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DIETARY ARTICHOKE (*CYNARA SCOLYMUS*) EXTRACT AMELIORATED THE GROWTH PERFORMANCE, HUMORAL IMMUNE PARAMETERS AND RESISTANCE AGAINST *AEROMONAS HYDROPHILA* IN GOLDFISH (*CARASSIUS AURATUS*)

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Abstract

This trial investigated the efficacy of artichoke (*Cynara scolymus*) extract (AE) on the growth performance, immunity, antioxidant parameters, and resistance against *Aeromonas hydrophila* of goldfish (*Carassius auratus*). For this purpose, a total number of 470 goldfish with initial weight 5.70±0.2 g were fed with four experimental diets including 0 (T0), 100 (T1), 150 (T2), and 200 (T3) mg kg^{-1} diet AE for 8 weeks. At the end of feeding trial, growth performances, serum immune parameters, and mucus antioxidant enzymes were measured. Fish were challenged with *A. hydrophila*, and the antioxidant and immunity-related gene expression were investigated. Based on the results, the highest final weight (FW) and weight gain (WG) were attained in T2 and T3 (P<0.05). Immune factors including ACH50, lysozyme, and total immunoglobulin in T2 and T3 showed the highest values (P<0.05). The expression of *GR*, *IL1β*, *TNFα*, *HSC70*, *HSP70*, and *HSP90β* genes in T1, T2, and T3 were higher than the control (P<0.05). The *GST* expression was significantly enhanced in T2 (P<0.05). The present study demonstrated that the administration of AE, especially at doses of 150 mg kg^{-1}, could improve the growth, immunity, and antioxidant parameters, as well as enhance disease resistance against *A. hydrophila* in goldfish.

Key word: artichoke extract, goldfish, immunity, antioxidative status, *Aeromonas hydrophila*

Aquaculture has become one of the most sustainable tactics to extend depleted aquatic and endangered species and terminate the gap between stock and demand for aquatic protein (Mourad et al., 2022; Hussein et al., 2023). Aquaculture efficiency is altered by several interconnected factors, including the aquatic environment, diet, and the farmed stock (El Basuini et al., 2022) and boosting these aspects is the base of sustainable aquaculture.

Goldfish (*Carassius auratus*) is one of the most popular aquarium fishes belonging to the Cyprinidae family, traded in nearly 100 countries (Trujillo-González et al., 2018). Recently, the global demand for aquarium fish has been increasing, resulting in significant growth of the import and export trade market. Ornamental fish farming success is highly dependent on a health promoting diet, necessary to provide all the essential nutrients for fish growth and health (Anuar et al., 2017). Although chemical-based treatments for the improvement of fish health are available, many of them have been criticized for potentially negative side effects such as drug-resistant bacteria development, water contamination, and the presence of chemical residues in the muscle (Rashmeei et al., 2020). Phytocompounds could be useful alternatives to chemicals, due to their unique properties, including growth and health promotion, disease resistance and appetite enhancement (Lillehoj et al., 2018; Vijayaram et al., 2024). Phytochemicals present in fruits and vegetables have been considered of significant nutritional importance in fish farming (Kari et al., 2022; Kuebutornye and Abarike, 2020; Tadese et al., 2022). Therefore, from a preventative perspective, a constant supply of phytochemicals could ameliorate defense mechanisms with general health condition improvement and consequent reduction of the risk of diseases in fish.

In recent years, with the growing interest in natural antioxidants, artichoke (*Cynara scolymus*), with its high phenolic compounds content, is gaining renewed inter-

est (Miccadei et al., 2008). Artichokes are popular vegetable food in many different countries, including the USA, South America, North Africa, and Asia (Frutos et al., 2019). Totally, artichokes are produced at a rate of 1,793,015 tons per year, with over 1,075,800 tons produced in Europe (El-Hadidy et al., 2022). This vegetable is rich in health-promoting soluble and insoluble fibers (Quintero Ruiz et al., 2021) and is one of the ideal suppliers of dietary antioxidants (Halvorsen et al., 2006). Artichoke is a main source of phenolic acids, flavonoid derivatives, and xanthophylls (Gouveia and Castilho, 2012). There are various studies reporting the health-beneficial effects deriving from artichoke consumption. It is indeed capable of exerting anti-hypercholesterolemic, prebiotic, anti-photoaging, anti-obesity, anti-ulcerogenic, antifungal, anti-inflammatory, hepatoprotective**,** anti-cancer, and anti-metabolic syndrome effects (El-Hadidy et al., 2022; Frutos et al., 2019; Miraj and Kiani, 2016; Sharma et al., 2021). Despite its importance on human health, no efforts have been made to test artichoke in the aquaculture industry.

Goldfish is an important model organism used for determining the effects of different toxicants (Acar et al., 2023), ambient water conditions (Yousefi et al., 2023) and/or feed additives (Zemheri-Navruz et al., 2019; Kesbiç et al., 2020). Therefore, the purpose of this study was to appraise the impacts exerted by artichoke extract, its beneficial effects on growth, immunity, and antioxidant parameters as well as its resistance against *Aeromonas hydrophila*.

Material and methods

Chemicals

Methanol, Water Chromasolv® Plus, chlorogenic acid, caffeic acid, cynarin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Commercial artichoke extract (AE) was supplied by EPO (Istituto Farmochimico Fitoterapico S.r.l., Via Stadera 19 – 20141 Milano, Italy). It consisted of *Cynara scolymus* L. leaf extract in powdered form. According to the producer's indications *Cynara scolymus* was cultivated in Western Europe and harvested in autumn, before flowering. The extraction was carried out with water and then dried. The particle size was 300 microns. Nutritional values were: carbohydrates: 90–95%; fat: 0–1%; protein: 0%; minerals: 5–7%. Energy value (Kcal/100 gr) was 405. Total content of polyphenols was 5%.

Attenuated Total Reflectance–Fourier Transformed Infrared (ATR-FTIR) analysis of artichoke extract

AE composition was analyzed by the ATR-FTIR. In brief, powdered samples of AE were analyzed by means of a spectrophotometer (Perkin-Elmer, USA), supplied with a DTGS (deuterated triglycine sulfate) detector. Untreated samples (about 10 mg) were accommodated on the germanium crystal and pressed with 70±2 psi. The ATR-FTIR spectrum was recorded in the mid-IR region (4000–650 cm^{-1}) at resolutions of 4 cm^{-1}. 32 scans were run for each sample. Samples were analyzed in triplicate.

High pressure liquid chromatography (HPLC) of artichoke extract

AE powder (10 mg) was mixed with methanol/water (80:20 v/v) (1 mL) and filtered by a 0.45 µm membrane filter (Millipore Corp.). 20 µl of extract were analyzed by an HPLC (LC-4000; JASCO, Japan) supplied with a pump (PU-2829 plus), an autosampler (AS-2059 plus), a column oven (CO-2060 plus), a UV/Vis photodiode array detector (MD-2818 plus), and operated by a ChromNAV software (JASCO, Japan). The column was a C18 Luna column 5-µm particle size, 25 cm × 3.00 mm I.D (Phenomenex, Torrance, CA, USA) with a guard cartridge of similar material. The running conditions were after Llorach et al. (2002). Briefly, the binary mobile phase was made of 5% formic acid and methanol. The flow rate was 0.8 mL min^{-1}. The linear gradient started with 5% methanol and 95% formic acid to reach 20% methanol and 80% formic acid at 5 min; 25% methanol and 75% formic acid at 50 min; 30% methanol and 70% formic acid at 60 min; and 80% methanol and 20% formic acid at 62 min. Chromatogram was registered in the range 200–650 nm.

Diets

A basal diet (Table 1) was supplemented with different concentration of AE as follows: 0 mg kg^{-1} AE (T0), 100 mg kg^{-1} AE (T1), 150 mg kg^{-1} AE (T2), and 200 mg kg^{-1} AE (T3). The diet ingredients were softly blended together without and with the different concentrations of AE, and water (30–40%) was combined. The dough was pelleted in an electric meat grinder and the feed was broken down into pellets according to the size of the fish's mouth. The pellets were air-dried and stored at –20°C until use.

Animals

All animal manipulations and handling were approved by the Ethical Committee for Animal Experiments of the Faculty of Veterinary Medicine, Semnan University. 480 healthy goldfish (initial weight 5.70±0.2 g) were provided by Technical and Vocational Center located in Gorgan, Iran. The fish were distributed into 12 tanks (500 L, 40 fish per tank) and allowed to adapt to the rearing conditions for two weeks. The basal diet (without AE) was provided twice a day during the initial two weeks. After two weeks, the tanks were allocated to the four experimental treatments with three replicates. During the feeding trial, hand feeding was executed *ad libitum* three times a day. Uneaten feed was collected one hour after each feeding time, dried at 60°C, and used in the calculations for feed intake, feed conversion ratio (FCR). The temperature and dissolved oxygen were measured by a mercury thermometer (Zomorodazma Company, Iran) and Cyberscan Eutech instruments (DO 110, Singapore).

Table 1. Dietary formulation and proximate composition of the control diet

Ingredients	%
Fish meal	40
Fish oil	6
Wheat flour	21
Soybean meal	13.5
Gluten	5.5
Soybean oil	6
Vitamin premix[a]	2
Mineral premix[a]	3
Binder[b]	2
Antifungal[c]	0.5
Antioxidant[d]	0.5
Chemical composition	
crude protein (%)	36.81
dry matter (%)	91.12
crude lipid (%)	11.33
ash (%)	3.5
fiber	11.58
NFE[e]	27.90
energy (kJ kg^{-1})[f]	17.90

[a]Premix detailed by Azarin et al. (2015).
[b]Amet binder™, Mehr Taban-e-Yazd, Iran.
[c]ToxiBan antifungal (Vet-A-Mix, Shenandoah, IA, USA).
[d]Butylated hydroxytoluene (Merck, Germany).
[e]Nitrogen-free extracts (NFE) = dry matter − (crude protein + crude lipid + ash + fiber).
[f]Gross energy (kJ kg^{-1}) calculated according to 23.6 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid, and 17.0 kJ g^{-1} for NFE (Brett, 1979).

Water quality parameters were kept as follows: temperature 20.15±0.4°C; dissolved oxygen 8.2±0.8 mg/L. Photoperiod was 12D:12L.

Growth efficiency

After eight weeks, feeding was stopped and fish were sacrificed after 24 hours. Benzocaine (120 mg L^{-1}) was used as an anesthetic (Mehdinejad et al., 2019). The fish were individually weighed. Growth parameters, namely weight gain, specific growth rate (SGR), feed conversion ratio (FCR), as well as survival rate were figured using standard formulas.

WG: W*eight gain (g) = Final weight (g) – Initial weight (g);*

SGR: Specific growth rate = 100 (Ln final weight − Ln initial weight) / the total experimental period days (56);

FI: Feed intake (g feed/fish) = the total feed amount given to each tank / fish number;

FCR: Feed conversion ratio = Dry feed consumed (g) / weight gain (g);

Survival rate (%) = 100 (No. of fish at the end / No. of fish at the start.

Blood sampling

Six fish per treatment were selected at random for blood sampling. Blood samples were placed in tubes and centrifuged at 1600×g, for 10 min. Thereafter, the serum was taken out of the tube and saved at –70°C for further investigation.

Serum immune response

The turbidimetric method was applied to detect lysozyme activity as reported in Ellis (1990), with slight modifications. The method of Yano et al. (1988) was employed to measure serum alternative complement (ACH50) activity. Total immunoglobulin content was determined according to Siwicki (Siwicki and Anderson, 1993) protocol using PEG (polyethylene glycol) precipitation.

Challenging test with *Aeromonas hydrophila*

A. hydrophila (ATCC 7966) was purchased from the Iranian Biological Resource Center (Tehran, Iran). The bacteria were cultured at 25°C overnight and then centrifuged (3000 × g for 15 min). The upper layer was removed and the cell pellet was cleaned with phosphate saline buffer (PBS, 0.1 M, pH 7.2) three times by centrifugation at 3000 × g for 15 min. Finally, the cell pellet was suspended in PBS. The bacteria concentration was measured with a spectrophotometer at the wavelength of 540 nm and adjusted to 10^8 CFU. After the eight-week feeding trial, 20 fish per tank (60 fish per treatment) were taken and injected with 0.1 mL of bacterium suspension at 10^8 CFU per fish (Yousefi et al., 2022). The dead fish were taken out and the mortality rate (%) was recorded after 14 days by dividing the number of dead fish by the total number of fish.

Tissue sampling and qPCR

After the challenging test five fish/treatment were randomly captured and anesthetized. The intestines were dissected, rapidly frozen in liquid nitrogen, and conserved at –80°C. Commercial kits were purchased to extract total RNA and synthesize complementary DNA (cDNA). Real-time PCR analysis was realized for the target genes indicated in Table 2 with GAPDH gene as the housekeeping gene. Our pre-works showed that more stable and appropriate reference gene arrangement was found as GAPDH> EF1AA> β-actin> 18S rRNA to be considered for intestines. PCR amplification of genes was conducted, and the RNA level was calculated based on the $2^{-\Delta\Delta CT}$ formulae.

Statistical analysis

All data were analyzed using SPSS version 22 software at a 95% confidence level. First, the normality and homogeneity of the variance were checked using Kolmogorov-Smirnov test and Levene's test respectively. After both conditions were satisfied, the one-way analysis of variance (ANOVA) was carried out, Tukey's test was chosen to evaluate the differences between treatments. All data were reported as mean ± SE. Survival rates in the challenge test were performed using the Kaplan–Meier survival analyses and a log-rank test at the 0.05 significance level.

Table 2. The list of selected genes and their primers

Gene	Primer	Length (pb)	Tm (°C)	NCBI Reference
GR	F: 5' AAGCAGTCTGTCTCAATGG 3' R: 5' CTTCACTGTCAATCCCATCC 3'	19 20	54.80 55.81	XM_026280896.1
GST	F: 5' GACTTTCAAGCACGGAGACA 3' R: 5' CAAACATTCGCTGGTAGACG 3'	20 20	58.13 57.21	XM_026232308.1
IL1β	F: 5' CTCAGTAACTCCAGACAGGG 3' R: 5' ATCAAAGCCAGCACTGTCAG 3'	20 20	56.66 58.47	XM_026274667.1
TNFα	F: 5' CGAGCTGAGGAGACTTTACA 3' R: 5' GTCTATTAGTCCTCGCAAAGC 3'	20 21	56.69 56.53	XM_026256406.1
HSC70	F: 5' GGCAAGATGTCAAAAGGTCC 3' R: 5' TCCTGTTCCCCTGGTCATT 3'	20 19	56.98 57.80	XM_026282033.1
HSP70	F: 5' C ACACGACTGGTCCATTTCTG 3' R: 5' GCTTAATTGCGGTTCCTTGT 3'	20 20	57.83 56.99	XM_026208615.1
HSP90β	F: 5' GTTCTCTCCTCTCTGCTTCC 3' R: 5' CGCATTTCTTCAGGCATCTTG 3'	20 21	57.04 58.21	XM_026241276.1
GAPDH	F: 5' TGGAAAGTACAAGGGTGAGG 3' R: 5' CACCAGTAGACTCCACGACA 3'	20 20	50.00 55.00	XM_026284269.1

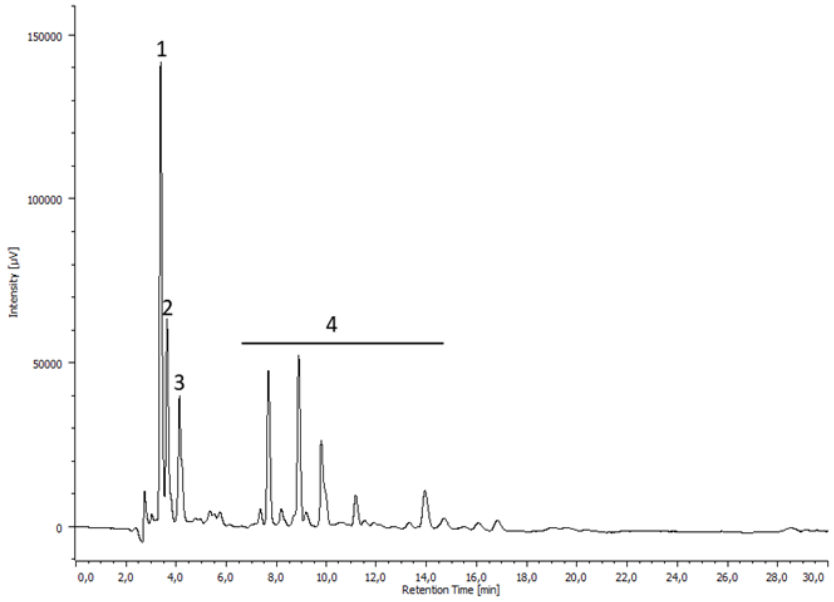


Figure 1. HPLC 1 chromatograms of artichoke (*Cynara scolymus*) extract. Peaks: (1) chlorogenic acid; (2) caffeic acid; (3) cynarin; (4) caffeic acid derivatives. Sample wavelength reading 280 nm

Results

ATR-FTIR analysis

Figure 1 illustrates the ATR-FTIR profile of the AE. Table 3 reports the peak identification, frequency assignments and the correspondent functional groups. The peak assignment is established on the data available in the literature (Abbas et al., 2017; Quintero Ruiz et al., 2021). The peaks arise from the vibrations of carbohydrates and phenolic compounds functional groups, except for the peak (2) at 2929 cm^{-1}, related to CH_2 stretching of the aliphatic hydrocarbons present in lipids. The broad peak (1) at 3290 cm^{-1} is related to the OH groups of the carboxylic acids (Socrates, 2004). The peaks (3–8) between the wavelengths of 1800–750 cm^{-1} are due to the biochemical composition of plants, given by carbohydrates, lipids, proteins and polyphenols.

HPLC analysis

The profile of the phenolic compounds of the AE determined by HPLC is reported in Figure 2. By comparison with the literature data and internal standards the peaks could be attributed to chlorogenic acid, caffeic acid and derivatives, and cynarin. The overlapping of the HPLC profiles acquired at 280 nm (phenolic acids) and 320 nm (flavonoids) reported in Figure 3 indicates that the phenolic compounds can be attributed mainly to phenolic acids.

Table 3. Functional groups and frequency assignments for artichoke (*Cynara scolymus*) extract from attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). Assignments are according to the literature data (Lu et al., 2011; Abbas et al., 2017; Quintero Ruiz et al., 2021)

Peak number	Wavelength (cm^{-1})	Assignment
1	3290	OH stretching; aromatic CH stretching
2	2929	CH2 asymmetric stretch of lipids
3	1580	Aromatic ring C=C stretching
4	1395	C–O stretching;
5	1280	OH deformation
6	1150	Saturated C-C chains stretching
7	1080	C-O and C-C stretching
8	1019	C-O C-C C-O-H of polysaccharides

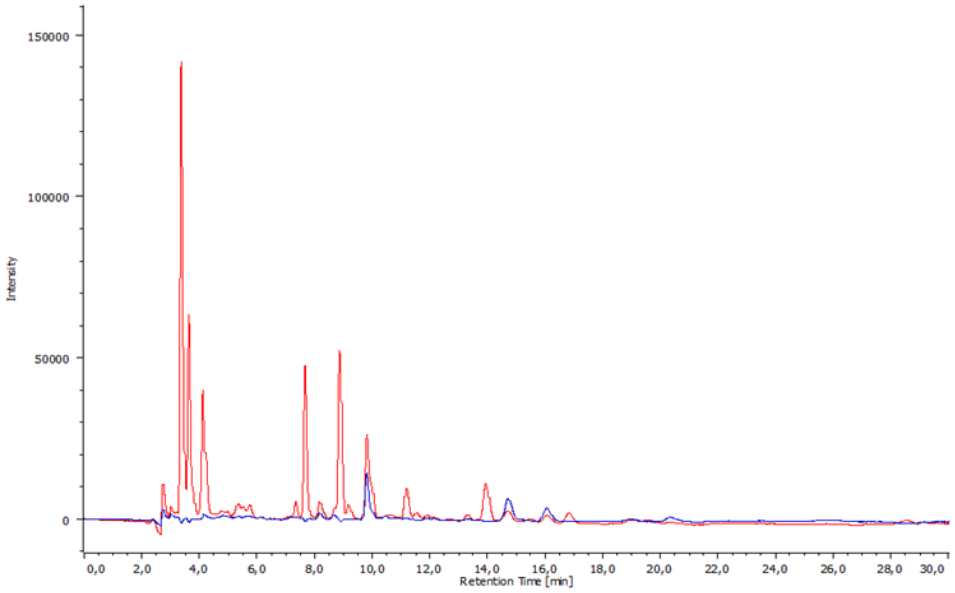


Figure 2. HPLC 2 overlapping of HPLC chromatograms of artichoke (*Cynara scolymus*) extract read at 280 nm (red line) and at 320 nm (blue line)

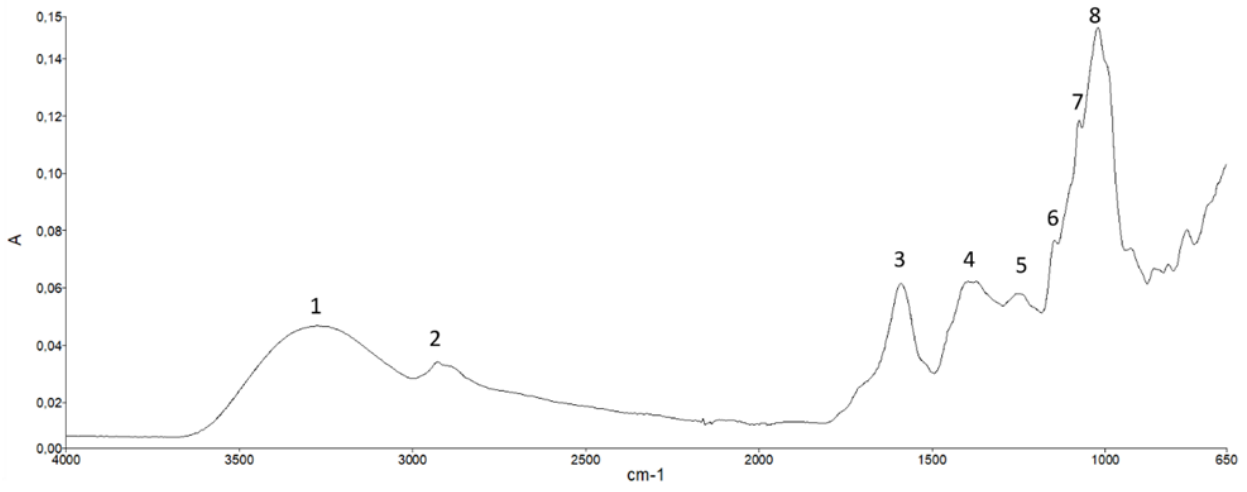


Figure 3. ATR-FTIR absorption spectra of artichoke (*Cynara scolymus*) extract in the wavelength 4000–650 cm^{-1} (on the X-axis). Absorbance is reported on the Y-axis

Table 4. Growth performance of goldfish (*Carassius auratus*) fed with 0 (T0), 100 (T1), 150 (T2), and 200 (T3) mg kg^{-1} artichoke (*Cynara scolymus*) extract for eight weeks

Treatment groups	Initial weight (g)	Final weight (g)	Weight gain (g)	FCR	SGR (%/day)	Survival rate %
T0	5.76±0.13 a	10.84±0.27 a	5.08±0.24 a	2.97±0.14 b	1.13±0.04 a	100
T1	5.74±0.12 a	11.05±0.35 ab	5.31±0.41 a	2.85±0.21 ab	1.17±0.08 a	100
T2	5.70±0.18 a	12.54±0.24 c	6.84±0.16 b	2.20±0.05 a	1.41±0.04 a	100
T3	5.79±0.21 a	12.19±0.15 bc	6.40±0.35 ab	2.36±0.05 ab	1.33±0.09 a	100

Different letters designate significant differences as determined by Tukey's post-hoc tests (Mean ± SE).

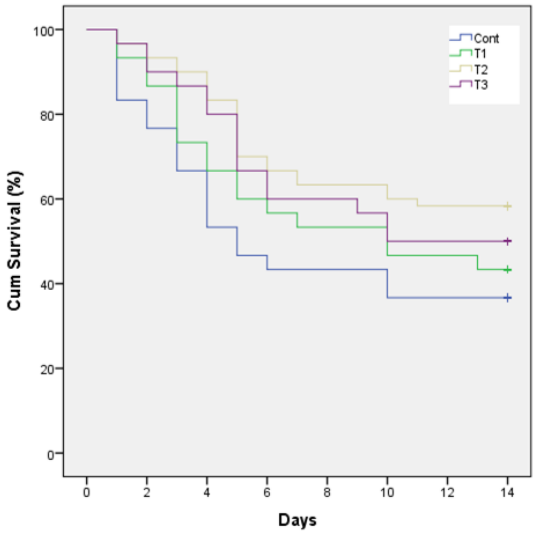


Figure 4. Kaplan-Meier survival curve of goldfish (*Carassius auratus*) fed with 0 (T0), 100 (T1), 150 (T2), and 200 (T3) mg kg^{-1} artichoke (*Cynara scolymus*) extract for eight weeks and subsequently challenged with *A. hydrophila* for 14 days

Table 5. Mortality rate, survival, and relative percentage survival (RPS) of goldfish (*Carassius auratus*) fed with 0 (T0), 100 (T1), 150 (T2), and 200 (T3) mg kg^{-1} artichoke (*Cynara scolymus*) extract for eight weeks and challenged with *A. hydrophila* for 14 days. Different letters designate significant differences as determined by pairwise comparisons (Mean ± SE)

Treatment groups	Mortality rate[1] (%)	Survival rate (%)	RPS2
T0	63.33 a	36.67 b	–
T1	56.67 ab	43.33 ab	10.53
T2	41.67 b	58.33 a	34.21
T3	50.00 b	50.00 a	21.05

[1]Mortality (%) = (number of dead fish / total number of fish) ×100
[2]RPS (%) = [1 – (cumulative mortality of the treated group/cumulative mortality of the control group)] × 100.

Growth efficiency

The results of the growth efficiency of goldfish after the eight-week feeding trial are presented in Table 4. FW and WG were notably higher in T2 and T3 than in T0 and T1 groups ($P<0.05$). A significant effect was detected in FCR between treatments with the lowest value observed in T2 group ($P<0.05$). SGR and survival rate were not altered by AE-supplemented diets ($P>0.05$). Low mortality (Figure 4) and high survival rate (Table 5) were evidenced in fish fed AE-supplemented diets compared to the control.

Serum immune response

Serum immune factors including ACH50 (Figure 5 A) and lysozyme (Figure 5 B) were higher in T2 and T3 than T0 and T1 groups ($P<0.05$). Similarly, total immunoglobulin (Figure 5 C) increased in T2 and T3 groups ($P<0.05$).

Gene expression

The relative expression of antioxidant-related genes (GR, Figure 6 A; GST, Figure 6 B) was up-regulated in fish fed 150 (T2) and 200 (T3) g kg^{-1} AE compared to fish fed 0 (T0), 100 (T1) g kg^{-1} AE ($P<0.05$). Immune-related genes including IL1β (Figure 6 C) and TNFα (Figure 6 D) were up-regulated in T2 and T3 compared to T0 and T1 groups ($P<0.05$). The expression of HSC70 gene (Figure 6 E) increased in fish fed AE, with the T3 group showing the highest levels ($P<0.05$). The expression of stress-related genes including HSP70 (Figure 6 F) and HSP90β (Figure 6 G) was up-regulated in the T2 group ($P<0.05$).

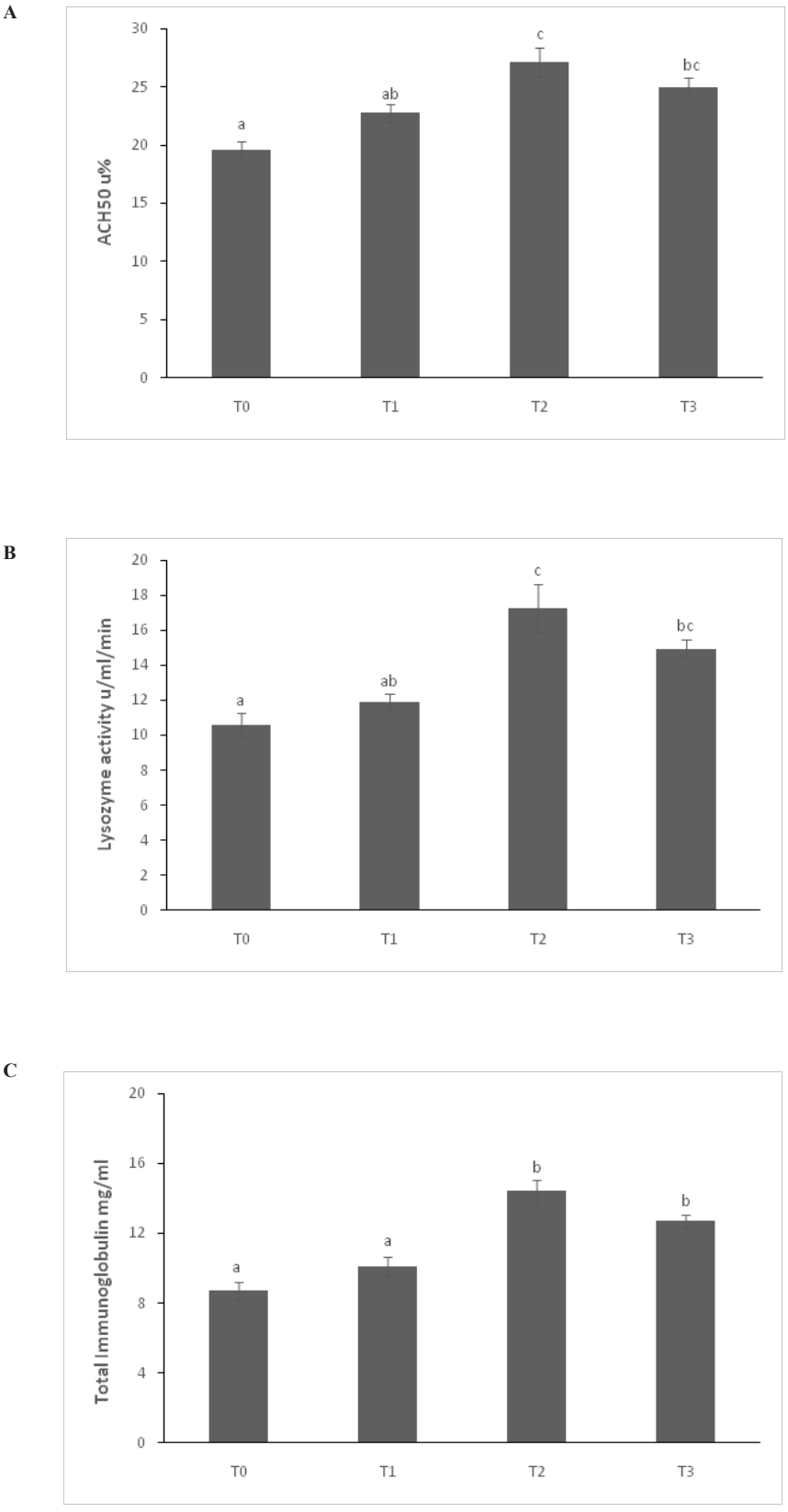
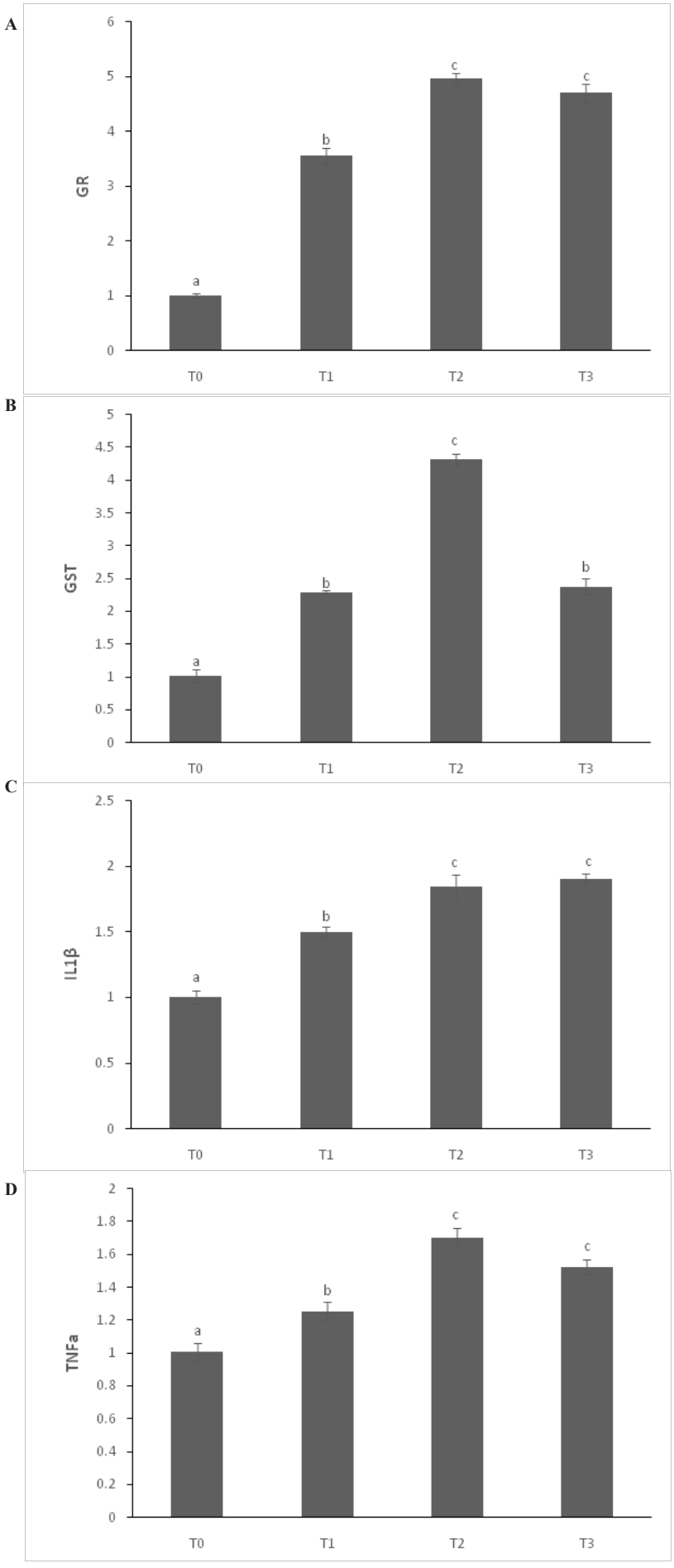


Figure 5. Serum ACH50 (A), lysozyme (B), and total immunoglobulin (C) of goldfish (*Carassius auratus*) fed with 0 (T0), 100 (T1), 150 (T2), and 200 (T3) mg kg^{-1} artichoke (*Cynara scolymus*) extract for eight weeks. Different letters designate significant differences as determined by Tukey's post-hoc tests (Mean ± SE)


A
GR
6
5
4
3
2
1
0
a
b
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c
T0
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T3
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GST
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IL1β
2.5
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T0
T1
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D
TNFa
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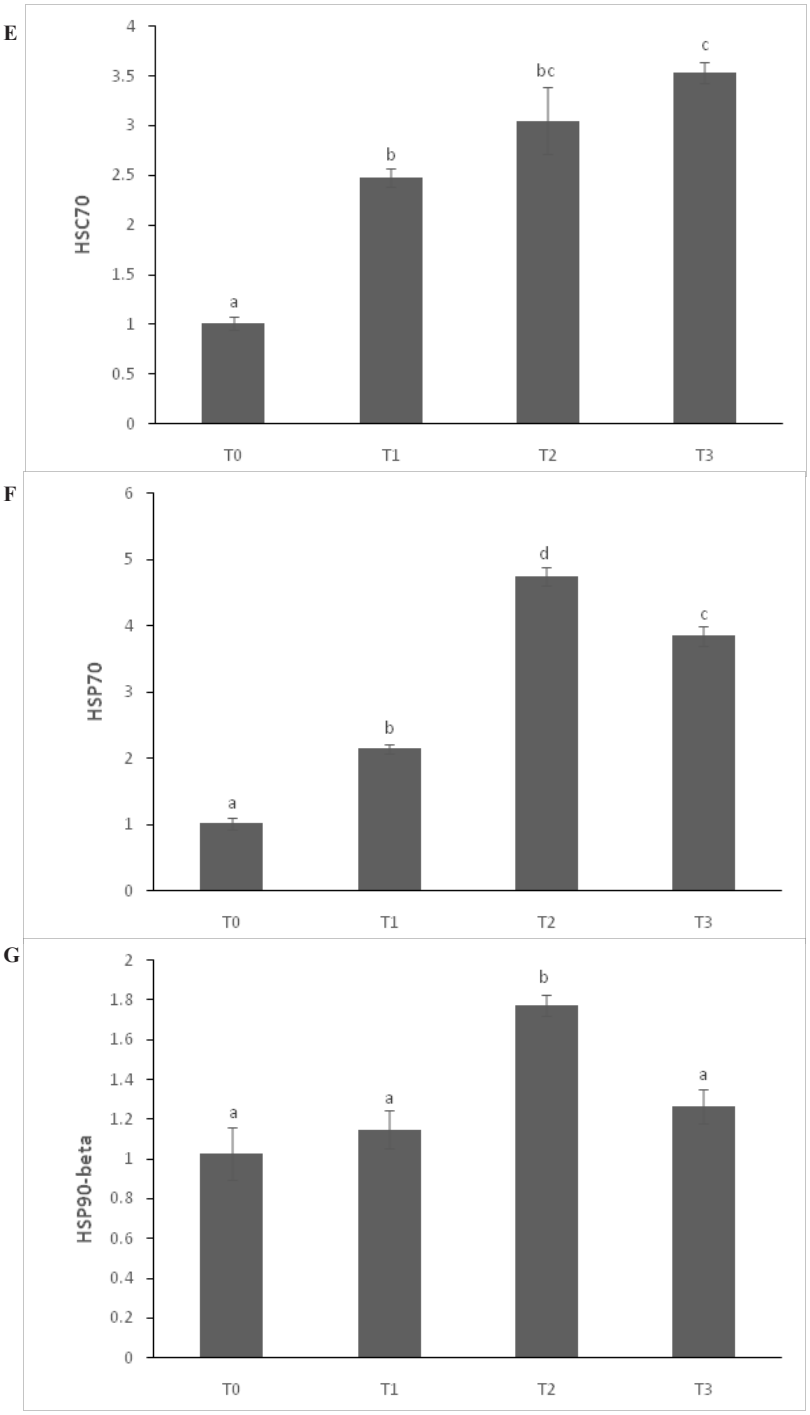

Figure 6. Relative expression of glutathione reductase (A, GR), glutathione s transferase (B, GST), interleukin-1 β (IL1β, C), tumor necrosis factor α (TNFα, D), HSC70 (E), HSP70 (F), and HSP90β (G) of goldfish (*Carassius auratus*) fed with 0 (T0), 100 (T1), 150 (T2), and 200 (T3) mg kg^{-1} artichoke (*Cynara scolymus*) extract for eight weeks and challenged with *A. hydrophila* for 14 days. Different letters designate significant differences as determined by Tukey's post-hoc tests (Mean ± SE)

Discussion

In this investigation, we report the influences of AE administration to goldfish on growth, immunity and antioxidant parameters, and disease resistance against *Aeromonas hydrophila*. After an eight-week feeding trial, fish growth was enhanced in the experimental diets, particularly at 150 mg of AE kg^{-1} feed. Even though no studies have been instructed to look into the effects of artichoke (*Cynara scolymus*) on fish growth performance, the beneficial effects of Jerusalem artichoke (*Helianthus tuberosus*), another member of the *Asteraceae*, on growth performance were shown in pangasius catfish (*Pangasius bocourti*) (Van Doan et al., 2016), common carp (Abedalhammed et al., 2020) and red tilapia (*Oreochromis* spp.) (Trullàs et al., 2022). Similar results were observed in rainbow trout fed Persian shallot (*Allium hirtifolium*) extract (Ghafarifarsani et al., 2022).

It has been suggested that the herbal efficacy is due to the presence of bioactive molecules, such as polyphenols, characterized by the presence of hydroxyl groups that confer the well-known, health promoting antioxidant properties (Rubió et al., 2013). The major constituents of artichoke are represented by the esters of caffeic acid known as caffeic acid derivatives (Mulinacci et al., 2004) which are attributed with many health promoting effects (da Cunha et al., 2004). The HPLC analysis of AE used in this study revealed a profile similar to the water and methanol artichoke extract reported by Llorach et al. (2002) which allows us to tentatively attribute to the AE used in this study, chlorogenic acid as the main peak, followed by caffeic acid and derivatives, and cynarin.

It has been reported that polyphenols improve growth parameters in different fish species (Awad and Awaad, 2017), however, the mechanism of action has not been yet clarified, although the studies performed indicate that polyphenols may have pleiotropic effects. Some studies show that polyphenol-rich herbs stimulated the growth of microbiota by means of their prebiotic effects while suppressing the growth of pathogenic flora (Gil-Cardoso et al., 2016; Milutinović et al., 2021). Similarly, dietary Jerusalem artichoke increased lactic acid bacteria and *Bifidobacterium* spp., but decreased *Vibrio* spp. in Nile tilapia (*Oreochromis niloticus*) (Boonanuntanasarn et al., 2018). Improvement of gut histomorphological indices, including the villi height, absorptive area and goblet cell count, following the administration of Jerusalem artichoke and synbiotics made of Jerusalem artichoke and *Lactobacillus rhamnosus* was observed in red tilapia (*Oreochromis* spp.) (Sewaka et al., 2019; Tiengtam et al., 2015). Further, growth promoting effects of phenolic compounds by enhancing digestive enzyme secretions were previously reported (Awad and Awaad, 2017). Finally, phenolic compounds from plant sources increased the flavor and palatability of the feed and thus improved the animal feed intake and growth performance (Mahfuz et al., 2021).

Fish culture intensification highlights the existence of various stressful stimuli such as infectious diseases, chemical and organic pollution, high density, and proinflammatory diets (Imperatore et al., 2022; Rehman et al., 2017). Fishes, as well as other vertebrates, fight against such potentially harmful conditions, producing free radicals that, if present in high amounts, can cause an imbalance with the antioxidant body reserves, generating the so called oxidative stress (Alkadi, 2020). Oxidative stress can cause damage to macromolecules such as DNA, lipids, and proteins, resulting in tissue damage (Özcan et al., 2015). Therefore, the consumption of natural antioxidants, such as plant extracts, may help neutralize the free radicals damage (Engwa, 2018).

In the current study, AE improved the antioxidant power and enzyme related antioxidant activity in goldfish skin mucus. Moreover, GSR and GR enzymes gene expressions were up-regulated in the intestine. Similarly, previous studies showed the beneficial effects of Jerusalem artichoke on the Nile tilapia antioxidant system (Trullàs et al., 2022). It may be possible that chlorogenic and caffeic acids contained in AE, suppressed the oxidative stress and improved the antioxidant enzyme gene expressions as reported in the Nile tilapia (Yilmaz, 2019), largemouth bass (*Micropterus salmoides*) (Yin et al., 2021), Koi carp (*Cyprinus carpio*) (Xu et al., 2022), and zebrafish (*Danio rerio*) (Liu et al., 2022).

Nonspecific immune responses are the first lines of defense which play crucial roles against pathogen invasion. Lysozyme, total immunoglobulin, and total protein, and complement system are used for evaluating the potential of dietary feed additives in fishes (Awad and Awaad, 2017). In the current study, these immune parameters were enhanced in fish treated with AE. Similar to our study, feed additives such as chlorogenic acid (Xu et al., 2022; Ghafarifarsani et al., 2023) and caffeic acid (Yilmaz, 2019; Ahmadifar et al., 2022) added to fish diets improved nonspecific immune responses.

In parallel with the increases in the nonspecific immune responses, immune-relevant gene expressions, including IL1β, TNFα, HSC70, HSP70 and HSP90β, were up-regulated in goldfish intestine. The intestine is an important organ in fish that oversees numerous events like digestion/absorption, water and electrolyte balance, and immunity (Ringø et al., 2003). Therefore, we selected the intestine as an organ for investigating the AE effects of immune-related gene expressions. Interleukin-1β (IL1β) and tumor necrosis factor alpha (TNFα) are important fish cytokines. This study observed that IL1β and TNFα gene expression levels increased in the intestines of goldfish receiving feeds containing AE. Similar to our study, adding 0.1 and 0.5% caffeic acid to the diet of Nile tilapia, *Oreochromis niloticus* increased the IL1β and TNFα expression levels in the livers (Yilmaz, 2019). It was also found that the addition of chlorogenic acid to the feed of Koi carp (Xu et al., 2022) up-regulated the gene expression.

Heat shock proteins (HSPs) protect cells against the adverse effects of stress (Wang et al., 2022). They play a critical role in maintaining fish health, especially in reducing trauma and physical stress, which are sources of stress in aquaculture activities such as transportation and vaccination (Roberts, 2010). In the present study, the HSP70, HSC70 and HSP90β gene expression levels were significantly increased in the intestine in the AE-supplemented groups. Similarly, Yilmaz (2019) found that adding 5 g/kg cafeic acid to the feed of Nile tilapia increased hsp70 gene expression in the liver after 60 days of feeding.

The mechanism of action of polyphenols on the immune system is not clearly determined. However, some studies suggest that polyphenols can boost the host immune capacity by manipulating the microbiota via exhibiting prebiotic effects and antimicrobial action against pathogenic flora (Ding et al., 2018; Tekin and Marotta, 2018; Kumar Singh et al., 2019; Orso et al., 2021).

Aeromonas hydrophila is one of the common freshwater pathogens which leads to severe economic loss in ornamental fish farming (Anjur et al., 2021). One of the approaches for disease prevention is fish vaccination. However, the problem that limits the development of commercial *A. hydrophila* vaccines is strain diversity and the failure of the current vaccines to confer protection to heterologous strains (Mzula et al., 2019). Therefore, alternative approaches, such as feed additives, are recommended by different researchers (Anjur et al., 2021; Vijayaram et al., 2022). The present study indicates that feeding AE increased the goldfish resistance against *A. hydrophila* challenge, which might be due to fish immune modulation, as suggested by the aforementioned immunological indices significantly increased in the treated goldfish. In agreement with such outcome, dietary administration of Jerusalem artichoke improved the resistance against *A. hydrophila* in catfish (*Pangasius bocourti*) (Van Doan et al., 2016).

Conclusion

The administration of AE, especially at the amounts of 150 mg kg^{-1} of feed could improve growth performance, and prompt antioxidant and immune indices. However, the detailed mechanism of action of this extract warrants further research.

Authors' contribution

Shalaleh Mousavi: methodology, and data curation; Sedigheh Mohammadzadeh*:* methodology and writing original draft and editing; Sara Mehdizadeh Mood: methodology and conceptualization; Ehsan Ahmadifar**:** conceptualization, methodology, data curation and editing; Najmeh Sheikhzadeh**:** writing and editing; Naser Kalhor: gene expression analysis; Mohsen Shahriari Moghadam: data analysis; Sevdan Yilmaz: reviewing and editing; Seyed Hossein Hoseinifar: editing; Marina Paolucci: methodology of polyphenol.

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Data availability

All data of this study are included in this article.

Competing interests

The authors declare no competing interests.

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