Aquaculture 563 (2023) 738878

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Anti-inflammatory and immunostimulant effect of different timing-related administration of dietary polyphenols on intestinal inflammation in zebrafish, *Danio rerio*

Roberta Imperatore [a], Graziella Orso [a], Serena Facchiano [a], Pierpaolo Scarano [a], Seyed Hossein Hoseinifar [b], Ghasem Ashouri [a], Carmine Guarino [a], Marina Paolucci [a,*]

[a] Department of Sciences and Technologies, University of Sannio, Via De Sanctis, snc, 82100 Benevento, Italy
[b] Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

ARTICLE INFO

Keywords:
Intestinal inflammation
Polyphenols
Molecular pathway
Cytokines
Antioxidant enzymes
Zebrafish

ABSTRACT

A short-term study was performed to assess the effects of diets supplemented with polyphenols (CSVP), extracted from the chestnut (*Castanea sativa*) shell, agri-food waste rich in tannins, and mullein (*Verbascum macrurum*), a perennial spontaneous plant rich in flavonoids, on a zebrafish (*Danio rerio*) model of k-carrageenan-induced intestinal inflammation. Morphological asset of intestine, immunodensity of the pro-inflammatory mediator Tumor necrosis factor α (TNFα) and Cyclooxygenase-2 (COX-2), immunoexpression of the two antioxidant enzymes Catalase (Cat) and Superoxide dismutase 2 (Sod2), quantitative gene expression of pro- and anti-inflammatory cytokines including TNFα, COX2A, Interleukin-1β (IL-1β) and IL-10, and the activated cell pathways were analyzed. Seven experimental diets were formulated including: peeled *A. salina* (Control group), peeled *A. salina* + 0.1% of k-carrageenan (Inflamed group), peeled *A. salina* + CSVP followed by k-carrageenan inflammatory diet (CSVP pre-treated group), peeled *A. salina* + 0.1% of k-carrageenan + CSVP (CSVP co-treated group), peeled *A. salina* + 0.1% of k-carrageenan followed by CSVP (CSVP post-treated group), peeled *A. salina* + CSVP (CSVP group) and peeled *A. salina* + 0.1% of k-carrageenan followed by 10 days of control diet (polyphenols control group or CP) to determine the efficacy of schedule administering polyphenols in preventing or ameliorating the intestinal inflammation induced in adult zebrafish by k-carrageenan with mean weight of 400 ± 100 mg (mean ± SD; total $n = 250$). The obtained results indicated that k-carrageenan caused morphological alterations such as the decrease of intestinal folds with consequent expansion of gut lumen, and increased number of goblet cells, as well as the over-regulation of pro-inflammatory markers and reduction of the anti-oxidant enzymes immunoexpression. Polyphenols supplementation ameliorated the inflammatory status, downregulated the pro-inflammatory markers, and upregulated IL-10, the main anti-inflammatory cytokine, and antioxidant activity when administered before the inflammation caused by k-carrageenan. The post- and co-treatment reverted only in part the morphological and physiological alterations. Moreover, the results showed that polyphenols modulated the anti-inflammatory effects acting on the Mitogen-activated protein kinase (MAPK) pathway, by inhibiting p38 phosphorylation and increasing extracellular-signal-regulated kinase (ERK) activation, which subsequently led to suppression of nuclear factor kappa B cells (NF-kB) pathway. Based on the findings achieved, the scheduled administration of bioactive phytochemicals like polyphenols could represent a suitable strategy for ameliorating intestinal inflammation in zebrafish and could be considered as a beneficial and sustainable feed additive in the aquaculture sector.

1. Introduction

Polyphenols are phytochemicals and secondary metabolites of plants, also abundant in agricultural waste, such as roots, leaves, and seeds (Panzella et al., 2020). Polyphenols include >8000 highly diverse compounds whose chemical structure is united by the presence of

* Corresponding author.
E-mail addresses: rimperatore@unisannio.it (R. Imperatore), graorso@unisannio.it (G. Orso), sfacchiano@unisannio.it (S. Facchiano), scarano@unisannio.it (P. Scarano), hoseinifar@gau.ac.ir (S.H. Hoseinifar), guarino@unisannio.it (C. Guarino), paolucci@unisannio.it (M. Paolucci).

https://doi.org/10.1016/j.aquaculture.2022.738878
Received 3 May 2022; Received in revised form 23 September 2022; Accepted 26 September 2022
Available online 1 October 2022

hydroxyl groups that make them powerful antioxidants, able to protect the organism against the oxidative stress caused by reactive oxygen species (ROS) or free radicals (Pandey and Rizvi, 2009; Rudrapal et al., 2022). The polyphenols antioxidant power is at the basis of their beneficial properties. Indeed, the consumption of polyphenol-rich diet is highly advisable to protect against numerous illnesses, among which cardiovascular and neurodegenerative diseases, inflammation, cancer, and diabetes (Vauzour et al., 2010; Rudrapal et al., 2022).

Due to their nutraceutical properties, polyphenols are used in animal nutrition (Huang et al., 2018; Correddu et al., 2020). Their addition to the diet improves the performance of farmed land-animals and the quality of animal products and has become a useful strategy to reduce the use of chemicals and antibiotics (Gessner et al., 2017; Correddu et al., 2020). The use of polyphenols in aquaculture is still in its infancy, although several studies conducted to date showed that they can positively affect growth, immune status, and resistance against diseases (Ahmadifar et al., 2021a, 2021b). Weight gain, specific growth rate, and feed conversion ratio were positively affected by dietary tea polyphenols in the juvenile black carp (*Mylopharyngodon piceus*) (Zhong et al., 2020), and Wuchang bream (*Megalobrama amblycephala*) (Long et al., 2017). Chestnut (*Castanea sativa*) and olive (*Olea europaea*) by-product polyphenols improved weight gain, specific growth rate, and feed conversion ratio in the common carp (*Cyprinus carpio L.*) (Jahazi et al., 2020), Beluga Sturgeon (*Huso huso*) (Safari et al., 2020) and Nile tilapia (*Oreochromis niloticus*) (van Doan et al., 2020). Moreover, polyphenols are natural immunostimulants, capable of modulating the innate immune system of fish (Ahmadifar et al., 2021a). Polyphenols from grapevine seeds, rich in catechins and epigallocatechins, administered with the diet to farmed European sea bass (*Dicentrarcus labrax L.*) increased the activity of the liver and kidney melano-macrophage centers, visceral pigmented cells playing an important role against pathogens and antigen stimulants (Agius and Roberts, 2003), lowered the intestinal proinflammatory cytokines, and induced large amounts of spleen IFN-γ (Arciuli et al., 2017; Magrone et al., 2018).

In the convict cichlid (*Amatitlania nigrofasciata*) humoral and mucosal immune response and serum antioxidant responses were significantly improved by chestnut and olive by-product polyphenols (Hoseinifar et al., 2020). Chestnut polyphenols administered to *O. niloticus* not only improved the humoral and mucosal immune response, but also protected against *Streptococcus agalactiae*, a common bacterial pathogen causing infectious diseases in farmed tilapia (van Doan et al., 2020).

In rearing conditions, fish are subjected to numerous stressors, ranging from the environmental conditions to the manufactured diet, with negative consequences on the general health status, the immune response, and intestinal physiology (Dawood, 2021). The decline in wild fish stocks as a source of fishmeal to be employed in farmed fish feed has forced the aquafeed industry to rely on plant-based feed ingredients whose negative impact on the fish's intestinal functions has now become apparent, limiting the absorption and utilization of nutrients, with consequent reduction of the growth and capability to resist infections and environmental stressors (Bjørgen et al., 2020; Dawood, 2021; Agboola et al., 2022). As natural products, phytochemicals are considered safe for fish and humans and can be used as feed additives in farmed fish species to counteract intestinal inflammation (Li et al., 2020; Li et al., 2022). Apple polyphenols administered to grass carp (*Ctenopharyngodon idellus*) fed low fish meal diet exerted beneficial effects on the inflamed intestine by decreasing the pro-inflammatory cytokines like IL-1β, IL-6, and TNFα and increasing the expression of the antioxidant enzymes Catalase (Cat) and Superoxide dismutase (Sod) (Yang et al., 2021). In the inflamed intestine of zebrafish (*Danio rerio*) polyphenols from chestnut shell counteracted the inflammatory status by modulating the microbiota, downregulating the pro-inflammatory factors TNFα, COX2A and increasing the expression of the anti-inflammatory cytokine IL-10 (Orso et al., 2021).

The employment of zebrafish as inflammatory model to define the optimal dose and the administration timing of nutraceuticals has become very important both in biomedicine and aquaculture (Brugman, 2016; Teame et al., 2019; von Jørgensen, 2020). It also appears as an excellent animal model to better understand the mechanisms of action of nutraceuticals and to define the safety doses and the timing schedule to prevent, maintain or recover the healthy status (Annona et al., 2021; Cirmi et al., 2021). Moreover, the zebrafish model allows the prescreening of feed components and the investigation of their mechanism of action permitting the evaluation of health parameters and live imaging analysis, which are difficult to reach in large and expensive aquaculture species (López Nadal et al., 2020).

In this frame, we examined the timing schedule and the mechanism of action of polyphenols extracted from chestnut shell (*C. sativa*) (agri-food waste), and *Verbascum macrurum* (spontaneous autochthonous plant), on a zebrafish model of intestinal inflammation induced by k-carrageenan.

2. Materials and methods

2.1. Fish husbandry

Male adult zebrafish, about 400 ± 100 mg (mean ± SD), were bred at the Gorgan University of Agricultural Sciences and Natural Resources (GUASNR) in the fish production center of Vakili Ornamental. The GUASNR guidelines for animal care were followed. Before the experiments, zebrafish were housed and acclimated for two weeks with a density of 1 individual l^{-1} in recirculating tanks under standard conditions of photoperiod and temperature (14:10 h light/dark phase, light on at 9:00 AM, 28 °C). Zebrafish were fed each day at 11:00 AM with peeled *A. salina* cysts purchased by Aqua Schwarz (22% fat, 44% proteins, 16% carbohydrates) at 2% of the body weight (Landgraf et al., 2017; Orso et al., 2021). All the main water parameters were daily supervised and maintained constant as follows: temperature: 26 ± 2 °C, dissolved oxygen: 7.8 ± 0.26 mg/l, nitrites: 0.05 ± 0.02 mg/l, nitrates: 5.6 ± 2.5 mg/l, and pH:7.2 ± 0.1. No medical treatments were performed. No deaths were detected before or during the experience.

2.2. Preparation of extracts

Chestnut (*C. sativa*) shell was obtained from the geographical area of Montella (Province of Avellino, Italy) and extracted as previously reported (Coccia et al., 2019). Specifically, shells were desiccated at Room Temperature (RT) and powdered. The powder was extracted on a magnetic stirrer in water (1:10 w / v) for 2 h at 75 °C. Plants of *V. macrurum* were collected in the Pietraroia area (Province of Benevento, Italy) and immediately transferred to the laboratory and washed to move away grimes and soil, reduced into small pieces, and desiccated at RT in dark condition. The plant material was extracted with ethanol: water (1:10 *w/v*), under standard temperature and pressure for 48 h, under stirring conditions in a closed beaker (Guarino, 2002). The extract was filtrated in a funnel with WHATMAN grade 42 filter paper and dried in a rotary evaporator at 35 °C. The extract recovered was placed in vials of 10 ml and further dried in a ventilated stove at a temperature of 37.5 °C, to eliminate any trace of solvent.

2.3. Total phenolic measurement

The Folin-Ciocalteu colorimetric assay was applied to calculate the total phenolic content, as mentioned in Coccia et al. (2019). The calibration curve was built by using the standard Gallic acid (GA) (Sigma, St. Louis, MO, USA). The absorbance was read at 765 nm. The phenolic content was showed as mg GA equivalent/g of extract (mg GAE/g). Analysis was conducted in triplicate.

2.4. HPLC analysis

Polyphenol analysis of *V. macrurum* extract was performed as described by Coccia et al. (2019). The HPLC equipment (LC-4000 Series Integrated, JASCO, Japan) was formed by an autosampler (AS-2059 plus), a column oven (CO-2060 plus), a UV/Vis Photodiode Array Detector (MD-2018 plus), an Intelligent Fluorescence Detector (PF-2020 plus), a liquid chromatography pump (PU-2089 plus) commanded by the software ChromNAV (JASCO, Japan). The column was a C18 25 cm × 3.00 mm I.D. (Phenomenex, Torrance, CA, USA) of 5-μm particle size equipped with a guard cartridge of the same material. The running temperature was 30 °C. The water–formic acid (99.80:0.20, *v*/v) and methanol were used as solvent A and solvent B, respectively, in the mobile phase. The following HPLC running conditions were used: 0–6 min, 35% B; 6–9 min, 35–60% B; 9–14 min, 60–80% B at 30 °C. A wash of 5 min with 100% B was performed after each run. Moreover, the starting mobile phase was used to equilibrate the system between runs. Analysis of samples was performed twice. The flowrate was 0.8 ml/min. 20 μl were injected. The standards employed were apigenin, quercetin, kaempferol, ellagic acid, and gallic acid (Sigma, St. Louis, MO, USA).

2.5. Experimental diets

A. salina cysts were peeled and used for the control diet, while a mix of peeled *A. salina* cysts and 0.1% of k-carrageenan was used to induce inflammation (Orso et al., 2021). The diet enriched in polyphenols contained peeled *A. salina* cystis mixed with 4 μg/d/zebrafish of chestnut shell extract and 2 μg/d/zebrafish of *V. macrurum* extract (CSVP). The polyphenols concentration was selected on the basis of prior work (Orso et al., 2021) and corresponded to the average intake in the common western diet (Mennen et al., 2005). The zebrafish were distributed into seven dietary groups ($n = 36$ zebrafish per group) fed once a day as follows: 1) Control group (C), zebrafish fed *A. salina* cysts; 2) Inflamed group (I), zebrafish fed *A. salina* added with 0.1% k-carrageenan for 10 days; 3) CSVP pre-treated group (CSVPpreI), zebrafish fed *A. salina* added with CSVP for 10 days followed by 10 days of *A. salina* added with 0.1% k-carrageenan; 4) CSVP co-treated group (CSVPwithI), zebrafish fed *A. salina* added with 0.1% k-carrageenan and CSVP for 10 days; 5) CSVP post-treated group (CSVPpostI), zebrafish fed *A. salina* added with 0.1% k-carrageenan for 10 days followed by *A. salina* added with CSVP for another 10 days; 6) CSVP group (CSVP), zebrafish fed with *A. salina* added with CSVP for 10 days; 7) control group of the CSVP post-treated group or control polyphenols (CP), zebrafish fed with *A. salina* with 0.1% k-carrageenan 10 days followed by 10 days of control diet. At the end of the feeding experiment zebrafish were sacrificed after a night of fasting.

2.6. Morphological analysis

For each group, the intestine of six zebrafish was collected for histological analysis. Fixation in 4% formalin, dehydration and clarification were performed before embedding the tissues in paraffin. Embedded samples were sliced into 5 μm sections and processed for Alcian blue and Hematoxylin-Eosin (H&E) staining. Being the mid intestine (MI), the site of nutrient absorption containing most immune cells and playing a main role in intestinal immune functions (Brugman, 2016), we focused our study on this segment. A light microscope Leica DMI6000 equipped with digital CCD camera Leica DFC340 (Leica Microsystems) was used to analyze histological sections and microscopic images were captured at 20× and 40× magnification. Score and goblet cells number were measured as described in Orso et al. (2021).

2.7. Immunohistochemical analysis

The avidin-biotin immunohistochemical technique was used to stain anatomically comparable sections of MI from six fish for each treatment. The primary antibodies used were mouse monoclonal antibody anti-TNFα (#ab1793, Abcam, Cambridge, UK), rabbit polyclonal antibody anti-COX-2 (#69720, NovaTeinBio, Woburn, MA.), rabbit polyclonal antibody anti-Cat (#GTX124357, GeneTex, USA), and rabbit polyclonal antibody anti-Sod2 (#GTX124294, GeneTex, USA). The sections were processed as reported in Imperatore et al. (2020). Briefly, the sections were treated with NGS (normal goat serum) (Vector Laboratories, UK) prepared at 10% in Tris-buffered saline (0.1 M, pH 7.6) with 0.3% Triton X-100 and then subjected to overnight incubation at 4 °C with primary antibodies prepared in NGS at a dilution of 1:200. Subsequently, the sections were incubated for 2 h at RT with the biotinylated secondary antibody (goat anti-mouse or goat anti-rabbit, Vector Laboratories) diluted 1:100 and then incubated for 1 h with the complex avidin-biotin (ABC Kit; Vectastain, Vector). At the end a solution of 0.05% of 3′-diaminobenzidine (DAB, Sigma Fast, Sigma-Aldrich) was used to reveal the immunosignal. A Leica DMI6000 microscope equipped with Leica DFC340 digital CCD camera (Leica Microsystems, Germany) was used to examine the histological sections and acquire the images at 20× and 40× magnification. The densitometric analysis of TNFα-, COX-2-, Cat- and Sod2-positive signal from digital images of MI sections ($n = 6$ zebrafish/goup; $n = 3$ sections/zebrafish, choosing each section at 50 μm distance) was achieved as defined in Imperatore et al. (2019). The software Image Pro Plus® 6.0 (Media Cybernetics) was utilized to analyze the images and measure the optical density working on absorbance by using a logarithmic scale. A blind independent operator performed the histological analyses.

2.8. Quantitative Real Time-PCR analysis

Quantitative RT-qPCR analysis of COX2A, IL-1β, IL-10 and TNFα gene expression was performed as mentioned in Orso et al. (2021). The intestinal total RNA (n = 6 zebrafish/goup) extraction was carried out with Trizol Reagent (Invitrogen, Thermo-Scientific, Waltham, Massachusetts, USA). RNA integrity was determined by electrophoresis in 1% agarose gels. Total RNA was quantified with a NanoDrop 1000 Spectrophotometer (Thermo-Scientific, Waltham, Massachusetts, USA). The cDNA was synthesized by 1 μg RNA with the SensiFAST™ cDNA Synthesis Kit (Bioline), as reported in the instructions of the manufacturer. A QuantStudio 5 System (Thermo Fisher Scientific) was used to carried out the RT-qPCR as described in Orso et al. (2021). The expression profiles of genes were normalized to actb1 and tuba1. The comparative Ct method formula $2^{-\Delta\Delta Ct}$ was used to perform the quantitative analysis. Three biological replicates and three technical repeats for each were analyzed. The primers sequences are listened in Table 1.

2.9. Western blot analysis

Western blotting was carried out on the intestine of control and inflamed zebrafish fed with CSVP and sacrificed 15 min and 1 h after feeding trials. The intestine was rapidly dissected on ice and washed with ice cold PBS. Intestines were saved at −80 °C until use.

Table 1
Primers sequences used for genes amplification through RT-qPCR.

Gene	Primer	Sequences 5′ → 3′	GeneBank n^{O}
actb1	F	TCTCTTAAGTCGACAACCCCC	NM_131031
	R	TCTGAGCCTCATCACCAACG	
COX2A	F	AGGGCGTGTGTTTATCCAAG	NM_153657.1
	R	ACCTGGACGTCCTTCATAAG	
IL-1β	F	TGGACTTCGCAGCACAAAATG	NM_212844.2
	R	GTTCACTTCACGCTCTTGGATG	
IL-10	F	CCCTATGGATGTCACGTCATG	AY887900.1
	R	CATATCCCGCTTGAGTTCCTG	
TNFα	F	GCTTATGAGCCATGCAGTGA	AY427649
	R	TGCCCAGTCTGTCTCCTTCT	
tuba1	F	CCTGCTGGGAACTGTATTGT	AF029250
	R	TCAATGAGTTCCTTGCCAAT	

Total tissue protein was extracted with ice-cold RIPA lysis buffer (150 mM NaCl, 50 mM Tris, pH 7.4, 1%NP-40, 0.1% SDS and 0.5% sodium deoxycholate) added with protease inhibitor cocktail (Roche Diagnostics SpA, Monza, Italy) and phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO). The lysate was centrifuged at 14,000 rpm for 20 min at 4 °C and the protein concentration was quantified with the Bradford Protein Assay Kit (Thermo Scientific, USA). Proteins were separated by SDS-PAGE (10%) and subsequently, electrotransferred to a nitrocellulose membrane (Millipore, USA). Membranes were immunoblotted overnight at 4 °C with p44/42 MAPK (ERK1/2) (#9107, Cell Signaling) or Phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204) (#9101, Cell Signaling) or IkBα (#55481S, ANASPEC, USA), Phospho-IkBα (Ser32/36) (#9246, Cell Signaling) or p38 (#315599, Biorbyt) or Phospho-p38 MAPK (Thr180/Tyr182) (#MA5–15182, Invitrogen) and β-actin (#8457, Cell Signaling) antibodies. The membranes were incubated with anti-mouse or anti-rabbit IgG (1:3000) (#7076 and #7074, Cell Signaling, respectively) as secondary antibody. The ChemiDoc (Bio-Rad) was used to detect signals by chemiluminescence with the clarity western ECL substrate (#1705061, Bio-Rad). Protein size was detected by using a pre-stained molecular weight ladder (Precision Plus Protein™ Dual Xtra Prestained Protein Standards #1610377, Biorad). Image Lab (Bio-Rad) software was used to quantify the western blot bands.

2.10. Statistical analysis

GraphPad Prism 6 software, version 6.05 (GraphPad, Inc.) was utilized to perform the statistical analyses. For normally distributed data the one-way ANOVA coupled to Bonferroni's post-hoc test was employed. Not-normally distributed data, such as that obtained by densitometric analysis, cytokines analysis and western blotting, were evaluated by Kruskal–Wallis ANOVA non-parametric test followed by Dunn's post hoc test. The differences were defined statistically significant at $p < 0.05$.

3. Results

3.1. Total phenolic content

The standard curve of gallic acid for determining the phenol content in the extracts of chestnut shell and *V. macrurum* is shown in Fig. 1. The concentration of gallic acid in the standard curve ranged from 0.02 to 0.15 mg/ml. The concentration of total phenolic content in chestnut shell and *V. macrurum* extracts was determined as milligrams of gallic acid equivalent (GAE) by extrapolation from the standard curve. The total content of phenols in the extracts, was 45 ± 2.5 mg/gr for chestnut shell and 4.3 ± 0.3 mg/gr for *V. macrurum*.

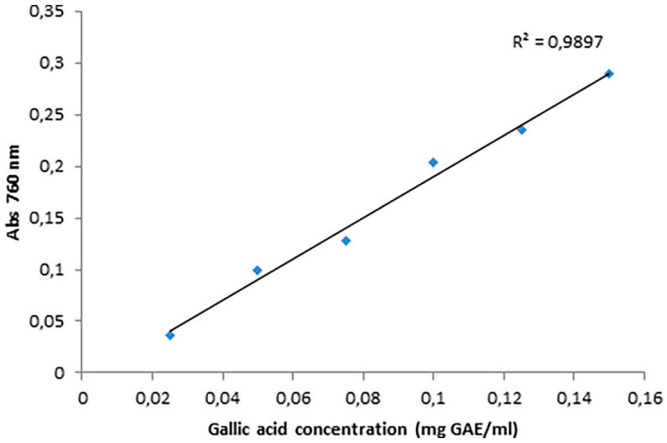

Fig. 1. Gallic acid calibration curve for total phenolic quantification.

3.2. HPLC analysis

Fig. 2 reports the HPLC profile of the *V. macrurum* extract. By comparing with the current literature data and the standards, the main peak was represented by apigenin. Other minor peaks identified were quercetin and ellagic acid.

3.3. CSVP effects on zebrafish intestinal histology

The histological status of the MI, shown in Fig. 3, was evaluated using the score system reported in Orso et al. (2021). The score analysis showed that the score number of the k-carrageenan enriched diet fed group was significantly increased compared to the control (C = 2 ± 0.26 vs $I = 10.73 \pm 0.24$; $p < 0.0001$), exhibiting strong signs of inflammation such as enhancement of goblet cells number and intraepithelial leucocytes infiltrates, mucosal thinning, expansion of the gut lumen with decrease of intestinal folds (villi) that looked thinner and irregular. Moreover, the Alcian blue staining showed, in the inflamed zebrafish, a significant higher number of positive goblet cells/villus with respect to the control (C = 7.92 ± 0.41 vs $I = 12.83 \pm 0.35$, $p < 0.0001$).

The different administration schedule of CSVP showed different effects. Specifically, the score number was significantly lower in CSVPpreI (3.53 ± 0.37, $p < 0.0001$), CSVPwithI (8.73 ± 0.32, $p < 0.001$), and CSVPpostI (9.16 ± 0.31, $p < 0.05$) compared to the inflamed group (10.73 ± 0.24), even if CSVPwithI and CSVPpostI showed a score number significantly higher than the control group (2 ± 0.26). On the contrary, the goblet cells number was significantly lower in CSVPpreI and CSVPwithI zebrafish with respect to I group (CSVPpreI = 9.08 ± 0.41 vs I = 12.83 ± 0.35, $p < 0.001$; CSVPwithI = 10 ± 0.64 vs I = 12.83 ± 0.35, $p < 0.05$), but no significant variations were detected for the CSVPpostI with respect to the I group. Moreover, the qualitative analysis of H&*E*-stained tissues indicated that the intestine of CSVPpreI zebrafish, as the control group, was characterized by compact villi and intact basal membrane. Observing the intestinal structure of CSVPpostI regular and well-organized villi could be noticed, even if their border was not well defined. Finally, the leukocytes infiltrate in the lamina propria and epithelium appeared reduced in CSVPpreI and CSVPpostI, but not in the CSVPwithI group. Zebrafish fed with the CSVP enriched diet did not show intestinal alterations and displayed a score number near to the control. The CP group showed a score and intestinal alteration similar to the inflamed group.

3.4. CSVP effect on pro-inflammatory markers in the zebrafish intestine

In addition to the morphological alterations, the k-carrageenan induced also an increased immunoexpression of pro-inflammatory factors TNFα and COX-2 in the epithelial and enteroendocrine cells of the MI as showed in Figs. 4 and 5. The zebrafish treated with CSVP showed a lower immunoexpression of TNFα and COX-2 with respect to the k-carrageenan enriched diet group. Specifically, by densitometric analysis a significantly higher optical density of TNFα was detected in the intestinal epithelium of inflamed zebrafish in comparison to the control (C = 0.16 ± 0.018 vs $I = 0.61 \pm 0.03$, $p < 0.0001$). The TNFα immunodensity decreased significantly in all the groups fed with CSVP enriched diet (I = 0.61 ± 0.03 vs CSVPpreI = 0.29 ± 0.015, $p < 0.0001$; I = 0.61 ± 0.04 vs CSVPwithI = 0.42 ± 0.027, $p < 0.0001$; I = 0.61 ± 0.04 vs CSVPpostI = 0.48 ± 0.021, $p < 0.05$). However, CSVPwithI and CSVPpostI zebrafish groups showed a TNFα optical density significantly higher than the control (Fig. 4).

COX-2 immunoexpression, even if mainly localized on the villus epithelium, showed a similar trend to TNFα (Fig. 5). Specifically, in inflamed zebrafish COX-2 displayed a strong immunosignal, confined on the apical side of the epithelial cells, with the optical density significantly higher than the control group (C = 0.16 ± 0.017 vs I = 0.6 ± 0.024, $p < 0.0001$). A significant lower optical density of COX-2 was found in CSVPpreI zebrafish compared to the inflamed zebrafish (I = 0.6

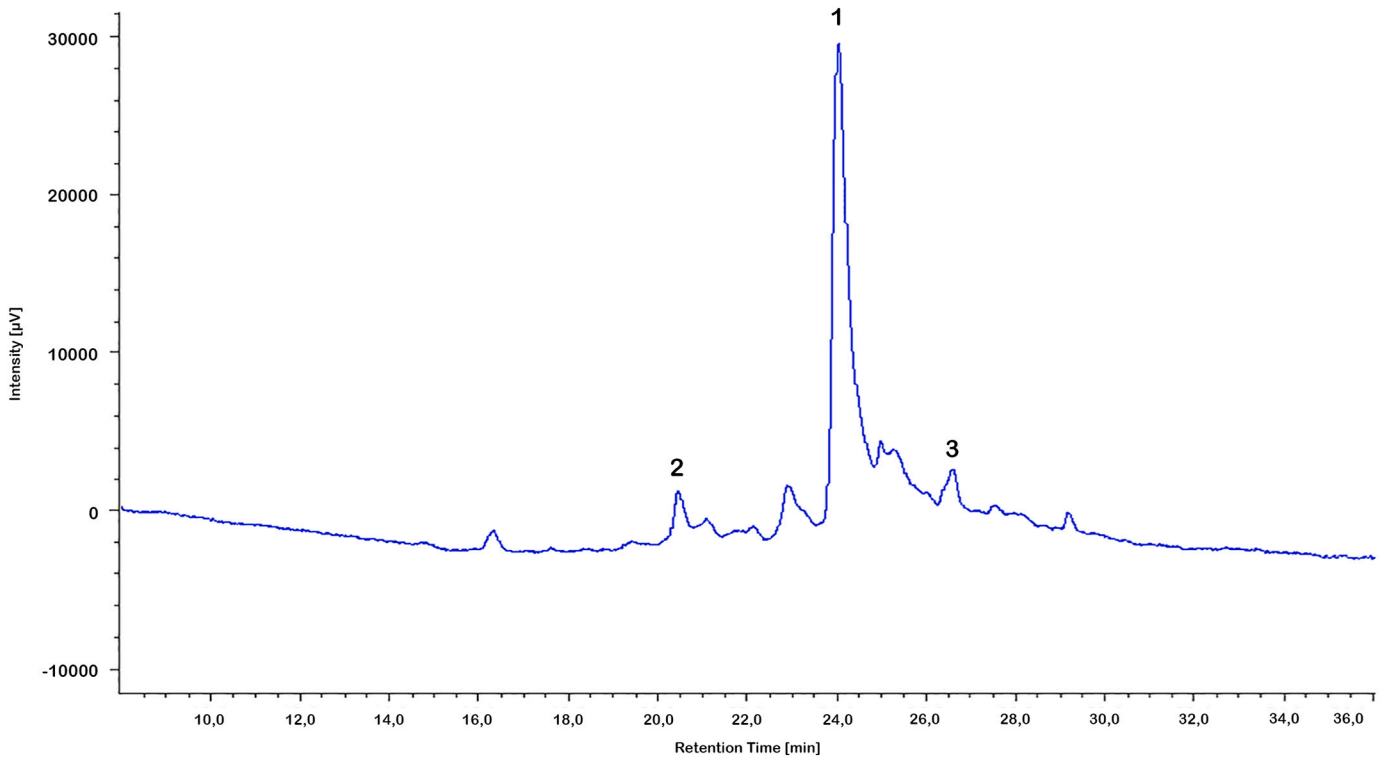

Fig. 2. Representative HPLC profile of *V. macrurum* extract. The peak identification is based on literature data analysis and standards. 1 = Apigenin; 2 = ellagic acid; 3 = quercetin.

± 0.024 vs CSVPpreI = 0.2 ± 0.011, $p < 0.0001$), as well as CSVPwithI and CSVPpostI (CSVPwithI = 0.28 ± 0.026, $p < 0.0001$; I = 0.6 ± 0.024 vs CSVPpostI = 0.3 ± 0.018, $p < 0.0001$), although COX-2 immunodensity in CSVPwithI and CSVPpostI groups increased significantly in comparison to the control (C = 0.16 ± 0.017 vs CSVPwith*I* = 0.27 ± 0.02, $p < 0.05$; C = 0.16 ± 0.017 vs CSVPpostI = 0.3 ± 0.018, $p < 0.001$).

Zebrafish fed with the CSVP enriched diet, and the CP group showed a TNFα and COX-2 immunoexpression similar to the control and inflamed groups respectively.

3.5. CSVP effect on antioxidative enzymes immunoexpression in the zebrafish intestine

K-carrageenan enriched diet induced a decrease of Sod2 and Cat immunoexpression in the MI of zebrafish. These antioxidative enzymes were increased by diet enriched with CSVP (Fig. 6). Specifically, a strong increase of Sod2 immunosignal was found in the epithelial cells of MI of CSVPpreI zebrafish group (I = 0.27 ± 0.021 vs CSVPpreI = 0.63 ± 0.025, $p < 0.0001$), while CSVPwithI and CSVPpostI showed only a slight enhancement of Sod2 immunoexpression with respect to the inflamed group (I = 0.27 ± 0.021 vs CSVPwithI = 0.38 ± 0.009, $p < 0.001$; I = 0.27 ± 0.021 vs CSVPpostI = 0.36 ± 0.01, $p < 0.05$).

On the contrary, observing Cat immunoexpression only a mild increase of the immunosignal was found in the epithelial cells of MI of CSVPpreI, CSVPwithI and CSVPpostI zebrafish groups with respect to the inflamed group (*I* = 0.32 ± 0.009 vs CSVPpreI = 0.54 ± 0.011, $p < 0.0001$; I = 0.32 ± 0.009 vs CSVPwithI = 0.39 ± 0.010, $p < 0.001$; I = 0.32 ± 0.009 vs CSVPpostI = 0.38 ± 0.015, $p < 0.05$). However, zebrafish fed with the CSVP enriched diet showed a significant increase of Cat immunosignal (Fig. 7).

3.6. CSVP effect on cytokines gene expression in the zebrafish intestine

The cytokines gene expression in the zebrafish MI is reported in Fig. 8. TNFα gene expression was significantly higher in the inflamed (6.92 ± 0.13, $p < 0.0001$), CSVPpostI (7.26 ± 0.02, $p < 0.0001$) and CSVPwithI (5.29 ± 0.2, $p < 0.0001$) groups than in the control. Conversely, TNFα expression significantly decreased in the CSVPpreI zebrafish with respect to the I group (I: 6.92 ± 0.13 vs CSVPpreI: 2.99 ± 0.19, $p < 0.05$). Also, the COX2A gene appeared significantly more expressed in the intestine of k-carrageenan enriched diet fed group (3.73 ± 0.15, $p < 0.0001$) than in the control, while it was significantly reduced in the CSVPpreI (1.22 ± 0.05, $p < 0.001$) and CSVPpostI (1.93 ± 0.1, $p < 0.05$) compared to the inflamed zebrafish. A significantly higher relative gene expression of IL-1β was evidenced in the inflamed group (5.53 ± 0.04, $p < 0.001$) in comparison to the control and significantly decreased in the CSVPpreI (0.79 ± 0.31, $p < 0.001$) with respect to the inflamed group. CSVPpostI (6.05 ± 0.17, $p = 0.992$) and CSVPwithI (4.59 ± 0.12, $p = 0.999$) did not showed significant differences. On the contrary, the expression of the anti-inflammatory cytokine IL-10 was higher in all the groups fed with CSVP enriched diet than in the control and inflamed zebrafish. Interestingly, the polyphenols control group showed a cytokines gene expression like the inflamed group, with TNFα, COX2A and IL-1β relative gene expression significantly higher than the control, accompanied by a low expression of IL-10 gene. CSVP group relative genes expression appeared like the control group.

3.7. CSVP effects on p38, ERK, and Nuclear Factor-Kappa B (NF-kB) Signaling Pathway in the zebrafish intestine

p38 mitogen-activated protein kinase (p38 MARK) and extracellular signal-regulated kinase (ERK) were evaluated by Western blotting. As shown in Fig. 9, the ERK phosphorylation was significantly promoted in the control and inflamed groups fed with CSVP enriched diet, both at 15 min and 1 h after treatment. On the contrary, after CSVP treatment the phosphorylation of p38 MAPK was significantly inhibited in inflamed

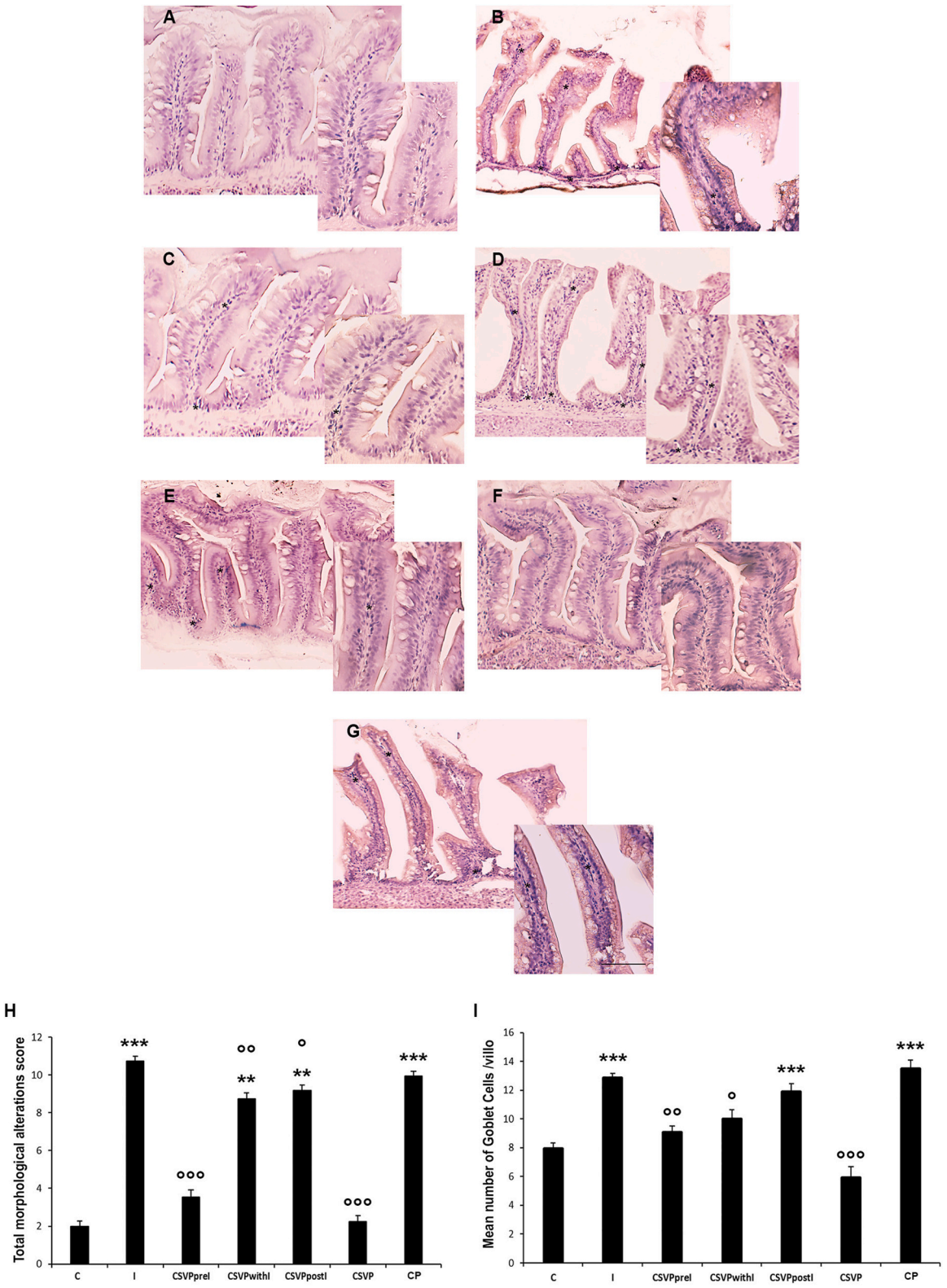

Fig. 3. Hematoxylin-eosin (H&E) staining of zebrafish (*D. rerio*) MI: (**A**) control (C), (**B**) inflamed (I), (**C**) I pre-treated with chestnut shell and *V. macrurum* extract (CSVPpreI), (**D**) I co-treated with chestnut shell and *V. macrurum* extract (CSVPwithI), (**E**) I post-treated with chestnut shell and *V. macrurum* extract (CSVPpostI), (**F**) fed with chestnut shell and *V. macrurum* extract enriched diet (CSVP), and (**G**) control polyphenols (CP). Asterisks show leukocytes infiltrate. Scale bar: 100 μm, and 50 μm for the higher magnifications. (**H**) Bar graph showing the total intestinal alteration score defined for each group. (**I**) Bar graph showing the number of goblet cells/villus for each group. Goblet cells were counted based on Alcian Blue staining. Data are expressed as mean ± SE. $^{***}p < 0.0001$, $^{**}p < 0.001$ compared to the control group; $^{\circ\circ\circ}p < 0.0001$, $^{\circ\circ}p < 0.001$ and $^{\circ}p < 0.05$ compared to the inflamed group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

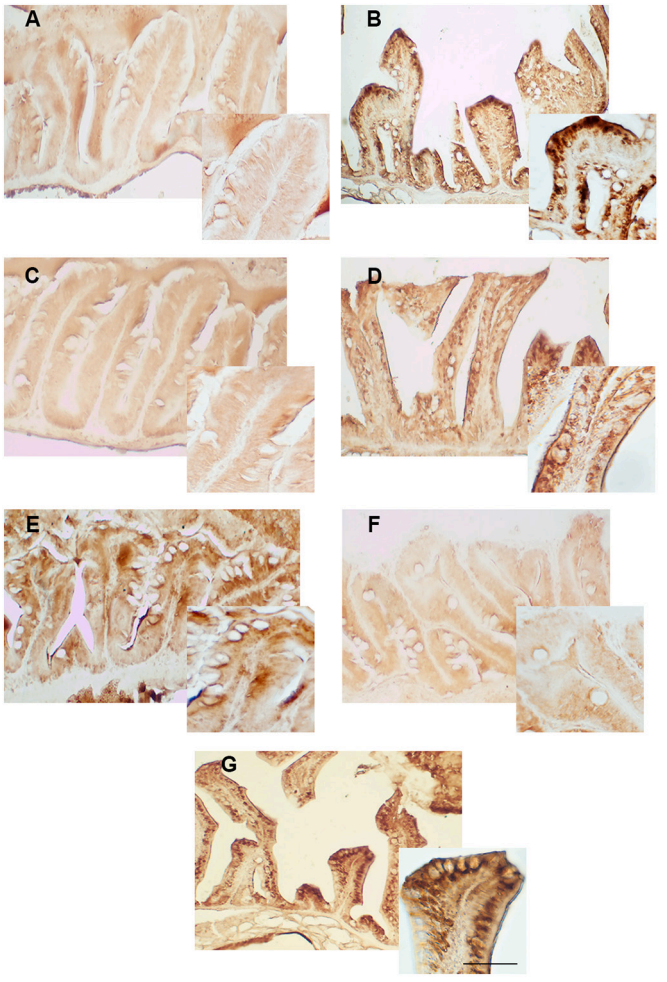

Fig. 4. TNFα immunostaining in the MI of zebrafish (*D. rerio*): (**A**) control (C), (**B**) inflamed (I), (**C**) I pre-treated with chestnut shell and *V. macrurum* extract (CSVPpreI), (**D**) I co-treated with chestnut shell and *V. macrurum* extract (CSVPwithI), (**E**) I post-treated with chestnut shell and *V. macrurum* extract (CSVPpostI), (**F**) fed with chestnut shell and *V. macrurum* extract enriched diet (CSVP), and (**G**) control polyphenols (CP). Scale bar: 100 μm, and 50 μm for the higher magnifications. (**H**) Bar graph showing TNFα optical density (O.D.) in the MI of each group. Data are expressed as mean ± SE. ***$p < 0.0001$, **$p < 0.001$ and *$p < 0.05$ compared to C group; °°°$p < 0.0001$, °$p < 0.05$ compared to the I group.

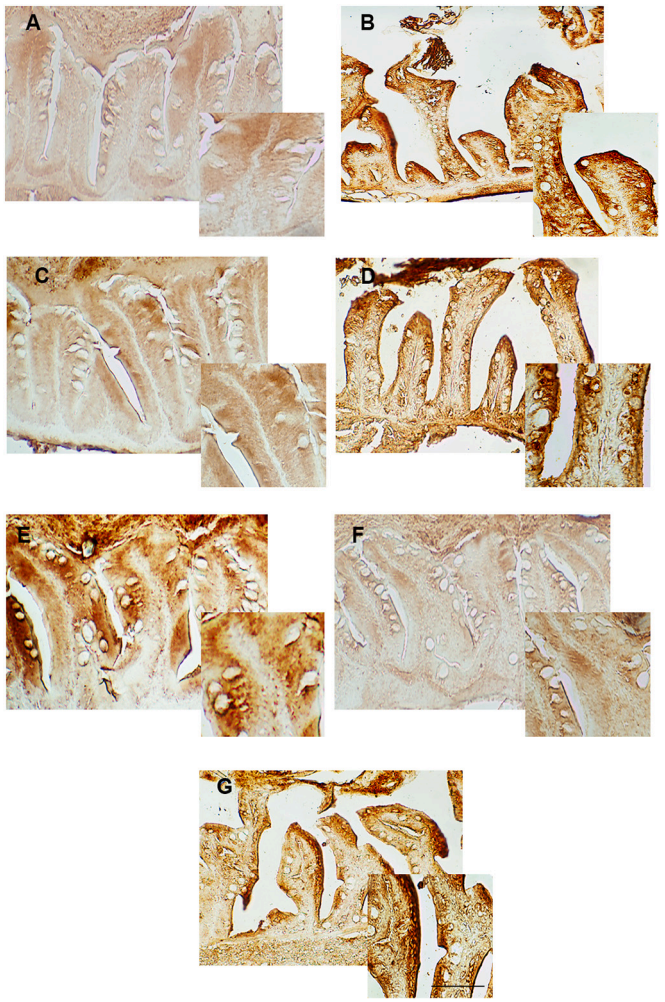

Fig. 5. COX-2 immunostaining in the MI of zebrafish (*D. rerio*): (**A**) control (C), (**B**) inflamed (I), (**C**) I pre-treated with chestnut shell and *V. macrurum* extract (CSVPpreI), (**D**) I co-treated with chestnut shell and *V. macrurum* extract (CSVPwithI), (**E**) I post-treated with chestnut shell and *V. macrurum* extract (CSVPpostI), (**F**) fed with chestnut shell and *V. macrurum* extract enriched diet (CSVP), and (**G**) control polyphenols (CP). Scale bar: 100 μm, and 50 μm for the higher magnifications. (**H**) Bar graph showing COX-2 optical density (O.D.) in the MI of each group. Data are expressed as mean ± SE. ***$p < 0.0001$, **$p < 0.001$ and *$p < 0.05$ compared to C group; °°°$p < 0.0001$ compared to the I group.

group starting from 15 min (Fig. 9A, 15 min: $p < 0.05$; Fig. 8B, 1h: $p < 0.001$), and in the control group after 1 h (Fig. 9B, $p < 0.05$).

To investigate the effect of CSVP on the NF-kB pathway, the IkBα phosphorylation was analyzed. As shown in Fig. 9, CSVP led to the inhibition of IkBα phosphorylation levels in inflamed group after 1 h of administration (Fig. 9B, $p < 0.001$). On the contrary, no significant effect was found after 15 min of treatment and in the control group fed with CSVP.

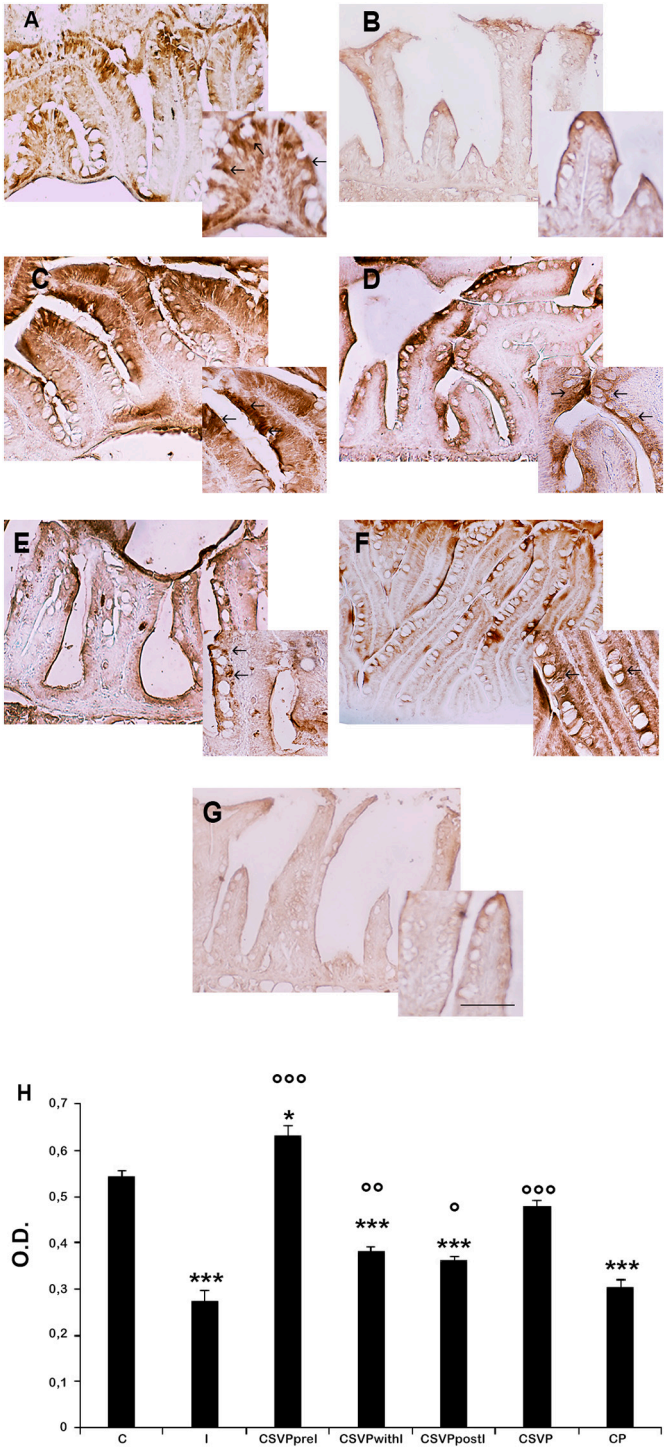

Fig. 6. Sod2 immunostaining in the MI of zebrafish (*D. rerio*): (**A**) control (C), (**B**) inflamed (I), (**C**) I pre-treated with chestnut shell and *V. macrurum* extract (CSVPpreI), (**D**) I co-treated with chestnut shell and *V. macrurum* extract (CSVPwithI), (**E**) I post-treated with chestnut shell and *V. macrurum* extract (CSVPpostI), (**F**) fed with chestnut shell and *V. macrurum* extract enriched diet (CSVP), and (**G**) control polyphenols (CP). Scale bar: 100 μm, and 50 μm for the higher magnifications. (**H**) Bar graph showing TNFα optical density (O.D.) in the MI of each group. Data are expressed as mean ± SE. $^{***}p < 0.0001$ and $^{*}p < 0.05$ compared to C group; $^{°°°}p < 0.0001$, $^{°°}p < 0.001$ and $^{°}p < 0.05$ compared to the I group.

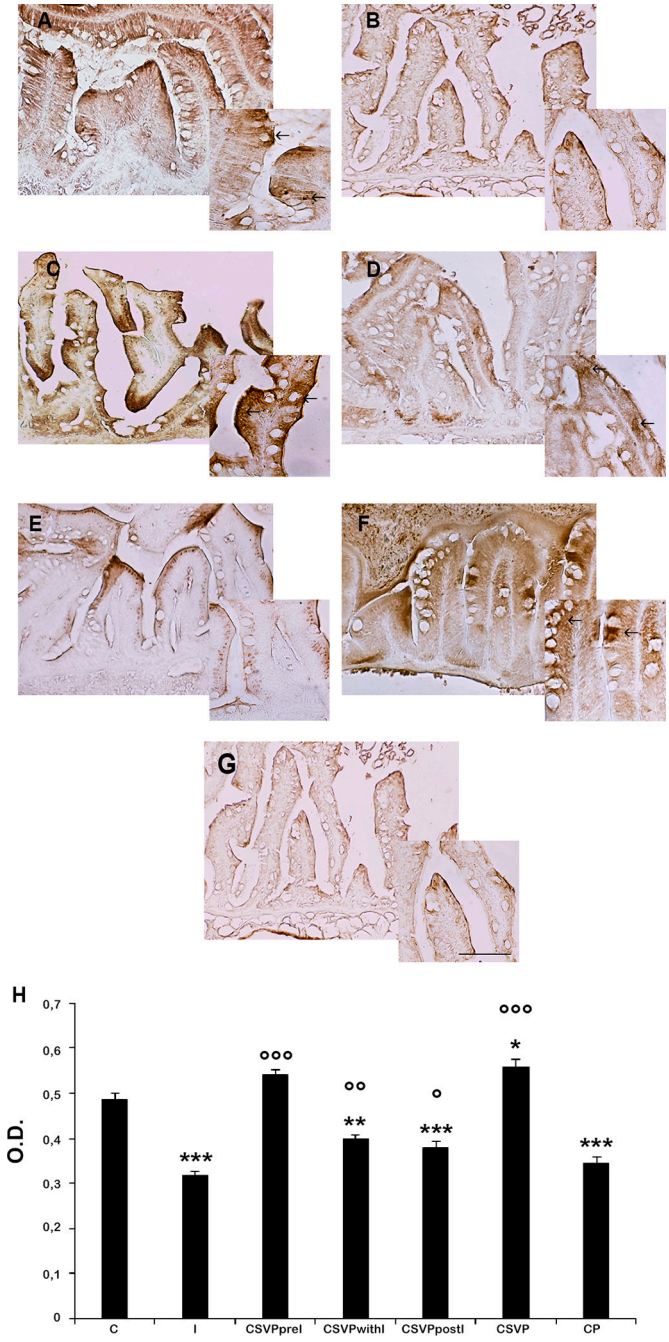

Fig. 7. Cat immunostaining in the MI of zebrafish (*D. rerio*): (**A**) control (C), (**B**) inflamed (I), (**C**) I pre-treated with chestnut shell and *V. macrurum* extract (CSVPpreI), (**D**) I co-treated with chestnut shell and *V. macrurum* extract (CSVPwithI), (**E**) I post-treated with chestnut shell and *V. macrurum* extract (CSVPpostI), (**F**) fed with chestnut shell and *V. macrurum* extract enriched diet (CSVP), and (**G**) control polyphenols (CP). Scale bar: 100 μm, and 50 μm for the higher magnifications. (**H**) Bar graph showing TNFα optical density (O.D.) in the MI of each group. Data are expressed as mean ± SE. $^{***}p < 0.0001$, $^{**}p < 0.001$ and $^{*}p < 0.05$ compared to C group; $^{°°°}p < 0.0001$, $^{°°}p < 0.001$ and $^{°}p < 0.05$ compared to the I group.

4. Discussion

In this study, the results demonstrated that the treatment with polyphenols prevented the severity of the intestinal inflammation induced in zebrafish by k-carrageenan, while the co-treatment and post-treatment only partially counteracted it. The polyphenols employed in

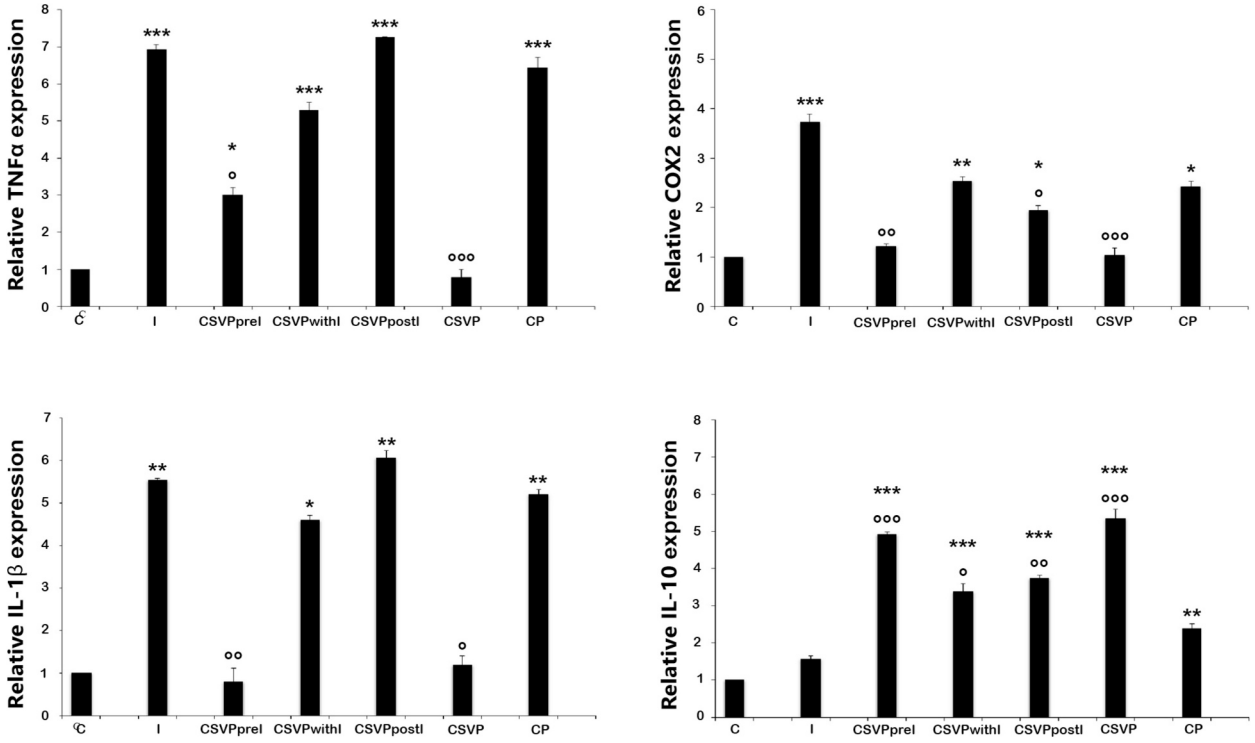

Fig. 8. Relative expression of TNFα, COX2A, IL-1β, and IL-10 genes in zebrafish (*D. rerio*) MI: control group (C), inflamed group (I), I pre-treated with chestnut shell and *V. macrurum* extract (CSVPpreI), I co-treated with chestnut shell and *V. macrurum* extract (CSVPwithI), I post-treated with chestnut shell and *V. macrurum* extract (CSVPpostI), group fed with chestnut shell and *V. macrurum* extract enriched diet (CSVP), and control polyphenols (CP). Values are presented as the mean ± SE. $n = 6$. $^{***}p < 0.0001$, $^{**}p < 0.001$ and $^{*}p < 0.05$ compared to control group; $^{\circ\circ\circ}p < 0.0001$, $^{\circ\circ}p < 0.001$ and $^{\circ}p < 0.05$ compared to the inflamed group.

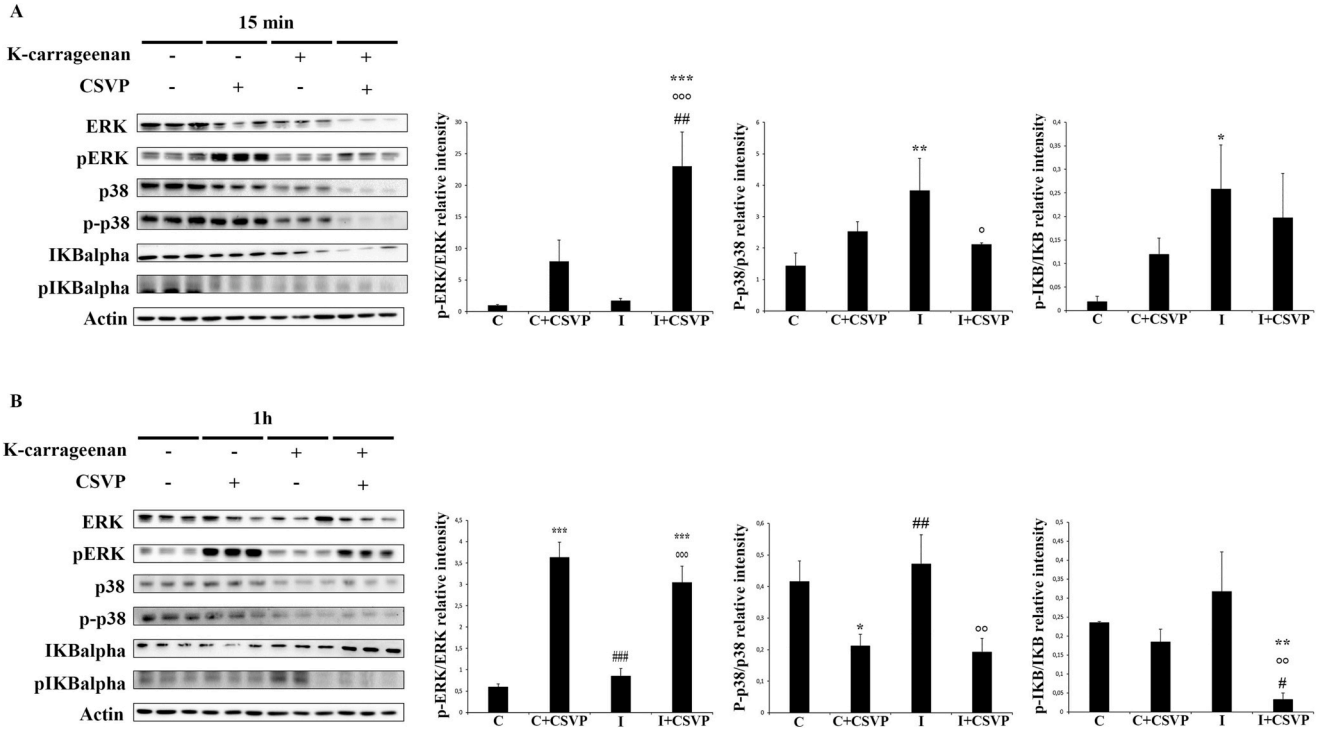

Fig. 9. Effect of chestnut shell and *V. macrurum* extract (CSVP) on MAPK and NF-kB signaling pathways in zebrafish (*D. rerio*) MI after 15 min and 1 h of treatment. (**A**) Protein expression analysis of NF-kB and MAPK signaling pathways evaluated using Western blot after 15 min; (**B**) Protein expression analysis of NF-kB and MAPK signaling pathways evaluated using Western blot after 1 h. The relative amounts of each protein were compared with β-actin. Values are presented as the mean ± SD. $n = 3$. $^{***}p < 0.0001$, $^{**}p < 0.001$ and $^{*}p < 0.05$, compared to control group; $^{\circ\circ\circ}p < 0.0001$, $^{\circ\circ}p < 0.001$ and $^{\circ}p < 0.05$ compared to inflamed group; $^{\#\#\#}p < 0.0001$, $^{\#\#}p < 0.001$ and $^{\#}p < 0.05$ compared to C + CSVP.

this research were extracted from chestnut (*C. sativa*) shell and *V. macrurum*, a perennial European spontaneous plant, easily found in cultivated lands and along roads (Frezza et al., 2019). Chestnut shell polyphenols were condensed tannins (CT), formed by monomeric units of epigallocatechin and catechin/epicatechin (Sorice et al., 2016). CT were characterized by a remarkable antioxidant activity (Deng et al., 2016) and the capacity to maintain intestinal health of fish (Peng et al., 2021). The polyphenols reported in several *Verbascum* species, were mainly flavonoids, such as apigenin, luteolin, quercetin and kampferol (Kahraman et al., 2011). In this study, apigenin was the major flavonoid found in ethanol:water extract (1:10) of *V. macrurum*, in agreement with Karalija et al. (2020) who reported apigenin as the major flavonoid in water extracts of *Verbascum* sp. Apigenin was reported to be one of the most potent anti-inflammatory flavonoids and may explain the use of *Verbascum* sp. as herbal drug against inflammation (Tatli and Akdemir, 2006; Tatli et al., 2008) and to ameliorate the clinical symptoms of colitis (Hausmann et al., 2007; Lenoir et al., 2011).

Very few studies directly addressed the timing issue of polyphenols administration. Cirmi et al. (2021), studying the anti-inflammatory role of a flavonoid-rich orange extract on a *Vibrio anguillarum*-induced enteritis in zebrafish, observed that the pretreatment reduced the intestinal villi damage and avoided the rise of the pro-inflammatory factors IL-1β, IL-6, and TNFα. Another study, carried out on dextran sulfate sodium (DSS)-induced colitis in mouse, underlined the capacity of luteolin to attenuate the symptoms, ameliorate tissue damage and inflammation both in post- and co-treated animals (Vukelić et al., 2020). Moreover, Gong et al. (2020) indicated that *Dendrobium candidum* polyphenols improved the oxazolone-induced intestinal inflammation in zebrafish, restored morphological intestinal structure and regulated the host immune system. In addition, Wang et al. (2019) evaluated the effects of tannins pretreatment on herbicide-induced oxidative stress in mouse, revealing that this class of bioactive compounds was able to improve intestinal morphology and ameliorate intestinal barrier acting on the tight junction. Finally, our results agree with Liu et al. (2020), who reported the tannin's protective actions on intestinal health and morphology. Thus, our study showed how polyphenols could effectively improve the tissue damage and intestinal mucosal injury in k-carrageenan-induced inflammation, indicating their enteritis preventing value and the capability to ameliorate the inflammatory status.

Recently, various studies revealed the polyphenol's capability to restrain intestinal inflammation by controlling cytokines expression (Santino et al., 2017; Xu et al., 2020), and suppressing COX2 expression (Li et al., 2020). The polyphenols employed in this study contained bioactive flavonoids, mainly apigenin, small amounts of quercetin, and CT. Previous findings showed that apigenin and quercetin could significantly lower the protein and mRNA levels of the proinflammatory marker COX2 both *in vitro,* in lipopolysaccharide (LPS)-induced macrophages, through the inhibition of inflammatory cytokines (Marzocchella et al., 2011) and *in vivo,* in DSS-induced chronic ulcerative mice model, by decreasing the infiltration of immune cells in colon tissues (Ai et al., 2017). Furthermore, apigenin suppressed the release of TNFα interfering with NF-kB pathway in human epithelial cells (O'Prey et al., 2003). In another study, Sadraei et al. (2017) observed that the pretreatment with apigenin showed anti-inflammatory effects comparable with those of prednisolone in acetic acid-induced colitis in rats. In agreement with the aforementioned authors, our results also proved that the pre-treatment with chestnut shell and *Verbascum* sp. polyphenols prevented the k-carrageenan-induced TNFα and COX2A increase, while the co- and post-treatment only partially modulated the pro-inflammatory cytokines expression, without being able to prevent or improve inflammation as showed by score number and morphological analysis. Furthermore, our results on immunoexpression emphasized the importance of using a mix of polyphenols. In fact, in the present study, the use of CSVP determined a more marked effect on the inhibition of immunoexpression in the intestine of inflamed zebrafish compared to a previous study, where only the chestnut shell polyphenols were used (Orso et al., 2021). This result confirmed the strong anti-inflammatory action of *Verbascum* sp. polyphenols and the importance of exploiting the synergy of these bioactive molecules.

Gut health is crucial to allow animal growth and is closely related to intestinal immune function. In piglets, for example, the supplementation of chestnut tannins enhanced the growth performances (Girard and Bee, 2020). The positive impact of tannin supplementation in monogastric animals is likely related to the antioxidant, antimicrobial, and anti-inflammatory properties (Biagi et al., 2010; Huang et al., 2018). Several *in vitro* studies indicated that tannins exerted an anti-inflammatory action through the decrease of the cytokines IL-1β and IL-6, and the prostaglandin synthesizing inducible enzyme COX-2 expression (Xiong et al., 2017; Khalilpour et al., 2019). Additionally, the anti-inflammatory action of tannins was reported in different tissues of human and livestock such as intestine, skeletal muscle, and lung (Fraga-Corral et al., 2021). González-Quilen et al. (2019) demonstrated how tannins reduced the proinflammatory cytokine TNFα expression, ameliorating intestinal health in diet-induced obese rats. Interestingly, due to the high number of hydroxyl groups, CT have a stronger antioxidant activity with respect to other simple phenolic compounds (Hagerman et al., 1998; Koleckar et al., 2008). In this study, Sod2 and Cat immunoexpression increased significantly in the polyphenols treated groups, especially in the pre-treatment one. Accordingly, tannins pretreatment ameliorated the oxidative stress status through the activation of the antioxidant enzymes in mice (Wang et al., 2019). Polyphenols were powerful stimulators of the antioxidant system in numerous fish species (Long et al., 2017; Hoseinifar et al., 2020; Ji et al., 2020). Quercetin and rutin enhanced the antioxidant capacity in silver catfish (*Rhamdia quelen*) (Pês et al., 2016), and caffeic acid dietary supplementation remarkably improved the Sod and Cat activities in rainbow trout (Yilmaz, 2019).

Cytokines are crucial in the pathogenesis of colitis and their increase induces inflammation with consequent tissue damage (Marafini et al., 2019; Martinez-Fierro et al., 2019). The downregulation of anti-inflammatory cytokines (like IL-10) and up-regulation of pro-inflammatory cytokines (like IL-1β) exacerbated inflammatory responses in fish (Wang and Secombes, 2013). Our findings showed that polyphenols significantly decreased the relative level of gene expression of pro-inflammatory cytokines TNFα, IL-1β, and COX2A, while increased the level of the anti-inflammatory cytokine IL-10. Since the increased expression of pro-inflammatory factors like TNFα and IL-1β is considered a distinctive feature of intestinal inflammation (Li et al., 2018), several authors mentioned that an important and logical therapeutic approach in the treatment of colitis is represented by the decrease of inflammatory cytokines (Sireswar et al., 2021; Sheng et al., 2020). Previous studies reported that polyphenols, such as apigenin, quercetin, and epigallocatechin gallate, could modulate the gene expression of cytokines in the intestine of rodents and zebrafish or in epithelial cell lines (Hoensch and Weigmann, 2018). Our results indicated that the pretreatment was able to simultaneously reduce the TNFα, IL-1β, COX2A, and increase IL-10. In agreement with our study, Cirmi et al. (2021) showed that flavonoid pretreatment was effective in the prevention of *V. anguillarum*-induced enteritis in zebrafish, hindering the rise of pro-inflammatory factors, like IL-1β, IL-6, and TNFα. Furthermore, apple polyphenols ameliorated intestinal inflammation by decreasing the pro-inflammatory cytokines expression in DSS-zebrafish model and the combined treatment of polyphenols with probiotics induced a significant enhancement in the anti-inflammatory cytokine IL-10 expression (Sireswar et al., 2021). Comparable results were identified in *in vitro* pretreatment studies with apigenin that significantly lowered the levels of COX-2, IL-1β, and TNFα and raised the level of IL-10 (Kumar et al., 2018). In addition, chestnut CT exhibited the anti-inflammatory and cytoprotective properties downregulating the IL-1β, TNFα and NF-kB expression in the neuroinflammatory cell model BV-2 microglia cells (Chiocchio et al., 2020). Therefore, CT inhibited the formation of pro-inflammatory cytokine in animal models (Xiong et al., 2017) and

downregulated NF-kB, iNOS, COX-2, TNFα, and IL-6 (Dobroslawa et al., 2009).

The signaling pathways NF-kB and MAPK play essential roles in the appearance and progression of inflammation (Gaestel et al., 2009). It was reported that polyphenols and their metabolites exerted anti-inflammatory activity through the modulation of such signaling pathways (Yahfoufi et al., 2018; Peng et al., 2019; Vukelić et al., 2020). Our results indicated that the CSVP modulated the MAPK pathway through the inhibition of p38 phosphorylation and activation of ERK. Moreover, CSVP suppressed the activation of NF-kB by blocking the phosphorylation of IkB. According to Gkouveris et al. (2014) ERK activation led to the reduction of inflammation by STAT3 suppression. It is worth noting that CSVP showed the beneficial effects faster (15′) and in the presence of intestinal inflammation, acting more rapidly on the MAPK pathway and subsequently, at 1 h, blocking the IkB phosphorylation leading to NF-kB pathway inhibition. In agreement with our findings, Maeng et al. (2006) reported that ERK directly blocked NF-kB signaling playing a pivotal role in the suppression of inflammation. Moreover, p38 played a key role in inflammation and triggered important intracellular responses in inflammatory diseases, such as regulating the transcription activity of NF-kB (Gao et al., 2019).

In conclusion, our results suggest that chestnut shell and *V. macrurum* polyphenols mixed extract (CSVP) exerts anti-inflammatory effects in k-carrageenan-induced intestinal inflammation, and the timing-related administration of polyphenols is essential to obtain more beneficial advantages and preserve or maintain the intestinal health status in zebrafish as animal model. Furthermore, in an era of circular economy, the presence of polyphenols easily extractable from renewable sources can be considered an advantageous and innovative strategy both for the aquafeed sector and for aquaculture.

Funding

This study was supported by Fondi Ricerca di Ateneo (FRA), University of Sannio.

Institutional review board statement

The authors confirm that the ethical considerations were met. This study has been done under Iran National Research Council's guidelines for the care and use of laboratory animals. The research board of GUASNR approved the main project on zebrafish as well as ethical considerations, No: 96-374-63.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

CRediT authorship contribution statement

Roberta Imperatore: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Visualization, Writing – review & editing. **Graziella Orso:** Methodology, Formal analysis, Investigation, Writing – original draft. **Serena Facchiano:** Investigation. **Pierpaolo Scarano:** Investigation. **Seyed Hossein Hoseinifar:** Investigation, Resources, Writing – review & editing. **Ghasem Ashouri:** Investigation, Writing – review & editing. **Carmine Guarino:** Resources, Writing – review & editing. **Marina Paolucci:** Conceptualization, Methodology, Funding acquisition, Resources, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that the research was carried out in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

Data availability

No data was used for the research described in the article.

References

Agboola, J.O., Chikwati, E.M., Hansen, J., Kortner, T.M., Mydland, L.T., Krogdahl, Å., Djordjevic, B., Schrama, J.W., Øverland, M., 2022. A meta-analysis to determine factors associated with the severity of enteritis in Atlantic salmon (*Salmo salar*) fed soybean meal-based diets. Aquaculture 555, 738214. https://doi.org/10.1016/J.AQUACULTURE.2022.738214.

Agius, C., Roberts, R.J., 2003. Melano-macrophage centres and their role in fish pathology. J. Fish Dis. 26 (9), 499–509. https://doi.org/10.1046/j.1365-2761.2003.00485.x.

Ahmadifar, E., Fallah, H.P., Yousefi, M., Dawood, M.A.O., Hoseinifar, S.H., Adineh, H., Yilmaz, S., Paolucci, M., van Doan, H., 2021a. The gene regulatory roles of herbal extracts on the growth, immune system, and reproduction of fish. Animals 11 (8), 2167. https://doi.org/10.3390/ani11082167.

Ahmadifar, E., Yousefi, M., Karimi, M., Fadaei Raieni, R., Dadar, M., Yilmaz, S., Dawood, M.A.O., Abdel-Latif, H.M.R., 2021b. Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. Rev. Fish. Sci. Aquacult. 29 (4), 478–511. https://doi.org/10.1080/23308249.2020.1818689.

Ai, X.Y., Qin, Y., Liu, H.J., Cui, Z.H., Li, M., Yang, J.H., Zhong, W.L., Liu, Y.R., Chen, S., Sun, T., Zhou, H.G., Yang, C., 2017. Apigenin inhibits colonic inflammation and tumorigenesis by suppressing STAT3-NF-κB signaling. Oncotarget 8 (59), 100216–100226. https://doi.org/10.18632/oncotarget.22145.

Annona, G., Tarallo, A., Nittoli, V., Varricchio, E., Sordino, P., D'Aniello, S., Paolucci, M., 2021. Short-term exposure to the simple polyphenolic compound gallic acid induces neuronal hyperactivity in zebrafish larvae. Eur. J. Neurosci. 53 (5), 1367–1377. https://doi.org/10.1111/ejn.15021.

Arciuli, M., Fiocco, D., Fontana, S., Arena, M.P., Frassanito, M.A., Gallone, A., 2017. Administration of a polyphenol-enriched feed to farmed sea bass (*Dicentrarchus labrax* L.): kidney melanomacrophages response. Fish Shellfish Immunol. 68, 404–410. https://doi.org/10.1016/j.fsi.2017.07.043.

Biagi, G., Cipollini, I., Paulicks, B.R., Roth, F.X., 2010. Effect of tannins on growth performance and intestinal ecosystem in weaned piglets. Arch. Anim. Nutr. 64 (2), 121–135. https://doi.org/10.1080/17450390903461584.

Bjørgen, H., Li, Y., Kortner, T.M., Krogdahl, Å., Koppang, E.O., 2020. Anatomy, immunology, digestive physiology and microbiota of the salmonid intestine: knowns and unknowns under the impact of an expanding industrialized production. Fish Shellfish Immunol. 107 (Pt A), 172–186. https://doi.org/10.1016/j.fsi.2020.09.032.

Brugman, S., 2016. The zebrafish as a model to study intestinal inflammation. Dev. Comp. Immunol. 64, 82–92. https://doi.org/10.1016/j.dci.2016.02.020.

Chiocchio, I., Prata, C., Mandrone, M., Ricciardiello, F., Marrazzo, P., Tomasi, P., Angeloni, C., Fiorentini, D., Malaguti, M., Poli, F., Hrelia, S., 2020. Leaves and spiny burs of *Castanea sativa* from an experimental chestnut grove: Metabolomic analysis and anti-neuroinflammatory activity. Metabolites 10 (10), 408. https://doi.org/10.3390/metabo10100408.

Cirmi, S., Randazzo, B., Russo, C., Musumeci, L., Maugeri, A., Montalbano, G., Guerrera, M.C., Lombardo, G.E., Levanti, M., 2021. Anti-inflammatory effect of a flavonoid-rich extract of orange juice in adult zebrafish subjected to *Vibrio anguillarum*-induced enteritis. Nat. Prod. Res. 35 (23), 5350–5353. https://doi.org/10.1080/14786419.2020.1758096.

Coccia, E., Siano, F., Volpe, M.G., Varricchio, E., Eroldogan, O.T., Paolucci, M., 2019. Chestnut shell extract modulates immune parameters in the rainbow trout *Oncorhynchus mykiss*. Fishes 4 (1), 18. https://doi.org/10.3390/fishes4010018.

Correddu, F., Lunesu, M.F., Buffa, G., Atzori, A.S., Nudda, A., Battacone, G., Pulina, G., 2020. Can agro-industrial by-products rich in polyphenols be advantageously used in the feeding and nutrition of dairy small ruminants? Animals 10 (1), 131. https://doi.org/10.3390/ani10010131.

Dawood, M.A.O., 2021. Nutritional immunity of fish intestines: important insights for sustainable aquaculture. Rev. Aquac. 13 (1), 642–663. https://doi.org/10.1111/raq.12492.

Deng, Y.T., Liang, G., Shi, Y., Li, H.L., Zhang, J., Mao, X.M., Fu, Q.R., Peng, W.X., Chen, Q.X., Shen, D.Y., 2016. Condensed tannins from *Ficus altissima* leaves: structural, antioxidant, and antityrosinase properties. Process Biochem. 51 (8), 1092–1099. https://doi.org/10.1016/j.procbio.2016.04.022.

Dobroslawa, B., Kasimsetty, S.G., Khan, S.I., Daneel, F., 2009. Urolithins, intestinal microbial metabolites of pomegranate ellagitannins, exhibit potent antioxidant activity in a cell-based assay. J. Agric. Food Chem. 57 (21), 10181–10186. https://doi.org/10.1021/jf9025794.

Fraga-Corral, M., Otero, P., Echave, J., Garcia-Oliveira, P., Carpena, M., Jarboui, A., Nuñez-Estevez, B., Simal-Gandara, J., Prieto, M.A., 2021. By-products of agri-food industry as tannin-rich sources: a review of tannins' biological activities and their potential for valorization. Foods 10 (1), 137. https://doi.org/10.3390/foods10010137.

Frezza, C., Bianco, A., Serafini, M., Foddai, S., Salustri, M., Reverberi, M., Gelardi, L., Bonina, A., Bonina, F.P., 2019. HPLC and NMR analysis of the phenyl-ethanoid glycosides pattern of *Verbascum thapsus L.* cultivated in the Etnean area. Nat. Prod. Res. 33 (9), 1310–1316. https://doi.org/10.1080/14786419.2018.1473398.

Gaestel, M., Kotlyarov, A., Kracht, M., 2009. Targeting innate immunity protein kinase signalling in inflammation. Nat. Rev. Drug Discov. 8 (6), 480–499. https://doi.org/10.1038/nrd2829.

Gao, W., Wang, C., Yu, L., Sheng, T., Wu, Z., Wang, X., Zhang, D., Lin, Y., Gong, Y., 2019. Chlorogenic acid attenuates dextran sodium sulfate-induced ulcerative colitis in mice through MAPK/ERK/JNK pathway. Biomed. Res. Int. 2019, 6769789. https://doi.org/10.1155/2019/6769789.

Gessner, D.K., Ringseis, R., Eder, K., 2017. Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. J. Anim. Physiol. Anim. Nutr. 101 (4), 605–628. https://doi.org/10.1111/jpn.12579.

Girard, M., Bee, G., 2020. Invited review: tannins as a potential alternative to antibiotics to prevent coliform diarrhea in weaned pigs. Animal 14 (1), 95–107. https://doi.org/10.1017/S1751731119002143.

Gkouveris, I., Nikitakis, N., Karanikou, M., Rassidakis, G., Sklavounou, A., 2014. Erk1/2 activation and modulation of STAT3 signaling in oral cancer. Oncol. Rep. 32 (5), 2175–2182. https://doi.org/10.3892/or.2014.3440.

Gong, X., Jiang, S., Tian, H., Xiang, D., Zhang, J., 2020. Polyphenols in the fermentation liquid of *Dendrobium candidum* relieve intestinal inflammation in zebrafish through the intestinal microbiome-mediated immune response. Front. Immunol. 11, 1542. https://doi.org/10.3389/fimmu.2020.01542.

González-Quilen, C., Gil-Cardoso, K., Ginés, I., Beltrán-Debón, R., Pinent, M., Ardévol, A., Terra, X., Blay, M.T., 2019. Grape-seed proanthocyanidins are able to reverse intestinal dysfunction and metabolic endotoxemia induced by a cafeteria diet in wistar rats. Nutrients 11 (5), 979. https://doi.org/10.3390/nu11050979.

Guarino, C., 2002. Antimicrobial activity of *Verbascum macrurum* Ten. (Scrophulariaceae). Boll. Chim. Farm. 141 (3), 238–242.

Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., Riechel, T.L., 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46 (5), 1887–1892. https://doi.org/10.1021/jf970975b.

Hausmann, M., Obermeier, F., Paper, D.H., Balan, K., Dunger, N., Menzel, K., Falk, W., Schoelmerich, J., Herfarth, H., Rogler, G., 2007. In vivo treatment with the herbal phenylethanoid acteoside ameliorates intestinal inflammation in dextran sulphate sodium-induced colitis. Clin. Exp. Immunol. 148 (2), 373–381. https://doi.org/10.1111/j.1365-2249.2007.03350.x.

Hoensch, H.P., Weigmann, B., 2018. Regulation of the intestinal immune system by flavonoids and its utility in chronic inflammatory bowel disease. World J. Gastroenterol. 24 (8), 877–881. https://doi.org/10.3748/wjg.v24.i8.877.

Hoseinifar, S.H., Jahazi, M.A., Nikdehghan, N., van Doan, H., Volpe, M.G., Paolucci, M., 2020. Effects of dietary polyphenols from agricultural by-products on mucosal and humoral immune and antioxidant responses of convict cichlid (*Amatitlania nigrofasciata*). Aquaculture 517, 734790. https://doi.org/10.1016/j.aquaculture.2019.734790.

Huang, Q., Liu, X., Zhao, G., Hu, T., Wang, Y., 2018. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Anim. Nutrition 4 (2), 137–150. https://doi.org/10.1016/j.aninu.2017.09.004.

Imperatore, R., D'angelo, L., de Girolamo, P., Cristino, L., Paolucci, M., 2019. Identification of orexin and endocannabinoid receptors in adult zebrafish using immunoperoxidase and immunofluorescence methods. J. Vis. Exp. 148 https://doi.org/10.3791/59308.

Imperatore, R., Tunisi, L., Mavaro, I., D'angelo, L., Attanasio, C., Safari, O., Motlagh, H. A., de Girolamo, P., Cristino, L., Varricchio, E., Paolucci, M., 2020. Immunohistochemical analysis of intestinal and central nervous system morphology in an obese animal model (*Danio rerio*) treated with 3,5-t2: a possible farm management practice? Animals 10 (7), 1131. https://doi.org/10.3390/ani10071131.

Jahazi, M.A., Hoseinifar, S.H., Jafari, V., Hajimoradloo, A., van Doan, H., Paolucci, M., 2020. Dietary supplementation of polyphenols positively affects the innate immune response, oxidative status, and growth performance of common carp, *Cyprinus carpio L.* Aquaculture. 517, 734709 https://doi.org/10.1016/j.aquaculture.2019.734709.

Ji, M., Gong, X., Li, X., Wang, C., Li, M., 2020. Advanced research on the antioxidant activity and mechanism of polyphenols from hippophae species-a review. Molecules 25 (4), 917. https://doi.org/10.3390/molecules25040917.

Kahraman, Ç., Ekizoglu, M., Kart, D., Akdemir, Z.S., Tatli, I., 2011. Antimicrobial activity of some verbascum species growing in Turkey. Fabad J. Pharmaceut. Sci. 36 (1), 11–15.

Karalija, E., Parić, A., Dahija, S., Bešta-Gajević, R., Ćavar Zeljković, S., 2020. Phenolic compounds and bioactive properties of *Verbascum glabratum* subsp. bosnense *(K. Malý)* Murb., an endemic plant species. Nat. Prod. Res. 34 (16), 2407–2411. https://doi.org/10.1080/14786419.2018.1538221.

Khalilpour, S., Sangiovanni, E., Piazza, S., Fumagalli, M., Beretta, G., Dell'Agli, M., 2019. In vitro evidences of the traditional use of *Rhus coriaria L.* fruits against skin inflammatory conditions. J. Ethnopharmacol. 238, 111829 https://doi.org/10.1016/j.jep.2019.111829.

Koleckar, V., Kubikova, K., Rehakova, Z., Kuca, K., Jun, D., Jahodar, L., Opletal, L., 2008. Condensed and hydrolysable tannins as antioxidants influencing the health. Mini-Rev. Med. Chem. 8 (5), 436–447. https://doi.org/10.2174/138955708784223486.

Kumar, K.S., Sabu, V., Sindhu, G., Rauf, A.A., Helen, A., 2018. Isolation, identification and characterization of apigenin from *Justicia gendarussa* and its anti-inflammatory activity. Int. Immunopharmacol. 59, 157–167. https://doi.org/10.1016/j.intimp.2018.04.004.

Landgraf, K., Schuster, S., Meusel, A., Garten, A., Riemer, T., Schleinitz, D., Kiess, W., Körner, A., 2017. Short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development of metabolically healthy versus unhealthy obesity. BMC Physiol. 17 (1), 4. https://doi.org/10.1186/s12899-017-0031-x.

Lenoir, L., Rossary, A., Joubert-Zakeyh, J., Vergnaud-Gauduchon, J., Farges, M.C., Fraisse, D., Texier, O., Lamaison, J.L., Vasson, M.P., Felgines, C., 2011. Lemon verbena infusion consumption attenuates oxidative stress in dextran sulfate sodium-induced colitis in the rat. Dig. Dis. Sci. 56 (12), 3534–3545. https://doi.org/10.1007/s10620-011-1784-x.

Li, J. Juan, Zhang, Han, Y., Wen, L., Tian, Q. Ping, He, Q. Xia, Wang, X. Min, Sun, C., Han, J., Liu, K. Chun, 2018. Tenacissoside H exerts an anti-inflammatory effect by regulating the NF-κB and p38 pathways in zebrafish. Fish Shellfish Immunol. 83, 205–212. https://doi.org/10.1016/j.fsi.2018.09.032.

Li, H., Christman, L.M., Li, R., Gu, L., 2020. Synergic interactions between polyphenols and gut microbiota in mitigating inflammatory bowel diseases. Food Funct. 11 (6), 4878–4891. https://doi.org/10.1039/d0fo00713g.

Li, M., Wei, D., Huang, S., Huang, L., Xu, F., Yu, Q., Liu, M., Li, P., 2022. Medicinal herbs and phytochemicals to combat pathogens in aquaculture. Aquac. Int. 30, 1239–1259. https://doi.org/10.1007/s10499-022-00841-7.

Liu, H., Hu, J., Mahfuz, S., Piao, X., 2020. Effects of hydrolysable tannins as zinc oxide substitutes on antioxidant status, immune function, intestinal morphology, and digestive enzyme activities in weaned piglets. Animals 10 (5). https://doi.org/10.3390/ani10050757, 757. 10.

Long, M., Lin, W., Hou, J., Guo, H., Li, L., Li, D., Tang, R., Yang, F., 2017. Dietary supplementation with selenium yeast and tea polyphenols improve growth performance and nitrite tolerance of Wuchang bream (*Megalobrama amblycephala*). Fish Shellfish Immunol. 68, 74–83. https://doi.org/10.1016/j.fsi.2017.07.017.

López Nadal, A., Ikeda-Ohtsubo, W., Sipkema, D., Peggs, D., McGurk, C., Forlenza, M., Wiegertjes, G.F., Brugman, S., 2020. Feed, microbiota, and gut immunity: using the zebrafish model to understand fish health. Front. Immunol. 11, 114. https://doi.org/10.3389/fimmu.2020.00114.

Maeng, Y.S., Min, J.K., Kim, J.H., Yamagishi, A., Mochizuki, N., Kwon, J.Y., Park, Y.W., Kim, Y.M., Kwon, Y.G., 2006. ERK is an anti-inflammatory signal that suppresses expression of NF-κB-dependent inflammatory genes by inhibiting IKK activity in endothelial cells. Cell. Signal. 18 (7), 994–1005. https://doi.org/10.1016/j.cellsig.2005.08.007.

Magrone, T., Magrone, T., Spagnoletta, A., Spagnoletta, A., Magrone, M., Magrone, M., Russo, M.A., Russo, M.A., Corriero, A., Corriero, A., Jirillo, E., Jirillo, E., Passantino, L., Passantino, L., 2018. Effects of polyphenol administration to European Farmed Sea bass (*Dicentrharcus labrax L.*): special focus on hepatopancreas morphology. Endocrine, metabolic & immune disorders drug. Targets 19 (4), 526–533. https://doi.org/10.2174/1871530318666181009111214.

Marafini, I., Sedda, S., Dinallo, V., Monteleone, G., 2019. Inflammatory cytokines: from discoveries to therapies in IBD. Expert. Opin. Biol. Ther. 19 (11), 1207–1217. https://doi.org/10.1080/14712598.2019.1652267.

Martinez-Fierro, M.L., Garza-Veloz, I., Rocha-Pizaña, M.R., Cardenas-Vargas, E., Cid-Baez, M.A., Trejo-Vazquez, F., Flores-Morales, V., Villela-Ramirez, G.A., Delgado-Enciso, I., Rodriguez-Sanchez, I.P., Ortiz-Castro, Y., Kantarçeken, B., 2019. Serum cytokine, chemokine, and growth factor profiles and their modulation in inflammatory bowel disease. Medicine (United States) 98 (38), e17208. https://doi.org/10.1097/MD.0000000000017208.

Marzocchella, L., Fantini, M., Benvenuto, M., Masuelli, L., Tresoldi, I., Modesti, A., Bei, R., 2011. Dietary flavonoids: molecular mechanisms of action as anti-inflammatory agents. Recent Patents Inflamm. Allergy Drug Discov. 5 (3), 200–220. https://doi.org/10.2174/187221311797264937.

Mennen, L.I., Walker, R., Bennetau-Pelissero, C., Scalbert, A., 2005. Risks and safety of polyphenol consumption. Am. J. Clin. Nutr. 81 (1 Suppl), 326S–329S. https://doi.org/10.1093/ajcn/81.1.326s.

O'Prey, J., Brown, J., Fleming, J., Harrison, P.R., 2003. Effects of dietary flavonoids on major signal transduction pathways in human epithelial cells. Biochem. Pharmacol. 66 (11) https://doi.org/10.1016/j.bcp.2003.07.007, 2075-88. 66.

Orso, G., Solovyev, M.M., Facchiano, S., Tyrikova, E., Sateriale, D., Kashinskaya, E., Pagliarulo, C., Hoseinifar, H.S., Simonov, E., Varricchio, E., Paolucci, M., Imperatore, R., 2021. Chestnut shell tannins: effects on intestinal inflammation and dysbiosis in zebrafish. Animals 11 (6), 1538. https://doi.org/10.3390/ani11061538.

Pandey, K.B., Rizvi, S.I., 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Med. Cell. Longev. 2 (5), 270–278. https://doi.org/10.4161/oxim.2.5.9498.

Panzella, L., Moccia, F., Nasti, R., Marzorati, S., Verotta, L., Napolitano, A., 2020. Bioactive phenolic compounds from agri-food wastes: an update on green and sustainable extraction methodologies. Front. Nutrition 7, 60. https://doi.org/10.3389/fnut.2020.00060.

Peng, J., Hu, T., Li, J., Du, J., Zhu, K., Cheng, B., Li, K., 2019. Shepherd's purse polyphenols exert its anti-inflammatory and antioxidative effects associated with suppressing MAPK and NF-κB pathways and heme oxygenase-1 activation. Oxidative Med. Cell. Longev. 2019, 7202695. https://doi.org/10.1155/2019/7202695.

Peng, K., Zhao, H., Wang, G., Chen, B., Wenyan, M.O.W., Huang, Y., 2021. Effect of condensed tannins on growth performance, intestinal immune capacity and bacterial microbiomes of Lateolabrax japonicus. Aquac. Res. 52 (11), 5321–5331. https://doi.org/10.1111/are.15402.

Pês, T.S., Saccol, E.M.H., Ourique, G.M., Londero, É.P., Gressler, L.T., Finamor, I.A., Rotili, D.A., Golombieski, J.I., Glanzner, W.G., Llesuy, S.F., Gonçalves, P.B.D., Neto, J.R., Baldisserotto, B., Pavanato, M.A., 2016. Effect of diets enriched with rutin on blood parameters, oxidative biomarkers and pituitary hormone expression in silver catfish (*Rhamdia quelen*). Fish Physiol. Biochem. 42 (1), 321–333. https://doi.org/10.1007/s10695-015-0140-z.

Rudrapal, M., Khairnar, S.J., Khan, J., Dukhyil, A. Bin, Ansari, M.A., Alomary, M.N., Alshabrmi, F.M., Palai, S., Deb, P.K., Devi, R., 2022. Dietary polyphenols and their role in oxidative stress-induced human diseases: insights into protective effects,

antioxidant potentials and mechanism(s) of action. Front. Pharmacol. 13, 806470 https://doi.org/10.3389/fphar.2022.806470.
Sadraei, H., Asghari, G., Khanabadi, M., Minaiyan, M., 2017. Anti-inflammatory effect of apigenin and hydroalcoholic extract of *Dracocephalum kotschyi* on acetic acid-induced colitis in rats. Res. Pharmaceut. Sci. 12 (4) https://doi.org/10.4103/1735-5362.212050, 322-329. 12.
Safari, R., Hoseinifar, S.H., Imanpour, M.R., Mazandarani, M., Sanchouli, H., Paolucci, M., 2020. Effects of dietary polyphenols on mucosal and humoral immune responses, antioxidant defense and growth gene expression in beluga sturgeon (*Huso huso*). Aquaculture 528, 735494. https://doi.org/10.1016/j.aquaculture.2020.735494.
Santino, A., Scarano, A., de Santis, S., de Benedictis, M., Giovinazzo, G., Chieppa, M., 2017. Gut microbiota modulation and anti-inflammatory properties of dietary polyphenols in IBD: new and consolidated perspectives. Curr. Pharm. Des. 23 (16), 2344–2351. https://doi.org/10.2174/1381612823666170207145420.
Sheng, K., He, S., Sun, M., Zhang, G., Kong, X., Wang, J., Wang, Y., 2020. Synbiotic supplementation containing: *Bifidobacterium infantis* and xylooligosaccharides alleviates dextran sulfate sodium-induced ulcerative colitis. Food Funct. 11 (5), 3964–3974. https://doi.org/10.1039/d0fo00518e.
Sireswar, S., Dey, G., Biswas, S., 2021. Influence of fruit-based beverages on efficacy of Lacticaseibacillus rhamnosus GG (*Lactobacillus rhamnosus GG*) against DSS-induced intestinal inflammation. Food Res. Int. 149, 110661 https://doi.org/10.1016/j.foodres.2021.110661.
Sorice, A., Siano, F., Capone, F., Guerriero, E., Picariello, G., Budillon, A., Ciliberto, G., Paolucci, M., Costantini, S., Volpe, M.G., 2016. Potential anticancer effects of polyphenols from chestnut shell extracts: modulation of cell growth, and cytokinomic and metabolomic profiles. Molecules 21 (10), 1411. https://doi.org/10.3390/molecules21101411.
Tatli, I.I., Akdemir, Z.Ş., 2006. Traditional uses and biological activities of Verbascum species. Fabad J. Pharmaceut. Sci. 31 (2), 85–96.
Tatli, I.I., Akdemir, Z.S., Yesilada, E., Kupeli, E., 2008. Anti-inflammatory and antinociceptive potential of major phenolics from *Verbascum salviifolium* boiss. Zeitschrift fur Naturforschung - Section C J. Biosci. 63 (3–4), 196–202. https://doi.org/10.1515/znc-2008-3-406.
Teame, T., Zhang, Z., Ran, C., Zhang, H., Yang, Y., Ding, Q., Xie, M., Gao, C., Ye, Y., Duan, M., Zhou, Z., 2019. The use of zebrafish (*Danio rerio*) as biomedical models. Anim. Front. 9 (3), 68–77. https://doi.org/10.1093/af/vfz020.
van Doan, H., Hoseinifar, S.H., Hung, T.Q., Lumsangkul, C., Jaturasitha, S., El-Haroun, Ehab, Paolucci, M., 2020. Dietary inclusion of chestnut (*Castanea sativa*) polyphenols to Nile tilapia reared in biofloc technology: impacts on growth, immunity, and disease resistance against Streptococcus agalactiae. Fish Shellfish Immunol. 105, 319–326. https://doi.org/10.1016/j.fsi.2020.07.010.
Vauzour, D., Rodriguez-Mateos, A., Corona, G., Oruna-Concha, M.J., Spencer, J.P.E., 2010. Polyphenols and human health: prevention of disease and mechanisms of action. Nutrients 2 (11), 1106–1131. https://doi.org/10.3390/nu2111106.
von Jørgensen, L.G., 2020. Zebrafish as a model for fish diseases in aquaculture. Pathogens 9 (8), 609. https://doi.org/10.3390/pathogens9080609.
Vukelić, I., Detel, D., Batičić, L., Potočnjak, I., Domitrović, R., 2020. Luteolin ameliorates experimental colitis in mice through ERK-mediated suppression of inflammation, apoptosis and autophagy. Food Chem. Toxicol. 145, 111680 https://doi.org/10.1016/j.fct.2020.111680.
Wang, T., Secombes, C.J., 2013. The cytokine networks of adaptive immunity in fish. Fish Shellfish Immunol. 35 (6), 1703–1718. https://doi.org/10.1016/j.fsi.2013.08.030.
Wang, M., Huang, H., Liu, S., Zhuang, Y., Yang, H., Li, Y., Chen, S., Wang, L., Yin, L., Yao, Y., He, S., 2019. Tannic acid modulates intestinal barrier functions associated with intestinal morphology, antioxidative activity, and intestinal tight junction in a diquat-induced mouse model. RSC Adv. 9 (55), 31988–31998. https://doi.org/10.1039/c9ra04943f.
Xiong, J., Grace, M.H., Esposito, D., Komarnytsky, S., Wang, F., Lila, M.A., 2017. Polyphenols isolated from Acacia mearnsii bark with anti-inflammatory and carbolytic enzyme inhibitory activities. Chin. J. Nat. Med. 15 (11), 816–824. https://doi.org/10.1016/S1875-5364(18)30015-3.
Xu, X., Hua, H., Wang, L., He, P., Zhang, L., Qin, Q., Yu, C., Wang, X., Zhang, G., Liu, Y., 2020. Holly polyphenols alleviate intestinal inflammation and alter microbiota composition in lipopolysaccharide-challenged pigs. Br. J. Nutr. 123 (8), 881–891. https://doi.org/10.1017/S0007114520000082.
Yahfoufi, N., Alsadi, N., Jambi, M., Matar, C., 2018. The immunomodulatory and anti-inflammatory role of polyphenols. Nutrients 10 (11), 1618. https://doi.org/10.3390/nu10111618.
Yang, G., Yu, R., Geng, S., Xiong, L., Yan, Q., Kumar, V., Wen, C., Peng, M., 2021. Apple polyphenols modulates the antioxidant defense response and attenuates inflammatory response concurrent with hepatoprotective effect on grass carp (*Ctenopharyngodon idellus*) fed low fish meal diet. Aquaculture 534, 736284. https://doi.org/10.1016/j.aquaculture.2020.736284.
Yilmaz, S., 2019. Effects of dietary caffeic acid supplement on antioxidant, immunological and liver gene expression responses, and resistance of Nile tilapia, *Oreochromis niloticus* to *Aeromonas veronii*. Fish Shellfish Immunol. 86, 384–392. https://doi.org/10.1016/j.fsi.2018.11.068.
Zhong, L., Hu, Yajun, Hu, Yi, Li, J., Tian, Y., Chen, J., Ai, Q., Xiao, T., 2020. Effects of dietary tea polyphenols on growth, immunity and lipid metabolism of juvenile black carp *Mylopharyngodon piceus*. Aquac. Res. 51 (2), 569–576. https://doi.org/10.1111/are.14403.