity (LYS) was determined according to Ellis (1990). The total myeloperoxidase (MPO) content in plasma has been determined, according to Quade and Roth (1997).

Analyses of cytokine gene expression

RNA was collected from each fish's head kidney, spleen, and intestine tissue samples (30 mg) by using the BIOLINE kit (ISOLATE II RNA Mini Kit) as previously described (Bilen and Elbeshti 2019). Complementary DNA was synthesized using 1 µg of total RNA using an Applied Biosystems kit (ThermoFisher™ high-capacity cDNA reverse transcription kit) after being treated with DNAase to remove genomic DNA (Bilen et al. 2021). The expression of cytokine genes such as *IL-1β*, *IL-6*, *IL-8*, *TNF-α*, *IL-10*, *TGF-β*, *COX-2*, and *Hepcidin* was determined with a real-time PCR detection system (Bio-Rad, USA) using SensiFAST SYBR No-ROX Kit PCR kit (BIOLINE). Gene expression levels were determined by the $2^{-\Delta\Delta C}{}_{T}$ method (Livak and Schmittgen 2001). Specific primer sequences are listed in Table 2.

Challenge test

At the end of the feeding trial, 11 fish, the remaining fish after sampling, were challenged with *Vibrio anguillarum* (LD_{50} dose was 1×10^7 in 100 µL PBS). The bacteria used in the experiment were injected

Table 2 Gene-specific primers with their sequences and references used for qRT-PCR in the study

Gene	Primer sequence (5′ − 3′)	References
β-actin	F: 5′ ATGTGGATCAGCAAGCAGG 3′ R: 5′ AGAAATGTGTGGTGTGGTCG 3′	Román et al. (2013)
IL-1β	F: 5′ ATTACCCACCACCCACTGAC 3′ R: 5′ TCTCTTCCACTATGCTCTCCAG 3′	Román et al. (2013)
IL-6	F: 5′ ACTTCCAAAACATGCCCTGA 3′ R: 5′ CCGCTGGTCAGTCTAAGGAG 3′	Sepulcre et al. (2007)
IL-8	F: 5′ GTCTGAGAAGCCTGGGAGTG 3′ R: 5′ GCAATGGGAGTTAGCAGGAA 3′	Sepulcre et al. (2007)
IL-10	F: 5′ ACCCCGTTCGCTTGCCA 3′ R: 5′ CATCTGGTGACATCACTC 3′	Picchietti et al. (2009)
TNF-α	F: 5′ AGCCACAGGATCTGGAGCTA 3′ R: 5′ GTCCGCTTCTGTAGCTGTCC 3′	Sepulcre et al. (2007)
TGF-β	F: 5′ GACCTGGGATGGAAGTGG 3′ R: 5′ CAGCTGCTCCACCTTGTG 3′	Picchietti et al. (2009)
COX-2	F: 5′ CATTCTTTGCCCAGCACTTCACC 3′ R: 5′ AGCTTGCCATCCTTGAAGAGTC 3′	Picchietti et al. (2009)
Hepcidin	F: 5′ GGAATCGTGGAAGATGCCGT 3′ R: 5′ CAGACACCACATCCGCTCAT 3′	Reyes-López et al. (2018)

intraperitoneally. The tanks were monitored daily, and dead fish were removed and counted daily. Mortality was recorded during eight days as per the treatment groups.

Statistical evaluation

The data obtained on growth performance, immune parameters, and cytokine responses at the end of the trial were first subjected to analysis of variance (ANOVA) and then to Duncan's post hoc test with the help of Statgraphics Centurion XVI (Manugistics Incorporated, Rockville MD, USA) statistical program (Zar 1999). All tests' significance level was $P < 0.05$ (95% confidence interval). Differences in challenge test results were determined according to the Kaplan–Meier survival analysis test. After analyzing the survival results with Kaplan–Meier, when significant differences were found ($P < 0.05$), a comparison among means was made with a log-rank test.

Results

The growth performance of the European seabass fed with celery extract is presented in Table 3. No significant difference was recorded in the FMW of the experimental groups ($P > 0.05$). The SGR was significantly increased in the AG0.05 group, while the FCR was decreased ($P < 0.05$).

On the 10th day, the RBA was ascertained as the lowest in the AG0.1 group ($P < 0.05$). On the 20th day, no differences were recorded in any groups ($P > 0.05$). At the end of the study, the RBA levels in all groups were significantly increased compared to the C ($P < 0.05$) (Fig. 1).

Table 3 Growth performance of the European seabass fed the celery aqueous methanolic extract-containing (AG) diets for 30 days

	C	AG0.01	AG0.05	AG0.1
Initial mean weight (g)	4.80 ± 0.12	4.78 ± 0.06	4.66 ± 0.04	4.83 ± 0.04
Final mean weight (g)	9.44 ± 0.12	9.45 ± 0.06	9.45 ± 0.10	9.51 ± 0.06
Specific growth rate (%/day)	2.26 ± 0.04^{a}	2.27 ± 0.02^{a}	2.35 ± 0.03^{b}	2.26 ± 0.01^{a}
Feed conversion ratio	1.72 ± 0.01^{b}	1.71 ± 0.01^{b}	1.67 ± 0.03^{a}	1.71 ± 0.01^{b}

Different letters in the same line indicate statistically significant differences ($P < 0.05$) among the groups

The LYS showed an increase in all AG groups compared to the C on the 10th day (Fig. 2), and the highest LYS was determined in the AG0.1 group. No differences were observed among the LYS of the experimental groups on the 20th and 30th days of the study ($P > 0.05$).

The MPO was determined similarly among celery groups and the C group in every sampling time of the study ($P > 0.05$) (Fig. 3).

In the kidney, the *IL-1β* gene was very highly upregulated on the 20th day of the study in the AG0.01 (> 664) compared to all other groups ($P < 0.05$) (Fig. 4). Also, *IL-1β* gene expression of all experimental groups was significantly increased compared to that of the C in the 30th day of the study ($P < 0.05$). On the 10th day of the trial in the spleen, upregulated *IL-1β* was ascertained in all celery groups ($P < 0.05$). Compared to other groups, this upregulation was determined on the 20th day of the study in the AG0.01 and AG0.05 groups ($P < 0.05$). No statistical difference in the *IL-1β* in the spleen was observed on the 30th day of the study ($P > 0.05$). *IL-1β* gene in the intestine of fish fed with celery diets showed no differences from C at any sampling time ($P > 0.05$).

In the kidney, *IL-6* expression of the AG.01 group was significantly higher (= 189) on the 20th day of the study ($P < 0.05$) (Fig. 5). On the 30th day of the study, all groups' *IL-6* expression was

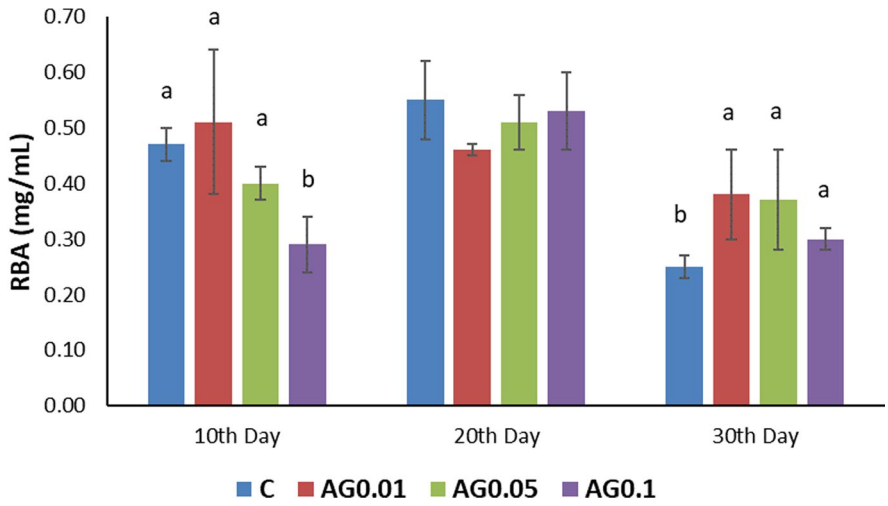


Fig. 1 Respiratory burst activity (RBA) of European seabass fed with experimental diets containing different doses of celery aqueous methanolic extract as 0 (C), 0.01 (AG0.01), 0.05 (AG0.0.5), and 0.1 (AG0.1) g/kg. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)

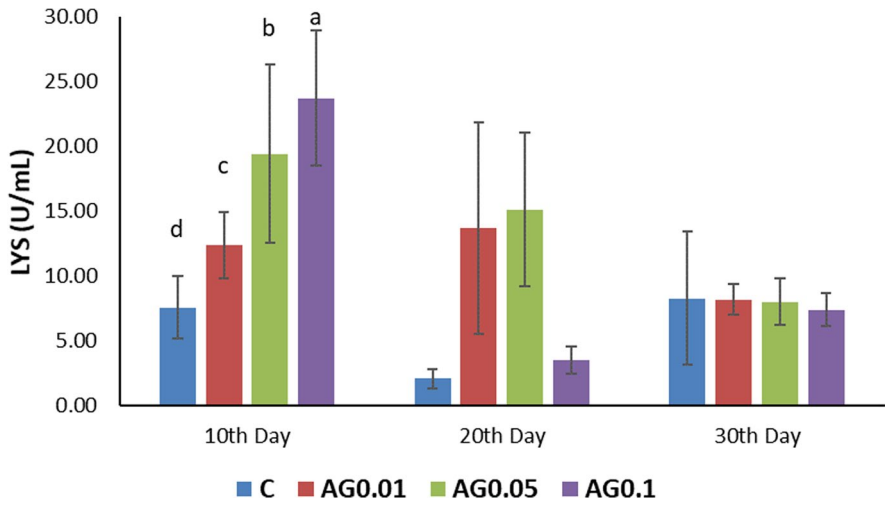


Fig. 2 Lysozyme activity (LYS) of European seabass fed with experimental diets containing different doses of celery aqueous methanolic extract as 0 (C), 0.01 (AG0.01), 0.05 (AG0.0.5), and 0.1 (AG0.1) g/kg. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)

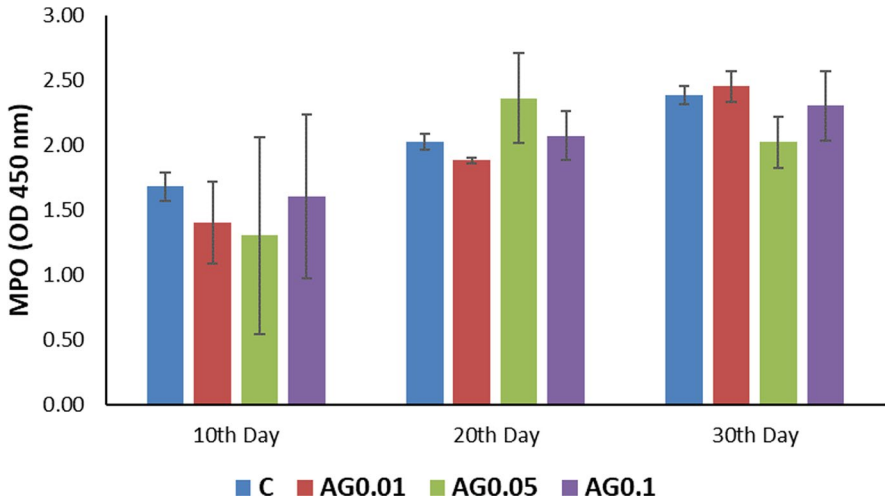


Fig. 3 Myeloperoxidase activity (MPO) of European seabass fed with experimental diets containing different doses of celery aqueous methanolic extract as 0 (C), 0.01 (AG0.01), 0.05 (AG0.0.5), and 0.1 (AG0.1) g/kg. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)

upregulated compared to the C ($P < 0.05$). In the spleen, elevated *IL-6* gene was only determined on the 20th day of the study in AG0.01 groups compared to other groups ($P < 0.05$). No significant differences were observed at any other sampling time ($P > 0.05$). However, *IL-6* gene expression was not affected in the intestine at any sampling time ($P > 0.05$).

Similar to *IL-6* results, in the kidney on the 20th day of the study, significantly increased *IL-8* gene expression (> 255 times) was observed in the AG0.01 group ($P < 0.05$) (Fig. 6). The elevated activity was also determined in all the AG groups compared to the C at the end of the study ($P < 0.05$). The *IL-8* in the spleen was increased in the AG0.01 group on the 20th day and the AG0.1 group on the 30th day ($P < 0.05$). *IL-8* gene was significantly upregulated in the fish intestine of the AG0.05 groups on the 20th day of the study ($P < 0.05$).

On the 20th day of the trial, *IL-10* in the kidney was determined as highly upregulated (> 197 times) in the AG0.01 group ($P < 0.05$) (Fig. 7). On the 30th day of the study, all celery groups' *IL-10* gene expression was significantly upregulated compared to the C group ($P < 0.05$). In the spleen, increased gene expression was observed only on the 20th day of the study in the AG0.01 group ($P < 0.05$). An increased *IL-10* gene expression was ascertained in the intestine of fish fed the AG0.05 diet on the 10th day of the study ($P < 0.05$). This increase was also established on the 20th day of the trial in the AG0.05 and AG0.1 groups compared to the C group ($P < 0.05$).

TNF-α gene expression level was elevated in the kidney of fish in all celery groups on the 20th and 30th days compared to the C ($P < 0.05$) (Fig. 8). This increase in *TNF-α* was determined only in the AG0.01 group fish on the 20th day of the study in the spleen ($P < 0.05$). *TNF-α* gene in the intestine of fish fed the AG0.05, and AG0.1 diets were upregulated compared to the C on the 20th day of the study ($P < 0.05$).

TGF-β gene in the kidney and the spleen of the AG0.1 group was significantly higher than other groups on the 10th and 30th days of the sampling. The AG0.01 was the highest on the 20th day ($P < 0.05$) (Fig. 9). TGF-β gene in the kidney of the AG0.1 group was the highest on the 30th day ($P < 0.05$), while no differences were recorded on the 10th and 20th sampling days ($P > 0.05$).

On the 20th day, increased expression was observed in the kidney in the AG0.01 and AG0.1 groups compared to the C ($P < 0.05$) (Fig. 10). Also, increased *COX-2* was determined in the spleen of fish fed all celery diets compared to the C on the 20th day of the study ($P < 0.05$). *COX-2* was upregulated in the intestine of the AG0.05 and AG0.1 groups on the 20th day of the study ($P < 0.05$).

In the kidney, the first sampling time, elevated activity was observed in the AG0.05 and AG0.1 groups ($P < 0.05$) (Fig. 11). This increase was determined on the 20th day of the study only in the AG0.01 group ($P < 0.05$). At the end of the trial, *Hepcidin* gene expression was raised in the AG0.01 and AG0.1 groups compared to the C group ($P < 0.05$).

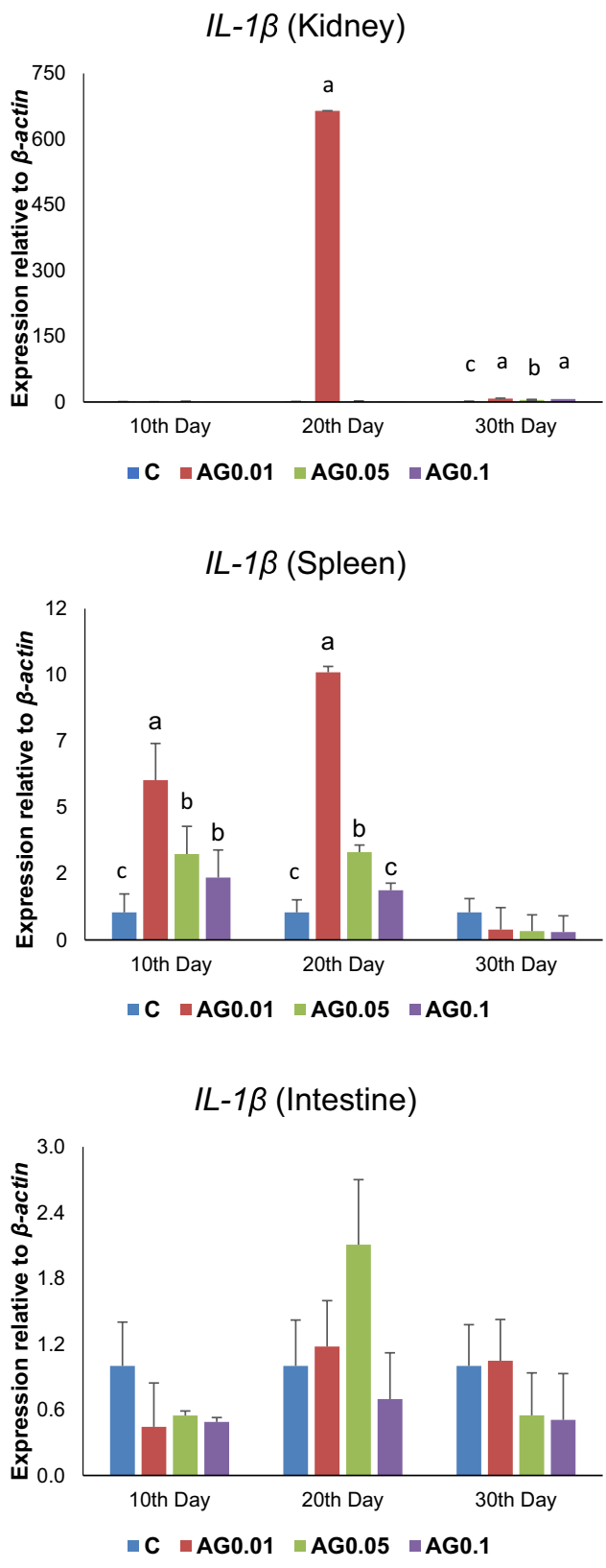


Fig. 4 Relative gene expression of *IL-1β* in head kidney, spleen, and intestine tissues of European seabass fed diet containing different levels of celery aqueous methanolic extract. C, AG0.01, AG0.05, and AG0.1 denote doses of celery extract at 0, 0.01, 0.05, and 0.1 g/kg diet, respectively. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)

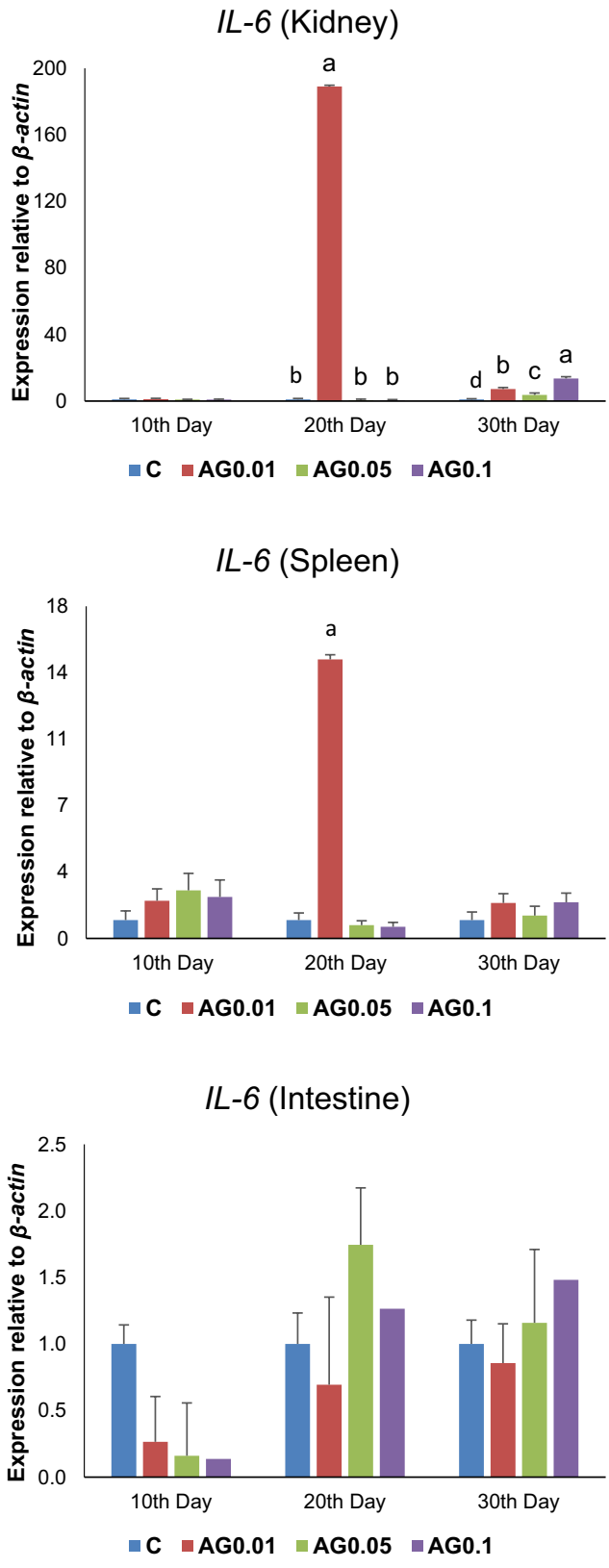
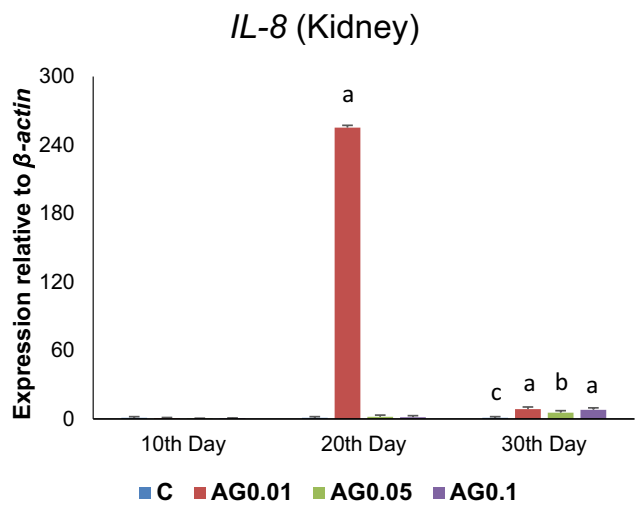
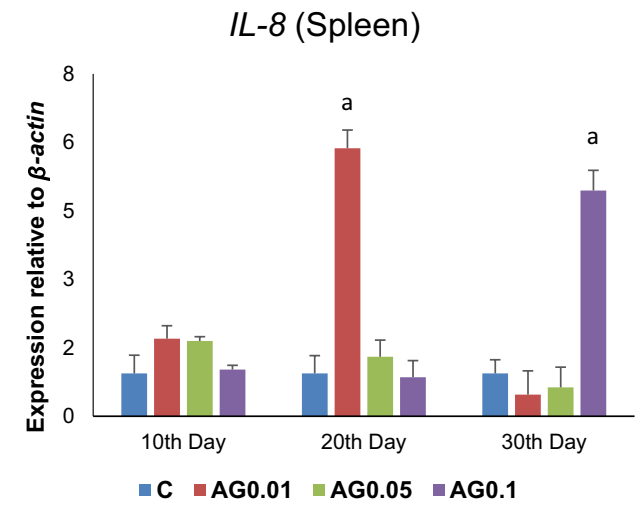


Fig. 5 Relative gene expression of *IL-6* in head kidney, spleen, and intestine tissues of European seabass fed diet containing different levels of celery aqueous methanolic extract. C, AG0.01, AG0.05, and AG0.1 denote doses of celery extract at 0, 0.01, 0.05, and 0.1 g/kg diet, respectively. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)





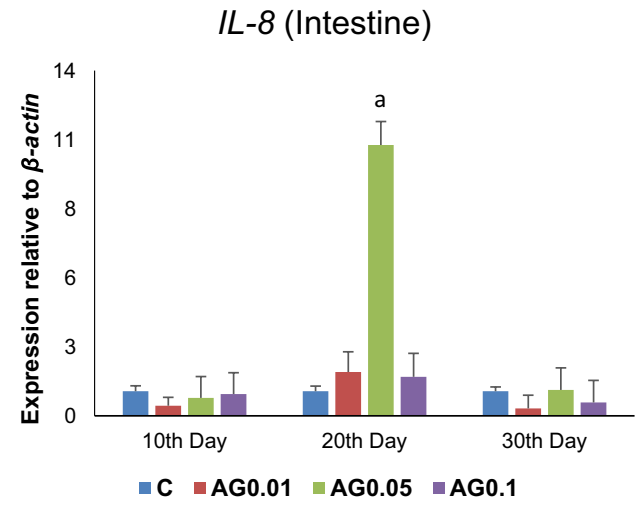


Fig. 6 Relative gene expression of *IL-8* in head kidney, spleen, and intestine tissues of European seabass fed diet containing different levels of celery aqueous methanolic extract. C, AG0.01, AG0.05, and AG0.1 denote doses of celery extract at 0, 0.01, 0.05, and 0.1 g/kg diet, respectively. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)

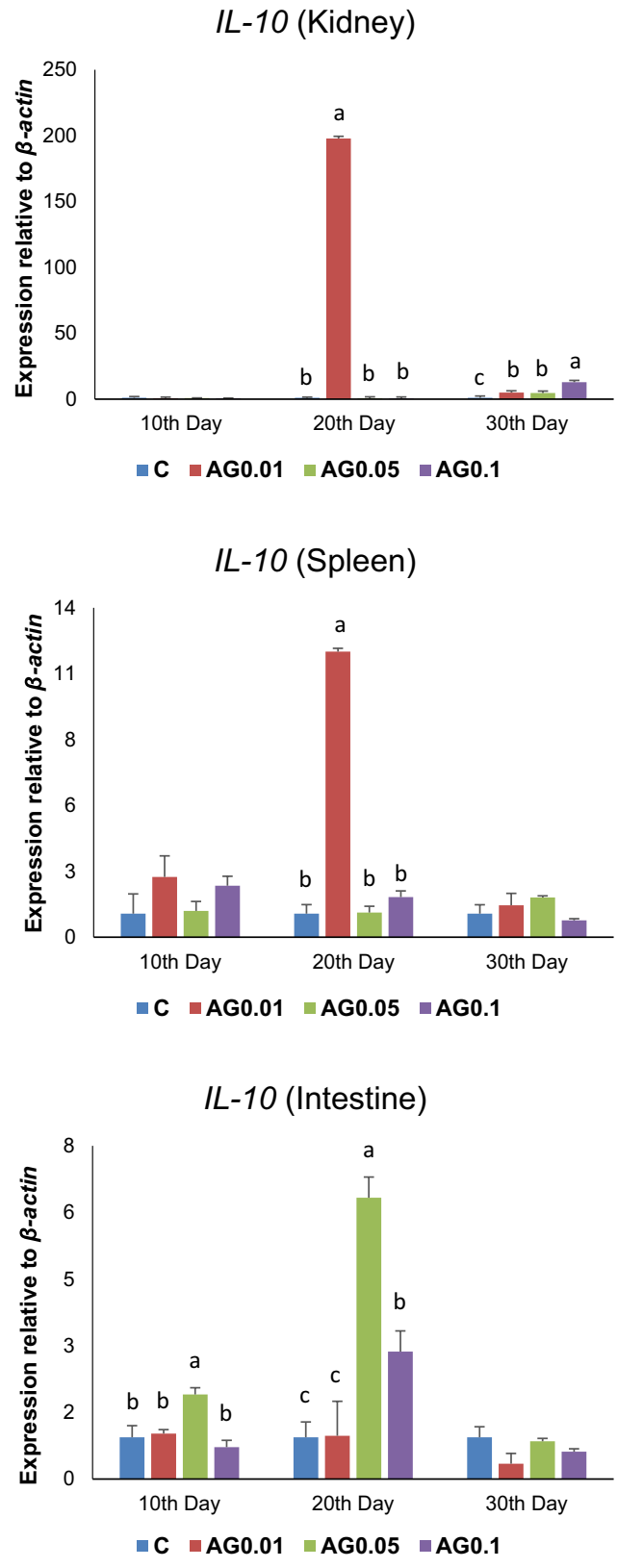


Fig. 7 Relative gene expression of *IL-10* in head kidney, spleen, and intestine tissues of European seabass fed diet containing different levels of celery aqueous methanolic extract. C, AG0.01, AG0.05, and AG0.1 denote doses of celery extract at 0, 0.01, 0.05, and 0.1 g/kg diet, respectively. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)

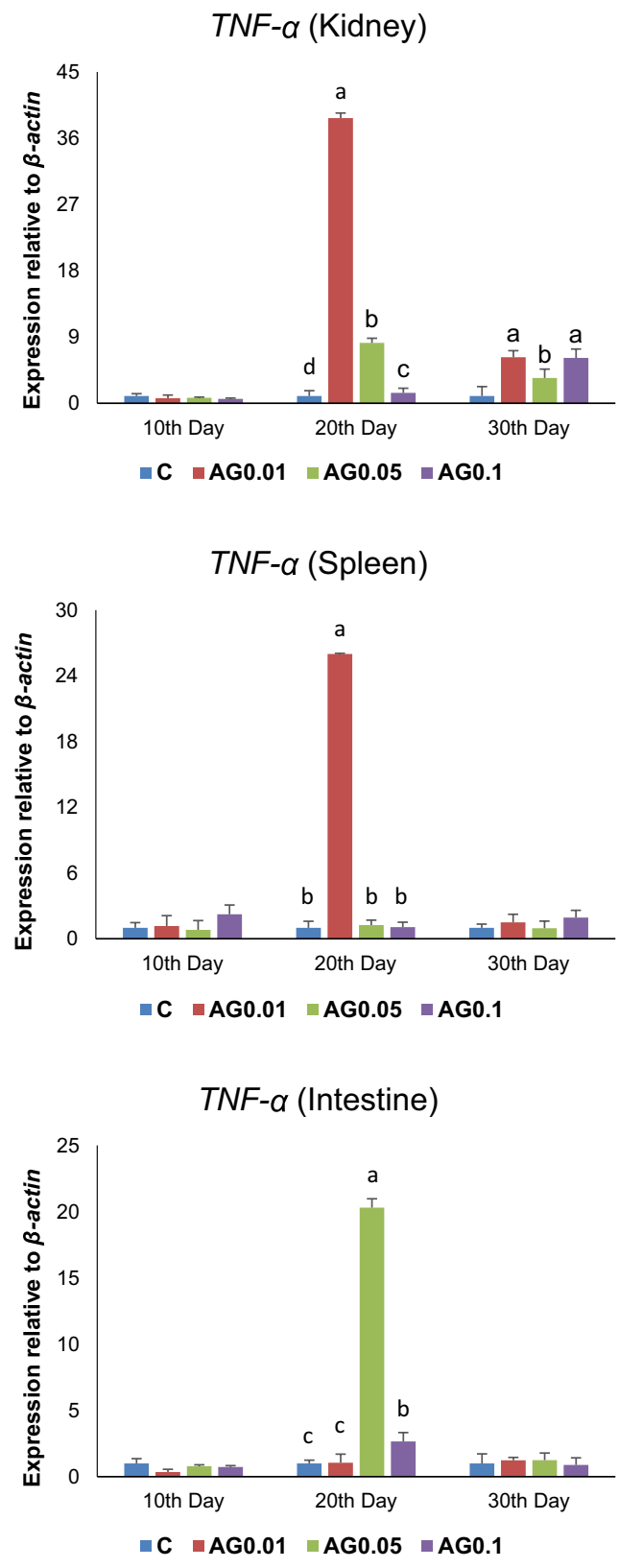


Fig. 8 Relative gene expression of *TNF-α* in head kidney, spleen, and intestine tissues of European seabass fed diet containing different levels of celery aqueous methanolic extract. C, AG0.01, AG0.05, and AG0.1 denote doses of celery extract at 0, 0.01, 0.05, and 0.1 g/kg diet, respectively. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)

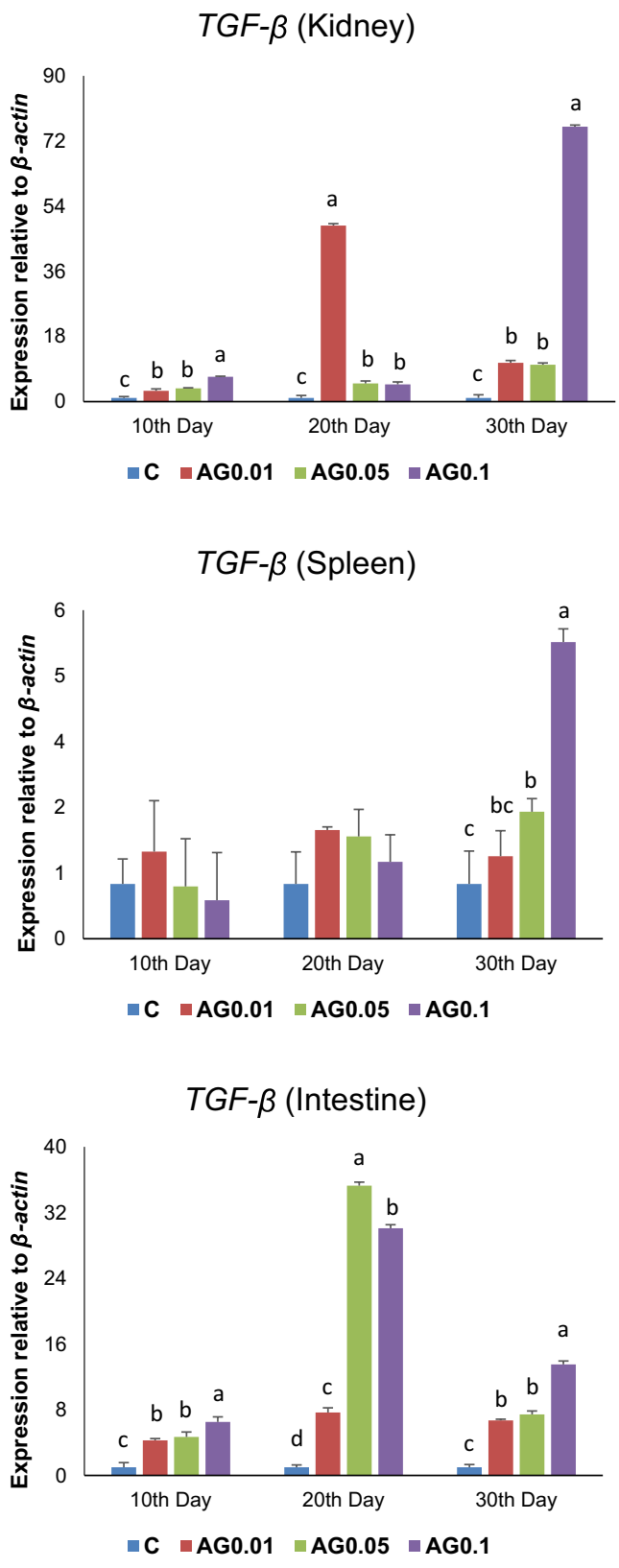


Fig. 9 Relative gene expression of *TGF-β* in head kidney, spleen, and intestine tissues of European seabass fed diet containing different levels of celery aqueous methanolic extract. C, AG0.01, AG0.05, and AG0.1 denote doses of celery extract at 0, 0.01, 0.05, and 0.1 g/kg diet, respectively. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)

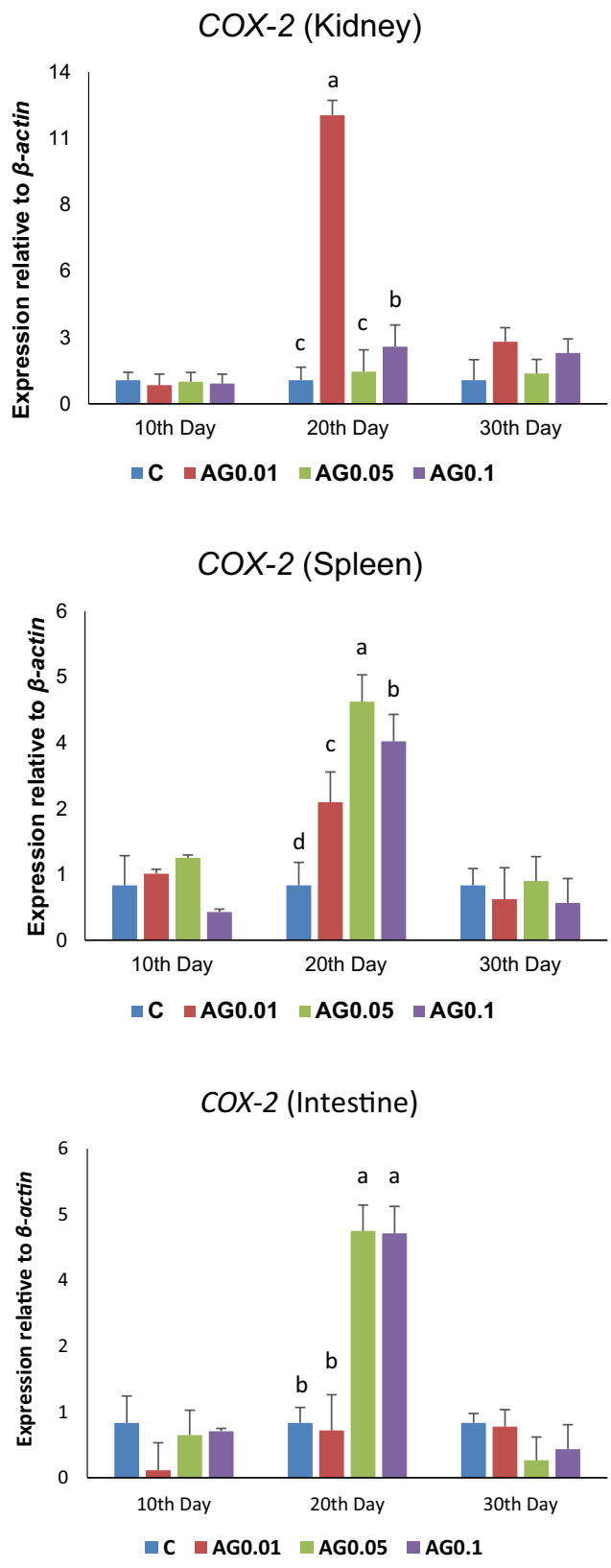


Fig. 10 Relative gene expression of *COX-2* in head kidney, spleen and intestine tissues of European seabass fed diet containing different levels of celery aqueous methanolic extract. C, AG0.01, AG0.05, and AG0.1 denote doses of celery extract at 0, 0.01, 0.05, and 0.1 g/kg diet, respectively. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)

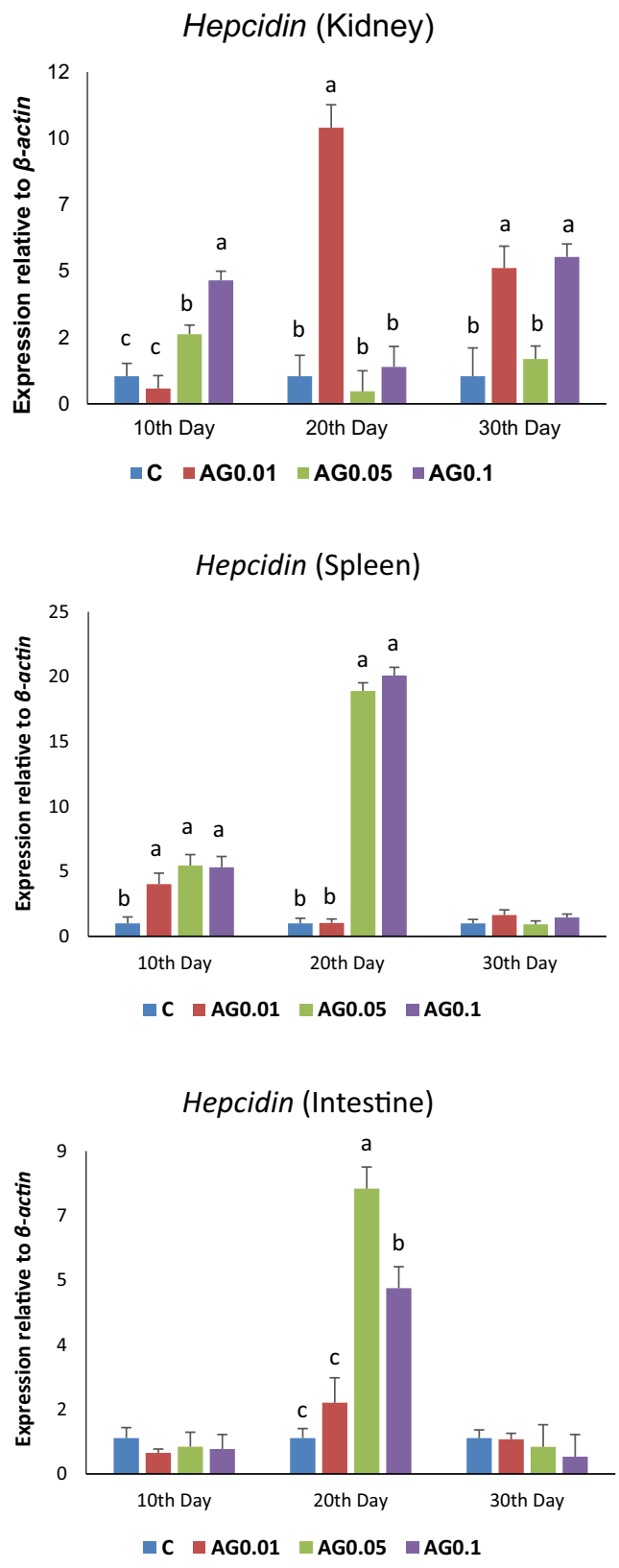


Fig. 11 Relative gene expression of *Hepcidin* in head kidney, spleen, and intestine tissues of European seabass fed diet containing different levels of celery aqueous methanolic extract. C, AG0.01, AG0.05, and AG0.1 denote doses of celery extract at 0, 0.01, 0.05, and 0.1 g/kg diet, respectively. Different letters above the error bars indicate significant differences ($P < 0.05$) among the groups. In the absence of letters above the error bars, there is no difference between groups ($P > 0.05$)

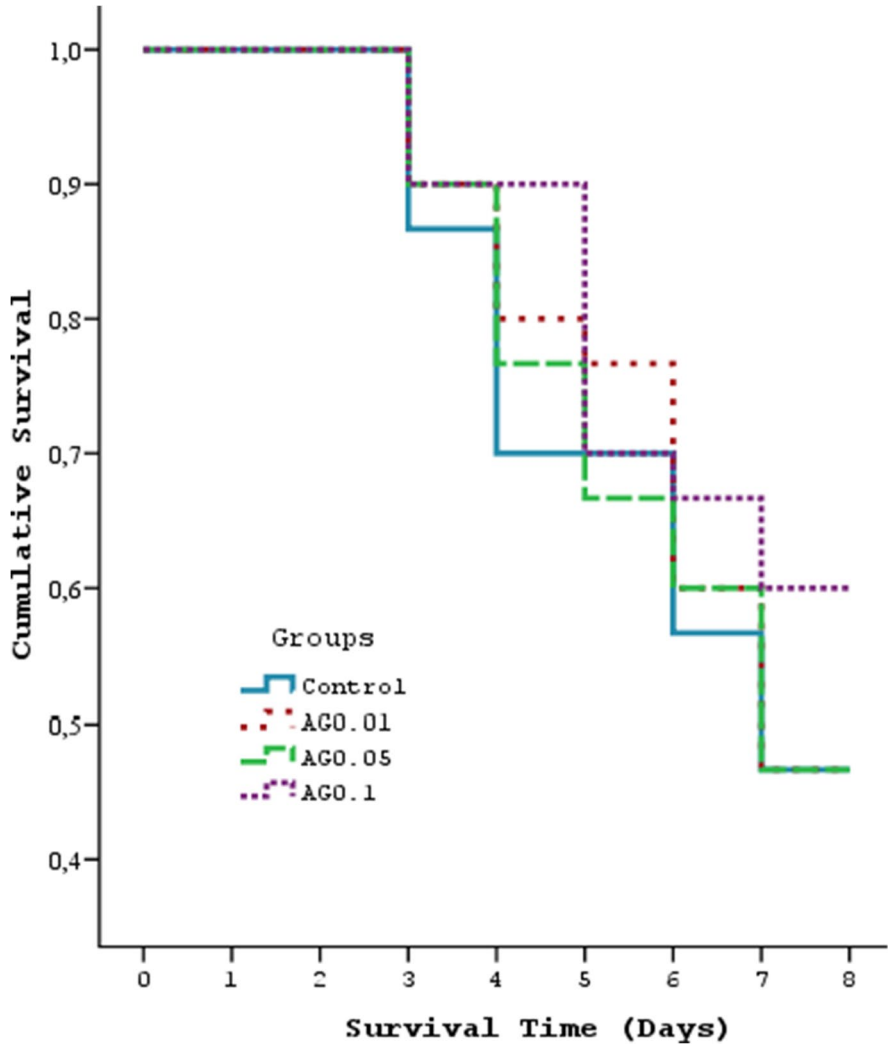


Fig. 12 Cumulative survival of the groups challenged with *Vibrio anguillarum* after administration of methanolic extract of celery. C, AG0.01, AG0.05, and AG0.1 denote doses of celery extract at 0, 0.01, 0.05, and 0.1 g/kg diet, respectively

In the spleen, elevated *Hepcidin* was observed in all experimental groups compared to the C at the first sampling time ($P < 0.05$). This increase was determined on the 20th day of the study in the AG0.05 and AG0.1 groups compared to other groups ($P < 0.05$). No differences in the spleen were observed on the 30th day of the trial among all the experimental groups ($P > 0.05$). *Hepcidin* in the intestine of the AG0.05 and AG0.1 diets were significantly increased compared to the C on the 20th day ($P < 0.05$). No differences in the intestine were observed at any other sampling times ($P > 0.05$).

In the challenge experiment with *Vibrio anguillarum*, the survival rate in the AG0.1 group was significantly higher than that of the control (Fig. 12). No differences in the survival rate of fish were observed among other celery extract-treated groups compared with the C group ($P > 0.05$).

Discussion

In this study, 0.05% of dietary celery extract added to the feed improved the growth performance of fish in terms of the SGR and the FCR. Previously celery extract was studied in carp (*Cyprinus carpio*) and black sharkminnow (*Labeo chrysophekadion*) and the results showed all growth parameters were affected positively (Mohamed et al. 2018; Sutthi et al. 2020). However, our study showed only a promoted activity on the SGR and FCR. Carps are herbivore fish, and differently,

European seabass is carnivorous. Peixoto et al. (2016) declared dietary *Ulva* sp. supplementation enhances immune responses in this species without compromising growth performance. Similar results were observed in rainbow trout (*Oncorhynchus mykiss*) after being fed with dandelion and lichen (Salem et al. 2021).

The RBA is an important indicator to determine the phagocytic cells' bacterial killing activity. The RBA in fish fed high doses of *A. graveolens* (AG0.01) showed a decrease on the 10th day of the study. However, at the end of the study, increased RBA was determined in all celery groups. Like our study, Mohamed et al. (2018) also found increased RBA in carp (*C. carpio*) after celery treatment and rainbow trout (*O. mykiss*) after cherry stem extract treatment (Amoush et al. 2022). Lysozymes is a peptide (Magnadóttir 2006) and acts as an opsonin by lysing bacterial walls (Alexander and Ingram 1992). The LYS was significantly increased at the first sampling time in all celery groups compared to the control group. However, no differences in the LYS activity were found at the end of the study. Our results agree with the findings from Bilen et al. (2020) that an increase in activity was detected in rainbow trout (*O. mykiss*) after the common mallow aqueous methanolic extract group. Elevated LYS activity was also determined in carp (*C. carpio*) fed supplemented with silver linden (*Tilia tomentosa*) methanolic extract diets (Almabrok et al. 2018). Moreover, the addition of prebiotics, probiotics, plant-based extracts, and seaweeds in the diets of European seabass (*D. labrax*) increased the LYS (Guardiola et al. 2016; Peixoto et al. 2016, 2019; Azeredo et al. 2017) as in the 10th day of the present study. The MPO is an enzyme expressed in neutrophils (Srivastava and Pandey 2015). It is an important immune response against pathogens by producing hydrogen peroxide, which is harmful to pathogens (Castro et al. 2008). In the present study, the MPO was not affected. Contrary to our research, elevated MPO activity in carp (*C. carpio*) after feeding the fish diet containing celery extract (Mohamed et al. 2018). The neutrophil is the primary source of MPO in fish. The study shows neutrophils did not stimulate after dietary celery treatment in the European seabass.

IL-1β gene expression in the intestine was not affected by any groups during the study. However, on the AG0.01 group, on the 20th day of the study, very high elevated expression (664 times) was found in the kidney. In addition, all celery groups' *IL-1β* were upregulated at the end of the study. Spleen, the seconder immune organ, showed an increase in *IL-1β* gene expression of all celery groups on the 10th and 20th days of the study. Macrophages are the primary sources of the *IL-1β* (Jørgensen et al. 2000), and the kidney and the spleen are the hosts of the macrophages. *IL-6* did not affect the intestine. However, on the 20th day of the study in AG0.01 groups, IL-6 gene expression was very high in the kidney and spleen. Also, *IL-6* gene expression was elevated in the kidneys in all celery groups at the end of the study. The spleen and especially the kidney was stimulated after celery treatment. *IL-6* is a pro-inflammatory cytokine and mediates inflammatory immune responses (Fu et al. 2016). However, the adverse effects of *IL-6* are well-known (Mu et al. 2018). In the present study, the non-inflammatory effects of celery were determined in the intestine. Because the spleen and the kidney are rich in immune-related cells, elevated activity in the kidney must be related to this.

Similarly, upregulated immune responses were recorded in European seabass (*D. labrax*) fed with date palm fruits (Guardiola et al. 2016) and seaweeds (*Ulva* sp. and *Gracilaria* sp.) (Peixoto et al. 2016, 2019) and rainbow trout (*O. mykiss*) fed with thin-skinned plum (*Prunus domestica*) (Terzi et al. 2021) and green tea (*Camellia sinensis*) (Nootash et al. 2013). *IL-8* is a pro-inflammatory cytokine as well and can stimulate the phagocyte. Increased *IL-8* gene expression was observed in all tissues on the 20th day and the kidney at the end of the study. *TNF-α* is a pro-inflammatory cytokine and is elaborated in inflammation and hematopoiesis (Secombes et al. 1996). *TNF-α* significantly increased on the 20th day of the study in all experimental tissues, especially kidneys. *COX-2* was also raised in all tissues on the 20th day of the trial. After dietary nettle administration, elevated *COX-2* was observed in rainbow trout (Mehrabi et al. 2020). *Hepcidin* has a duty on iron regulation (Shike et al. 2004) and is an essential antimicrobial peptide (Campoverde et al. 2017). *Hepcidin* in the spleen and kidney showed increased activity on the first two samplings, and the intestine showed only on the 20th day. Only the kidney showed an increase in all sampling times. This increased *Hepcidin* indicated that the regulated immune response of the gene was stimulated by celery extract. *IL-10* was significantly

increased in the first two sampling times in the intestine. The intestine of the fish showed an anti-inflammatory response after feeding with celery-containing diets. There was an increase in TGF-β activation in the intestines of fish fed with celery extract-containing feeds. These pro-inflammatory results suggest a stimulation in the head kidney, which affected the inflammatory responses by celery extract. Downregulation of the pro-inflammatory cytokine in the intestine showed an anti-inflammatory activity of the celery in European seabass.

There were no deaths from feeding or handling fish before the *Vibrio anguillarum* was injected. The survival rate in fish provided the 0.1% dietary celery extract was higher than the other groups after the fish was challenged with *Vibrio anguillarum*. Many studies showed an increased survival rate in the fish after medicinal plant application (Abdel-Tawwab et al. 2020; Sutthi et al. 2020; Bilen et al. 2021; Sönmez et al. 2021; Lakwani et al. 2022). Previous dietary celery research conducted with black sharkminnow (*L. chrysophekadion*) demonstrated decreasing in cumulative mortality after *Aeromonas hydrophila* challenge test (Sutthi et al. 2020). In our study, increased activity was observed in the highest dose of the experiment. All immune parameters generally increased in the AG0.1 group, especially gene expression. Despite a considerable increase in pro-inflammatory cytokine gene expression, the results did not support our survival rates. It is expected to determine also increased survival rate in the groups. However, significantly increased survival was only defined in the highest dose. In contrast to our study, Talpur and Ikhwanuddin (2013) observed an increased survival rate in the Asian seabass (*Lates calcarifer*) after neem leaf (*Azadirachta indica*) extract application against *Vibrio harvei*.

Consequently, the growth parameters and gene expression responses indicate that the inclusion of 0.05 g/kg dietary artichoke extract benefits European seabass. However, bacterial resistance also strengthened with the addition of 0.1 g/kg artichoke extract to the European seabass diets. In conclusion, it is suggested that it is possible to use diets containing 0.05% or more celery extract for European seabass. These levels increased the health parameters of the species, and it was concluded that it could prevent chronic fish inflammation as an anti-inflammatory.

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Author contribution DG is the project leader, managed the experiment, samplings, and analyses, wrote original draft, reviewed, and edited. BG consulted the formal analyses, collected samples, and reviewed the original draft. SB consulted the experiment and methodology, collected data, wrote original draft, reviewed, and edited. ONK analyzed and tested samples and collected data. İŞ made experimental feeds, reared fish, and collected data. ET consulted the experiment and collected data. OK reared fish and reviewed and edited original draft. SM reared fish, made analyses, and collected data.

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Data availability All data generated and analyzed during this study are presented in this article.

Code availability Not applicable.

Declarations

Ethics approval The experimental protocol was approved by the local ethics committee of Kastamonu University, Kastamonu, Turkey, under protocol number KUHADYEK 2019/10.

Concent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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