



Effect of diet with plant-derived bioactive compounds on physiological and tissular responses in the common carp (*Cyprinus carpio*)

Efecto de la dieta con compuestos bioactivos de origen vegetal en las respuestas fisiológicas y tisulares de la carpa común (*Cyprinus carpio*)

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Abstract

The aim of this study was to investigate some physiological responses to the influence of raw pomegranate peel (*Punica granatum*) (RPP) and alcoholic extract (PPE) on hematological parameters, immunological responses, and antioxidants with its histological examination of common carp (*Cyprinus carpio*) fingerling (13.5 ± 0.1 g). Treatments included two concentrations of RPP and PPE (control, 0.5 and 1 mg·kg⁻¹ of diet) of the commercial diet (~35 protein) for 70 days. At the end of the experiment, all fish were anaesthetized for morphometric measurements and blood sampling. Significant increases in hemoglobin (HGB), hematocrit (Hct), and red blood cells (RBCs) were observed in RP and PPE from the diet ($P < 0.05$). Show up the effectiveness of lysozyme improved significantly in all diets containing PPE compared to the control ($P < 0.05$). An improvement in catalase and glutathione peroxidase (GPx) activity were observed ($P < 0.05$). Histological examination showed a normal liver with lipid vacuoles. In summary, the present study revealed an overall improvement in blood, immune response, antioxidant status and maintenance of liver efficiency of PPE in the diet.

Keywords: antioxidants; phenols; pomegranate peel; common carp.

Resumen

El objetivo de este estudio fue investigar algunas respuestas fisiológicas a la influencia del epicarpio crudo (cáscara) de la granada (*Punica granatum*) (RPP) y su extracto alcohólico (PPE) en parámetros hematológicos, respuestas inmunológicas y antioxidantes, junto con su examen histológico en alevines de carpa común (*Cyprinus carpio*) ($13,5 \pm 0,1$ g). Los tratamientos incluyeron dos concentraciones de RPP y PPE (testigo, 0,5 y 1 mg·kg⁻¹ de dieta) en la dieta comercial (~35% de proteína) durante 70 días. Al final del experimento, todos los peces fueron anestesiados para realizar mediciones morfométricas y toma de muestras de sangre. Se observaron aumentos significativos en la hemoglobina (HGB), hematocritos (Hct) y glóbulos rojos (RBCs) en los peces alimentados con RPP y PPE en la dieta ($P < 0,05$). La efectividad de la lisozima mejoró significativamente en todas las dietas que contenían PPE en comparación con el testigo ($P < 0,05$). Se observó una mejora en la actividad de la catalasa y la glutatión peroxidasa (GPx) ($P < 0,05$). El examen histológico mostró un hígado normal con vacuolas lipídicas. El presente estudio revela una mejora general en la sangre, la respuesta inmune, el estado antioxidante y el mantenimiento de la eficiencia hepática con PPE en la dieta.

Palabras clave: antioxidantes; fenoles; cáscara de granada; carpa común.



Introduction

A variety of environmental stresses have an impact on fish species in the intensive aquaculture sector. When subjected to damaging stimuli including temperature changes, pH shifts, drops in oxygen levels, rises in ammonia levels, handling, transportation, and osmotic changes in water, fish experience stress reactions (Pickering, 1993; Everly and Lating, 2013).

The integrated stress response of the fish consists of behavioral, neurological, hormonal, and physiological components, can affect health status, and reduce its resistance to illnesses and stress, and it takes the fish some time to even return to normal (Lebelo et al., 2001; Suljević et al., 2016). Despite their unfavorable side effects, antibiotics are widely used to treat fish diseases. As a result, researchers have sought out natural alternatives that are more affordable, secure, and effective, such as herbs, vegetables, and other food plants, to use as growth or immune boosters (Badrey et al., 2019).

In this scenario, phytogetic are defined as environmentally friendly plant-derived bioactive compounds used as functional feed additives that show positive effects on animal growth and health. Phytogetic often comprise aromatic plants extracts, and essential oils characterized by its richness in biologically active compounds (Suphoronski, et al., 2019; Christaki et al., 2020). In farmed fish, a wide spectrum of phytogetics has been increasingly studied mainly due to their wide repertoire of properties, including growth promotion, and antimicrobial, immunostimulant, antioxidant, anti-inflammatory and sedative activities (Reverter et al., 2021).

In particular, phytogetics derived from pomegranate peel, products manufactured from pomegranate (*Punica granatum* L.) peel are much more antioxidant-rich than pomegranate juice, making them desirable candidates for use as a dietary supplement in animal feed (Badawi and Gomaa, 2016). However, Türkyılmaz et al. (2013) reported that pomegranate peel also contains flavonoids, phenolic acids, and tannins (ellagitannins such as punicalin, punicalagin, gallic acid and ellagic acid).

This makes pomegranate peel a possible candidate for the discovery of novel natural compounds with varied biological functions, antibacterial action, and potential health advantages (Kaderides et al., 2015). It is well known that pomegranate (*P. granatum*) peel contains phenolic chemicals in quantities roughly 10 times greater than those found in pulp (Li et al., 2006). The antibacterial, antioxidant, anti-inflammatory, antiproliferative, hypolipidemic, and hypoglycemic characteristics of pomegranates have been extensively studied in both edible and inedible sections of the plant, including the peel, seeds, and blossoms (Akhtar et al., 2015; Banihani et al., 2017; Danesi and Ferguson, 2017; Tortora et al., 2017; Bassiri-Jahromi, 2018; Altieri et al., 2019; Hou et al., 2019).

In order to take advantage of their health-promoting qualities, peel byproducts have been targeted as potential natural additives for food preservation and quality enhancement, as well as components of food supplements and nutraceuticals (Akhtar et al., 2015). With this background, pomegranate peel (*P. granatum*) was chosen as the study's subject with the intention of examining its effects on hematological parameters, oxidative state, immunological responses, and liver health of common carp (*Cyprinus carpio*).

Materials and methods

Preparation of pomegranate peel extract

According to the Gülçin et al. (2003) method, the preparation involved mixing 25 mg of powdered pomegranate peel with 250 mL of ethanol (96%), stirring the mixture for 24 hours on a magnetic stirrer, and then filtering the liquid twice through gauze. Once the filtrate was collected using filter paper (Whatmann No. 1), it was concentrated using a rotary evaporator, dried at a temperature of 40 °C, put in sealed opaque bottles, and kept in the refrigerator until needed. The process was then repeated using the same steps and conditions until enough of the extract was obtained.

Determination of total phenolics content

Was determined using the Folin Ciocalteu reagent (Chun et al., 2003). In a nutshell, 0.5 mL of the extract and 0.5 mL of the Folin-Ciocalteu reagent were combined. Before adding two mL of sodium carbonate solution 7.5% and increasing the volume to eight mL with water, the solution was maintained at 25 °C for 5-8 minutes.

The absorbance was determined by spectrophotometer (LKB 4050, England) at 725 nm after two hours of incubation. A calibration curve using gallic acid was realized. The results were expressed in gallic acid equivalents for fresh mass ($\text{mg}\cdot\text{g}^{-1}$). The results were computed using the following formula and expressed as a percentage w/w: $\text{GAE}\times\text{V}\times\text{D}\times10-6\times100/\text{W}$ = total phenolic content (% w/w), where GAE is the gallic acid equivalent ($\mu\text{g}\cdot\text{mL}^{-1}$), V is the total sample volume (mL), D is the dilution factor, and W is the sample weight (g) (Chun et al., 2003).

Determination of total flavonoids content

A colorimetric test was used to determine the total flavonoid concentration (Zhishen et al., 1999). Distilled water (4 mL) of were mixed with 100 microliters of the extract. Then 0.3 mL of sodium nitrite solution at 5% was added. Aluminum chloride (0.3 mL of 10%) was added after five minutes. Sodium hydroxide (2 mL of one M) was added to the mixture in six minutes. Distilled water

(3.3 mL) was added right away, and the liquid was properly mixed after being diluted.

At 510 nm, the absorbance was measured in comparison to a blank. Rutin served as the calibration curve's standard. Rutin equivalents per gram of sample ($\text{mg}\cdot\text{g}^{-1}$) was used to express the total amount of flavonoids in the extract. Ten milligrams of rutin were diluted to 10, 20, 40, 80, or 160 $\mu\text{g}\cdot\text{mL}^{-1}$ after being dissolved in 100 mL of methanol (80%) (Lin et al., 2007). The 0.5 mL diluted standard solutions were combined with 1.5 mL of 95% methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of diluted water in separate batches. Using a spectrophotometer, the absorbance of the reaction mixture was measured at 415 nm following a 30-minute incubation period at room temperature. The same volume of distilled water was used in place of 10% of aluminum chloride in the blank (Zhishen et al., 1999).

Experimental fish

The Aquaculture Department of the College of Agriculture provided the common carp fingerlings used in the research. Fish that were large or extremely stressed were not included. On an electronic scale, each fish was weighed to the nearest 13.51 g while submerged in a bowl of water. Five treatments with three replicates each were applied to the fish (5 fish). Before the experiment began, the fish were given two weeks to get acquainted to the laboratory environment. When the experiment began, the fish were placed in $60 \times 40 \times 50$ cm aquaria, which served as the experimental units.

Experimental procedure

A commercial Iranian diet with known chemical composition was utilized in table 1 as the control (C), and pomegranate peel powder (RPP) and alcohol extracts (PPE) at 0.5% and 1%, respectively, were added to four experimental diets. For 70 days, diets were given to the fish twice daily (at 9 am and 4 pm) at a rate of 2% of their body weight. During the trial period, measurements of the water quality parameters ($\text{pH}= 8.78$, $\text{EC}= 2.71 \text{ ds}\cdot\text{cm}^{-1}$, $\text{DO}= 9.43 \text{ ppm}$, $\text{Temp}= 24.34 \text{ }^{\circ}\text{C}$, $\text{Sal}= 1.30 \text{ psu}$) were made.

Table 1. The proximate chemical composition of the commercial diet used in the experiment.

Nutrition	Amount
Moisture	10%
Crude protein	35%
Ash	12%
Metabolizable energy	3700 Kcal $\cdot\text{kg}^{-1}$
Crude fiber	5.5%
Crude fat	6%
Total volatile nitrogen (TVN)	50 mg $\cdot 100 \text{ g}^{-1}$
Essential amino acids	
Lysine	1.8%
Methionine	0.48%
Threonine	1.15%

Composition: wheat flour, barley, corn gluten, vegetable meal, fish meal, yeast, fish oil, vegetable oil, choline chloride, lysine, methionine, threonine, vitamin premix, special mineral premix, anti-oxidant, inositol.

Hematological analysis

Blood samples were drawn through the heart by a tow ml glass syringe, and the drawn blood was placed in test tubes in two groups, the first group free of anticoagulant, to obtain serum by using centrifuge (Labofuge 400 E, 600 g; Heraeus, Os-terode, Germany) at 3000 rpm for 15 minutes and kept in refrigeration until biochemical analysis. The Mindrarry- BC-30S hematology analyzer was used to measure the hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells (RBC) and white blood cells (WBC).

Immune responses and antioxidant activity

Lysozyme activity was analyzed based on turbidity measurements. In this test, serum (10 μL) was added in cuvettes to 200 μL of *Micrococcus* suspension (35 mg of *Micrococcus* dry powder in 95 mL of 0.15 M phosphate buffer + 5.0 mL of NaCl solution). The change in extinction was measured immediately at 546 nm at the beginning of the reaction and after a 20-minute incubation at 40 $^{\circ}\text{C}$. Lysozyme content was determined based on the calibration curve and the extinction was measured according to Schäperclaus et al. (1992). Catalase, and glutathione peroxidase, activities were determined by using commercial kits (Cayman 707002 Catalase assay kit, and Cayman 703102 glutathione peroxidase assay kit, respectively).

Histopathological analysis

Two fish from each aquarium were randomly selected and sacrificed ($n=$ six per treatment). The head and tail of each fish were removed, and the viscera and liver were dissected and stored for 48 hours in 10% neutral buffered formalin (Thermo Fisher, Kalamazoo, MI). The liver samples were fixed in formalin for 48 hours. Then samples were washed and dried in successive grades of ethyl alcohol. After then the samples were routinely treated to obtain four μm thick paraffin slices using a Cambridge Rocking microtome. All tissues were sectioned longitudinally. For the microscopic analysis, hematoxylin and eosin stains (H&E) were used to stain the slices (Bancroft and Layton, 2013).

Statistical analysis

Data were presented as mean $\pm$ SD. The results were subjected to one-way analysis of variance (ANOVA) to test the effect of treatment inclusion on fish performance. Data were analyzed using IBM SPSS (2013) program, Version 22. Differences between means were compared using LSD's multiple range tests at $P<0.05$ level.

Results and discussion

The health benefits of flavonoids have been subjected by recent studies, and their potent antioxidant effects were proven (Orak et al., 2012). As can be seen in table 2, higher antioxidant activity and phenolic contents were found in pomegranate peel. Based on previous studies that report antioxidant activity and total phenolic and flavonoid content, the peel extracts are more potent



(nearly tenfold) than the pulps, indicating that peel extract has more potentially effective compounds (Ardekani et al., 2011).

The antioxidant results were consistent with those from studies by Nuamsetti et al. (2012) and Badawi and Gomaa (2016), which found that the alcohol extract contained phenols ($185 \text{ mg} \cdot \text{mg}^{-1} \text{ GAE} \cdot \text{g}^{-1}$) and flavonoids ($32 \text{ mg RE} \cdot \text{g}^{-1}$), as well as a total of $166.83 \text{ mg GAE} \cdot \text{g}^{-1}$, $152.6 \text{ mg GAE} \cdot \text{g}^{-1}$, and $85.48 \text{ mg GAE} \cdot \text{g}^{-1}$ of phenols, respectively. Hence, if we compare this plant's antioxidant content to that of many other plants, it is high. Although, differences between antioxidant content in other studies it must be considered that variations in the total phenolic contents of a pomegranate can be influenced by the solvent used for extraction (Ambigaipalan et al., 2016).

Table 2. Total phenolic and flavonoid contents of pomegranate peel extract (n= 3).

	Total phenolic (mg GAE·g ⁻¹)*	Total flavonoid (mg RE·g ⁻¹)**
Alcoholic extract	175.95±1.41	42.89±1.30

*(GAE)·g⁻¹ -mg gallic acid equivalents ** (RE)·g⁻¹ -mg rutin equivalents.

Lysozyme enzyme showed an increase in all treatments (figure 1). Badawi and Gomaa (2016) explained that lysozyme is one of the non-specialized agents that are considered the first line against pathogens, and the study indicated that adding PPE increased lysozyme levels, and the same result was indicated by Monir et al. (2020), where they noted improvement in lysozyme levels in serum of Nile tilapia (*Oreochromis niloticus*) after adding moringa (*Moringa oleifera*) leaves extract and PPE. The results of the study by Harikrishnan et al. (2012) showed an increase in lysozyme levels after adding PPE to Olive flounder (*Paralichthys olivaceus*) diets.

Hamed and Abdel-Tawwab (2021) confirmed that lysozyme levels increased in Nile tilapia fed on diets containing RPP. The study of Sönmez et al. (2022) showed a continuous increase in lysozyme activity during the 40 days of the experiment in Rainbow Trout (*Oncorhynchus mykiss*). However, the present results agree with the study of Badrey et al. (2019). Nevertheless, these results may be related to the chemical components of pomegranate peels and extracts such as flavonoids, calutanine, and ellagic acid derivatives (Dahham et al., 2010).

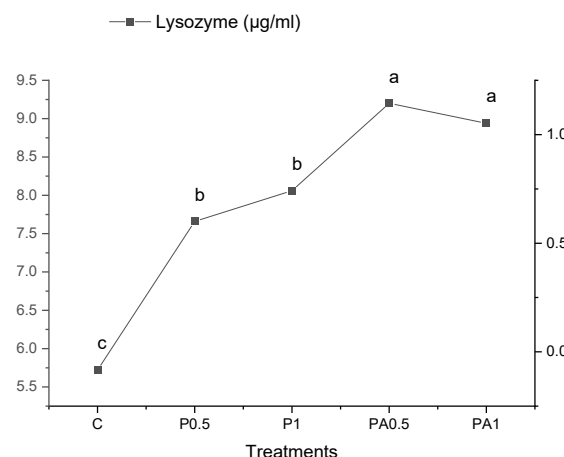


Figure 1. Lysozyme activity in serum of common carp (*Cyprinus carpio*) fed with RPP and PPE. Values are presented as mean±SD. Different letters above line express significant differences between treatments (P<0.05). C: Control, P0.5: Pomegranate peel powder 0.5%, Pomegranate peel powder 1%, PA0.5: Alcohol extracts 0.5%, PA1: Alcohol extracts 1%.

Both antioxidant activities (Catalase and Gpx) were found to be significantly increased (P<0.05) in the serum blood of fed common carp meals containing different concentrations of RPP and PPE presented in figure 2. Antioxidant activities (Catalase and Gpx) were found to be significantly increased (P<0.05) in the serum blood of fed common carp meals containing different concentrations of RPP and PPE are presented in figure 2.

However, the increase of Catalase may be attributed to the presence of some phenolic compounds in pomegranate peels such as protocatechuic acid, gallic acid, pyrogallol, p-coumaric acid, catechine, rosmarinic acid, rutin, naringeen, myrcetin scoplatin, and hisperdin, which have antioxidant activities through reducing the stress caused by the generation of free radicals and lipid peroxidation (Mashkor and Muhson, 2014).

Also, phenolic compounds enhance GPx enzyme activity (Moskaug et al., 2005). PPE may be an important factor in protecting tissues from oxidative damage by increasing the removal of free radicals by Catalase and GPx enzymes (Abdel-Moneim, 2012).

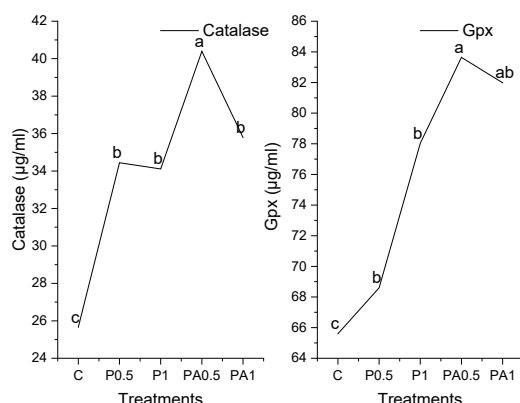
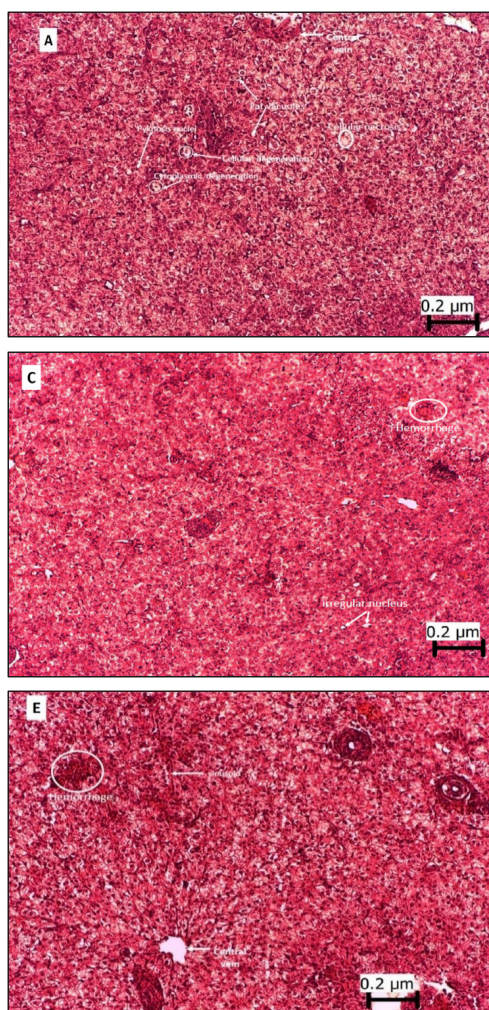


Figure 2. Catalase and GPx activity in serum of common carp (*Cyprinus carpio*) fed with RPP and PPE. Values are presented as mean±SD. Different letters above lines express significant differences between groups ($P<0.05$). C: Control, P0.5: Pomegranate peel powder 0.5%, Pomegranate peel powder 1%, PA0.5: Alcohol extracts 0.5%, PA1: Alcohol extracts 1%.



Histological studies are very important to assess the health status of fish; through it, the pathological effects that can be caused by nutrients are evaluated, in addition to understanding the mechanisms of digestion, metabolism, and monitoring the health status of fish. In general, the shape and structure of the alimentary canal in fish are closely related to fish food and feeding habits (Banan, 2012; Bonvini, 2017).

The results from the conducted histological study are presented in figure 3 for common carp that were fed RPP and PPE supplements. In the control treatment, we observed in figure 3A vacuolization in cells and a kind of necrosis and, additionally, degeneration in the cytoplasm. The liver histological with RPP and PPE showed a relatively normal liver morphology appearance, and the hepatic structure was characterized by compactly arranged hepatocytes disposed in a simple layer aligned with sinusoids. The parenchyma itself was primarily composed of polyhedral hepatocytes, typically with central nuclei, densely stained chromatin margins, and a prominent nucleolus. We also observed the pancreatic mass, which was situated around the branches of the hepatic portal veins.

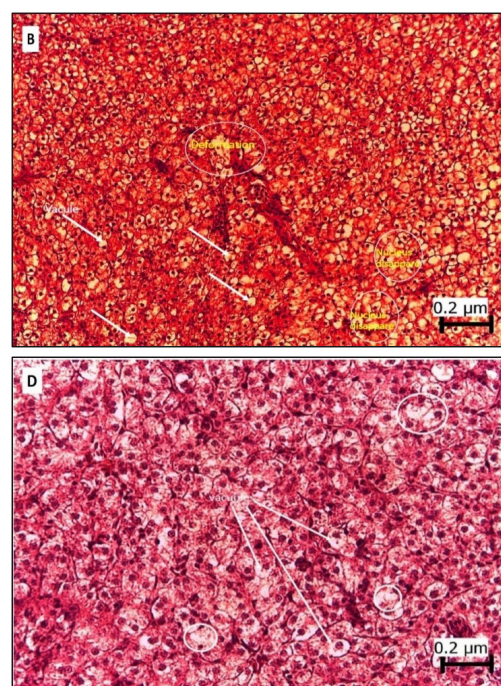


Figure 3. Photomicrographs of the liver of common carp fed RPP (P0.5%, P1%), PPE (PA0.5%, PA1%), and control treatment (c). (A) Degeneration and necrosis of hepatocytes (white arrows) and fat vacuoles. (B) fat vacuoles (white arrows), and small number of peripherally displaced, deformation (white circle). (C) Hemorrhage (white circle) and irregular nucleus (white arrows). (D) and (E) fat vacuoles (white arrows). H&E; bar= 20 µm.

Figure 3B displayed vacuolation and the nucleus disappeared. While, normal histological appearance with lipid accumulation in the cytoplasm of hepatocytes could be noticed in figures C, D, and E. Overall, it was discovered that the addition of RPP and PPE improved the liver's normal tissue. The development of lipid vesicles during alcohol treatments is depicted in figure 3. Perhaps, lipids and glycogen, which are related to the normal metabolic operation of the liver, may be present in vacuoles in the cytoplasm of hepatocytes.

The creation of vacuoles in the liver indicates an imbalance between the pace at which chemicals are synthesized in parenchyma cells and their rate of release into the circulation. Hepatocytes can produce vacuoles as a sort of cellular protection against dangerous compounds, and this defense mechanism may be in charge of gathering toxic molecules and keeping them from interfering with this cell's basic functions (Ayadi et al., 2015).

Moreover, the morphometric assessment of the liver revealed that in P0.5%, a small number of peripherally displaced nuclei were observed. According to Caballero et al. (1999) enlarged nuclei of hepatocytes and nuclear displacement to the hepatocyte periphery, depending on the experimental conditions, are considered a pathological situation or might be regarded as an adaptive mechanism of hepatocytes. In the presented study, the morphology of hepatocytes was similar to the analyzed carp diets.

Therefore, the observed hepatic morphology reflected a well-fed status rather than a pathological situation. The present findings are due to the active compounds in pomegranate peels, reported similar results by Hussein et al. (2022).

Conclusion

The outcomes of the current study demonstrated that including pomegranate peel alcohol extract in the diet increased blood parameters, immunity, antioxidants, and liver safety. More research should be done to figure out the optimal ratios for fish feed in order to improve the resistance and health of fish farmed using phytochemicals rather than using antibiotics.

Conflict of interest

The authors declare that they have no conflicts of interest in this publication in any of its phases.

Bibliographic references

Abdel-Moneim, A. E. (2012). Evaluating the potential role of pomegranate peel in aluminum-induced oxidative stress

and histopathological alterations in brain of female rats. *Biological Trace Element Research*, 150(1), 328-336.

Akhtar, S., Ismail, T., Fraternali, D. and Sestili, P. (2015). Pomegranate peel and peel extracts: Chemistry and food features. *Food Chemistry*, 174, 417-425.

Altieri, F., Cairone, F., Giamogante, F., Carradori, S., Locatelli, M., Chichiarelli, S. and Cesa, S. (2019). Influence of ellagitannins extracted by pomegranate fruit on disulfide isomerase PDIA3 activity. *Nutrients*, 11(1), 186. <https://doi.org/10.3390/nu11010186>

Ambigaipalan, P., De Camargo, A. C. and Shahidi F. (2016). Phenolic compounds of pomegranate byproducts (outer skin, mesocarp, divider membrane) and their antioxidant activities. *Journal of Agricultural and Food Chemistry*, 64(34), 6584-6604. <https://doi.org/10.1021/acs.jafc.6b02950>

Ardekani, M. R. S., Hajimahmoodi, M., Oveisi, M. R., Sadeghi, N., Jannat, B., Ranjbar, A. M., Gholam, N. and Moridi, T. (2011). Comparative antioxidant activity and total flavonoid content of persian pomegranate (*Punica granatum* L.) cultivars. *Iranian Journal of Pharmaceutical Research*, 10(3), 519. <https://doi.org/10.22037/ijpr.2011.948>

Ayadi, I., Monteiro, S. M., Regaya, I., Coimbra, A., Fernandes, F., Oliveira, M. M., Peixoto, F. and Mnif, W. (2015). Biochemical and histological changes in the liver and gills of Nile tilapia *Oreochromis niloticus* exposed to Red 195 dye. *RSC Advances*, 5(106), 87168-87178. <https://doi.org/10.1039/C5RA13127H>

Badawi, M. E. and Gomaa, A. M. (2016). Influence of diets supplemented with pomegranate peel extract on performance in *Oreochromis niloticus*. *Japanese Journal of Veterinary Research*, 64 (Supplement 2), S87-S94. <http://hdl.handle.net/2115/62021>

Badrey, A. A., Osman, A., Farrag, S. M., Toutou, M. M. M. and Moustafa, M. (2019). Influences of diets supplemented with pomegranate peel on haematology, blood biochemistry and immune status in monosex Nile tilapia, *Oreochromis niloticus*. *Egyptian Journal of Aquatic Biology and Fisheries*, 23(2), 133-144. <https://dx.doi.org/10.21608/ejabf.2019.33559>

Banan Khojasteh, S. M. (2012). The morphology of the post-gastric alimentary canal in teleost fishes: a brief review. *International Journal of Aquatic Science*, 3(2), 71-88.

- Bonvini, E. (2017). Development of innovative feed products and feed concepts for marine species in aquaculture. Ph.D. Thesis. University of Bologna. 100 p.
- Bancroft, J. D. and Layton, C. (2013). The hematoxylin and eosin. pp. 172-214. In: Suvarna, S. K., Layton, C. and Bancroft, J. D. (Eds.). *Theory and practice of histological techniques*. 7th Ed., Churchill Livingstone of El Sevier, Philadelphia, Ch. 10 and 11.
- Banihani, S., Swedan, S. and Alguraan, Z. (2013). Pomegranate and type 2 diabetes. *Nutrition Research*, 33(5), 341-348. <https://doi.org/10.1016/j.nutres.2013.03.003>
- Bassiri-Jahromi, S. (2018). *Punica granatum* (Pomegranate) activity in health promotion and cancer prevention. *Oncology Reviews*, 12(1), 345,351. <https://doi.org/10.4081/oncol.2018.345>
- Caballero, M. J., López-Calero, G., Socorro, J., Roo, F. J., Izquierdo, M. S. and Fernández, A. J. (1999). Combined effect of lipid level and fish meal quality on liver histology of gilthead seabream (*Sparus aurata*). *Aquaculture*, 179(1-4), 277-290. [https://doi.org/10.1016/S0044-8486\(99\)00165-9](https://doi.org/10.1016/S0044-8486(99)00165-9)
- Christaki, E., Giannenas, I., Bonos, E. and Florou-Paneri, P. (2020). Innovative uses of aromatic plants as natural supplements in nutrition. pp. 19-34. In: Florou-Paneri, P., Christaki, E. and Giannenas, I. (Eds.). *Feed additives*. Cambridge, Massachusetts. Academic Press.
- Chun, O. K., Kim, D. O., Moon, H. Y., Kang, H. G. and Lee, C. Y. (2003). Contribution of individual polyphenolics to total antioxidant capacity of plums. *Journal of Agricultural and Food Chemistry*, 51(25), 7240-7245. <https://doi.org/10.1021/jf0343579>
- Dahham, S. S., Ali, M. N., Tabassum, H. and Khan, M. (2010). Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L.). *American-Eurasian Journal of Agricultural and Environmental Sciences*, 9(3), 273-281.
- Danesi, F. and Ferguson, L. R. (2017). Could pomegranate juice help in the control of inflammatory diseases?. *Nutrients*, 9(9), 958. <https://doi.org/10.3390/nu9090958>
- Everly, G. S. and Lating, J. M. (2013). The anatomy and physiology of the human stress response. pp. 17-51. In: Everly, G. S. and Lating J. M. (Eds.). *A clinical guide to the treatment of the human stress response*. Springer, New York, NY.
- Gülçin, İ., Oktay, M., Kireççi, E. and Küfrevioğlu, Ö. İ. (2003). Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chem.*, 83(3), 371-382.
- Hamed, H. S. and Abdel-Tawwab, M. (2021). Dietary pomegranate (*Punica granatum*) peel mitigated the adverse effects of silver nanoparticles on the performance, haemato-biochemical, antioxidant, and immune responses of Nile tilapia fingerlings. *Aquaculture*, 540, 736742. <https://doi.org/10.1016/j.aquaculture.2021.736742>
- Harikrishnan, R., Kim, J. S., Kim, M. C., Balasundaram, C. and Heo, M. S. (2012). Pomegranate enriched diet enhances the hematology, innate immune response, and disease resistance in olive flounder against *Philasterides dicentrarchi*. *Veterinary Parasitology*, 187(1-2), 147-156. <https://doi.org/10.1016/j.vetpar.2011.12.006>
- Hou, C., Zhang, W., Li, J., Du, L., Lv, O., Zhao, S. and Li, J. (2019). Beneficial effects of pomegranate on lipid metabolism in metabolic disorders. *Molecular Nutrition and Food Research*, 63(16), 1800773. <https://doi.org/10.1002/mnfr.201800773>
- Hussein, E. E., Fatan, N., El-Betagy, A. E. D. and Naeem, M. A. (2022). Enhancement of the nutritional value, antioxidant properties and growth performance in mono-sex Nile tilapia, *Oreochromis niloticus* fries by pomegranate peel supplementation. *ResearchSquare*. <https://doi.org/10.21203/rs.3.rs-1717922/v1>
- Kaderides, K., Goula, A. M. and Adamopoulos, K. G. (2015). A process for turning pomegranate peels into a valuable food ingredient using ultrasound-assisted extraction and encapsulation. *Innovative Food Science and Emerging Technologies*, 31, 204-215. <https://doi.org/10.1016/j.ifset.2015.08.006>
- Lebelo, S. L., Saunders, D. K. and Crawford, T. G. (2001). Observations on blood viscosity in striped bass, *Morone saxatilis* (Walbaum) associated with fish hatchery conditions. *Kansas Academy of Science*, 104, 183194. [https://doi.org/10.1660/0022-8443\(2001\)104\[0183:OOBVIS\]2.0.CO;2](https://doi.org/10.1660/0022-8443(2001)104[0183:OOBVIS]2.0.CO;2)
- Li, Y., Guo, C., Yang, J., Wei, J., Xu, J. and Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry*, 96(2), 254-260. <https://doi.org/10.1016/j.foodchem.2005.02.033>
- Lin, J. Y. and Tang, C. Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chemistry*, 101(1), 140-147.
- Mashkor, I. M. A. A. and Muhson, A. A. (2014). Total phenol, total flavonoids and antioxidant activity of pomegranate peel. *International Journal of ChemTech Research*, 6(11), 4656-4661.
- Monir, W., Abdel-Rahman, M. A., Hassan, S. E. D. and Awad, S. M. (2020). Pomegranate peel and moringa-based diets enhanced biochemical and immune parameters of Nile tilapia against bacterial infection by *Aeromonas*

- hydrophila*. *Microbial Pathogenesis*, 145, 104202. <https://doi.org/10.1016/j.micpath.2020.104202>
- Moskaug, J. Ø., Carlsen, H., Myhrstad, M. C. and Blomhoff, R. (2005). Polyphenols and glutathione synthesis regulation. *The American Journal of Clinical Nutrition*, 81(1), 277S-283S.
- Nuamsetti, T., Dechayuenyong, P. and Tantipaibulvut, S. (2012). Antibacterial activity of pomegranate fruit peels and arils. *Science Asia*, 38(3), 319-322.
- Orak, H. H., Yagar, H. and Isbilir, S. S. (2012). Comparison of antioxidant activities of juice, peel, and seed of pomegranate (*Punica granatum* L.) and inter-relationships with total phenolic, tannin, anthocyanin, and flavonoid contents. *Food Science and Biotechnology*, 21(2), 373-387. <https://doi.org/10.1007/s10068-012-0049-6>
- Pickering, A.D. (1993). Growth and stress in fish production. In *Genetics in aquaculture*. *Aquaculture*, 111, 51-63. <https://doi.org/10.1016/B978-0-444-81527-9.50010-5>
- Reverter, M., Tapissier-Bontemps, N., Sarter, S., Sasal, P. and Caruso, D. (2021). Moving towards more sustainable aquaculture practices: A meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. *Review in Aquaculture*, 13(1), 537-555. <https://doi.org/10.1111/raq.12485>
- Sönmez, A. Y., Bilen, S., Özdemir, K. Y., Alagöz, K. and Özçelik, H. (2022). Effect of aqueous methanolic extract of pomegranate peel (*Punica granatum*) and veratrum (*Veratrum album*) on oxidative status, immunity and digestive enzyme activity in rainbow trout (*Oncorhynchus mykiss*). *Journal of Agricultural Sciences*, 28(2), 159-170. <https://doi.org/10.15832/ankutbd.870923>
- Suphoronski, S. A., Chideroli, R., Facimoto, C. T., Mainardi, R. M., Souza, F. P. and Lopera-Barrero, N. M. (2019). Effects of a phytogenic, alone and associated with Potassium diformate, on tilapia growth, immunity, gut microbiome and resistance against *Francisella*. *Scientific Reports*, 9(1), 6045. <https://doi.org/10.1038/s41598-019-42480-8>
- Suljević, D., Alijagić, A., Mitrašinić-Brulić, M., Fočak, M. and Islamagić, E. (2016). Comparative physiological assessment of common carp (*Cyprinus carpio*) and crucian carp (*Carassius carassius*) based on electrolyte and hematological analysis. *Macedonian Journal of Animal Science*, 6(2), 95-100. <http://dx.doi.org/10.54865/mjas1662095>
- Tortora, K., Femia, A. P., Romagnoli, A., Sineo, I., Khatib, M., Mulinacci, N., Giovannelli, L. and Caderni, G. (2018). Pomegranate by-products in colorectal cancer chemoprevention: effects in apc-mutated pirc rats and mechanistic studies in vitro and ex vivo. *Molecular Nutrition & Food Research*, 62(2), 1700401. <https://doi.org/10.1002/mnfr.201700401>
- Türkyılmaz, M., Tağı, Ş., Dereli, U. and Özkan, M. (2013). Effects of various pressing programs and yields on the antioxidant activity, antimicrobial activity, phenolic content and colour of pomegranate juices. *Food Chemistry*, 138(2-3): 1810-1818. <https://doi.org/10.1016/j.foodchem.2012.11.100>
- Zhishen, J., Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555-559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)

Author's contribution statement according to the CRediT classification

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