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Effects of artichoke (*Cynara scolymus*) polyphenolic extract on growth, antioxidants and some immune parameters in common carp (*Cyprinus carpio*)

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Abstract

In this research, *Cyprinus carpio* juveniles were fed with experimental diets containing different levels of artichoke polyphenolic leaf extract (ALE) 0 (Control), 0.5, 1, and 2 g/kg over eight weeks. After eight weeks, haemato-immunological parameters, growth performance, immune parameters and antioxidant defense were measured. The administration of varying levels of ALE had no significant effects on growth parameters compared to the control group. However, haematological parameters were significantly elevated compared to the control group. Immune parameters such as serum lysozyme, mucus lysozyme, serum and mucus total immunoglobulin, as well as serum alternative complement activity and bactericidal activity of ALE-treated carps, showed a notable increase in comparison to the CTRL group. Also, the agglutination titer of serum and mucus in ALE fed carps were significantly higher than CTRL group. Feeding with varying levels of ALE (0.5, 1, and 2 g/kg) significantly enhanced the activities of serum antioxidant enzymes, including superoxide dismutase in all groups, catalase in the 0.5 and 2 g/kg groups, and glutathione peroxidase in the 1 and 2 g/kg groups, compared to the control group. The results of this study demonstrate that dietary supplementation with ALE at 0.5–2 g/kg positively influences immune and antioxidant factors in *Cyprinus carpio*, with the most pronounced effects observed at 1 and 2 g/kg. These findings suggest that artichoke polyphenolic extract, particularly at higher dosages, has potential as an effective feed additive to enhance immunity and antioxidant capacity in carp farming.

Keywords *Cynara scolymus*, Growth, Antioxidant, Immunity, *Cyprinus carpio*

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Introduction

Aquaculture plays a vital role in providing healthy food and a very high-quality and rich source of protein for humans [1]. Along with the increase in world population (expected to reach 9.7 billion by 2050), there will be increasing reliance on aquaculture as a sustainable source of protein as food [2, 3]. To meet the demands, the intensive and super-intensive aquaculture systems are rapidly expanding [3]. Intensive farming is known to cause stress, immunosuppression, and increased incidence of disease, resulting in heavy economic losses [4]. Besides this, the overuse of antibiotics for preventing and curing diseases has resulted in the super challenge of antibiotic resistance and a search for new ways of improving health and growth in fish [5]. Over the past years, there has been increasing attention toward promising environment-friendly feed additives such as polyphenols to improve fish health and growth performance [6, 7]. Polyphenols are bioactive compounds with well-documented antioxidant, anti-inflammatory, and immunomodulatory properties that can improve the health and performance of fish [8, 9]. Artichoke (*Cynara scolymus*) leaf extract served as a promising polyphenol due to phenolic, flavonoid and acid compounds [10]. The main compounds of this plant are caffeine and esters of quinic acid, chlorogenic and pseudo-chlorogenic acid, neochlorogenic acid, cynarin and caffeoylquinic acid [11]. These compounds exhibit potent antioxidant and immunostimulatory effects, which can help mitigate oxidative stress and enhance immune responses in fish [12]. For instance, studies have demonstrated that polyphenols from olive waste and chestnut wood improve growth performance, antioxidant status, and immune function in common carp and other species [8, 13]. Despite the growing interest in polyphenols as feed additives, there is a gap in existing knowledge regarding the possible effects of artichoke leaf polyphenolic extract (ALE) in aquaculture, particularly in common carp (*Cyprinus carpio*), one of the most widely farmed species globally. Given the susceptibility of common carp to disease outbreaks and suboptimal growth performance in intensive farming systems, there is a critical need to explore sustainable strategies to enhance their health and productivity. Therefore, this study aimed to evaluate the effects of dietary supplementation with ALE on growth performance, haemato-immunological parameters, and antioxidant defense in common carp, providing insights into its potential as a natural feed additive for sustainable aquaculture.

Table 1 The nutritional value of ALE*

Parameters	Value
Carbohydrates	90–95 (%)
Protein	0 (%)
Fat	0–1 (%)
Minerals	5–7 (%)
Energy	100 (Kcal / 100 gr)
Total content of polyphenols (TCP)	5 (%)

* Based on the manufacturer (Istituto Farmochimico Fitoterapico S.r.l., Via Stadera 19–20141 Milano, Italy)

Materials and methods

Preparation and extraction of artichoke leaf extract (ALE)

Polyphenolic extract of *C. scolymus* L. leaves was kindly supplied by EPO (Istituto Farmochimico Fitoterapico S.r.l., Via Stadera 19–20141 Milano, Italy). The nutritional value of this extract is shown in Table 1. Dried *Cynara scolymus* leaves were extracted with ethyl alcohol at 60 °C to produce the artichoke leaf polyphenolic extract (ALE). The dried leaves became powder, which was extracted under 60 °C heat using 70% ethanol and two hours of stirring. After filtration, the extract went through vacuum concentration until we obtained powdered material using a rotary evaporator and freeze-drying methods. Our previous report described how high-performance liquid chromatography (HPLC) determined the amount of polyphenol substances in the extract [14]. The HPLC test showed that the extract contains essential bioactive components like caffeic acid, chlorogenic acid, and cynarin. The ATR-FTIR technique measured the chemical substance and functional group composition of the extract. To avoid redundancy, ATR-FTIR and HPLC procedures and results are not presented here in detail (see our recently published paper) [14].

Experimental design

Two hundred and forty common carp fingerlings (10 ± 1 g) were bought from a private farm in Golestan province (Iran). After the initial acclimation (for 14 days), they were randomly divided into four groups, including control and three treatments repeated in triplicates (each tank containing 20 fish). The experimental units were 100 L fibreglass tanks (Fig. 1). The duration of the feeding trial was eight weeks, and carps were fed with experimental diets containing 0 (control; CTRL), 0.5, 1 and 2 g/kg ALE. To prepare the experimental diet, the selected doses of ALE were added to a basal diet that was commercial feed (Faradane Company, Shahrekord, Iran) containing 40–43% protein, 8 – 4% fat, 11 – 5% moisture, 3–6% fiber, 7–11% ash and 1- 1.5% phosphorus. Briefly, different levels of ALE were dissolved separately in distilled water (30 mL) and then sprayed on the food. After complete drying of the extract, 2% gelatin solution was added to different levels of ALE (to prevent leaching before consumption of diets by carps). The experimental diets were kept in a refrigerator at 4 °C until use. Carps were fed daily (two times a day) with approximately 3% of body weight. Every two weeks after weighing each group, the feeding rates were calculated and adjusted.

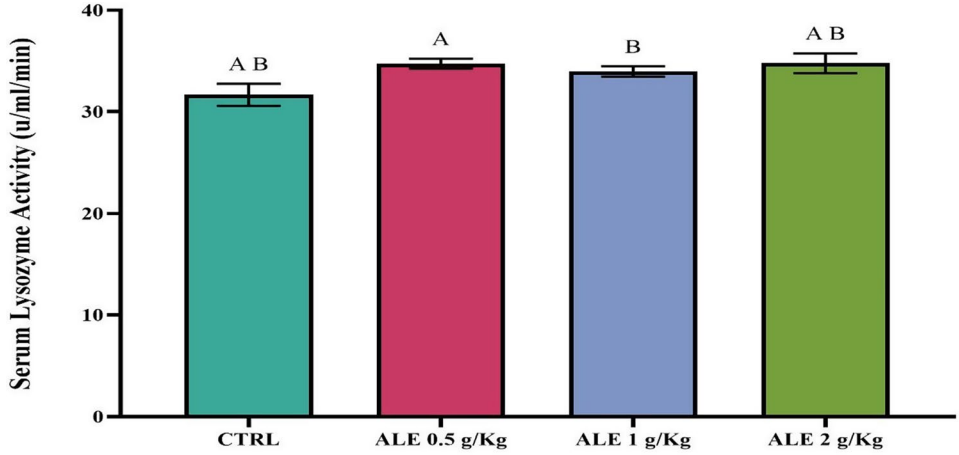

Fig. 1 Effect of different levels of ALE on serum lysozyme activity in *C. carpio* ($n=9$ fish per group. CTRL: control; ALE: artichoke leaf extract. Identical Latin letters denote no significant difference, whereas different letters represent a significant difference. Significance levels were considered to be $P<0.05$

The physicochemical parameters of water, including water temperature (26 ± 1 °C), pH (7.53 ± 0.15), and DO (6.95 ± 0.35 mg per L), were assayed every week. To maintain water quality, 30% of the tank's water was exchanged for fresh water.

Measurement of growth parameters

At the end of the feeding trial, the fish were weighed, and growth parameters, including weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR), were calculated using the following formulas.

$$\text{Weight Gain}\,(\%) = \frac{Final\,Weight\,(g) - Initial\,Weight\,(g)}{Initial\,Weight} \times 100$$

$$Specific\,Growth\,Rate\,(\%day-1) = \frac{[Ln\,(Final\,Weight) - Ln\,(Initial\,Weight)]}{\text{Days}} \times 100$$

$$Food\,Conversion\,Ratio\,(g/g) = \frac{Feed\,Consumed}{Weight\,Gain}$$

$$Survival\,Rate\,(\%) = \frac{Number\,of\,Survived\,fish}{Initial\,Number\,of\,fish} \times 100$$

Blood and skin mucus collection

In order to collect blood, the fish were anesthetized using clove powder at a concentration of 150 mg/L and returned to rearing conditions after blood collection [15]. Three fish from each replicate ($n=9$ from each treatment) were randomly selected, and blood was collected from the caudal vein. Blood samples were divided into heparinised (for haematological parameters) and non-heparinized (to measure serum immune and antioxidant parameters). The samples without heparin were centrifuged at 3500 × rpm for 10 min at 4 °C, and the serum was kept at -80 °C until further analysis. To collect skin mucus samples, the fish were randomly selected and transferred to plastic bags (containing 50 mM NaCl). Then, for better mucus extraction, the fish were shaken in the bags (1 min). In the next step, mucus was transferred to tubes and centrifuged (1500 × rpm, 15 min; 4 °C). Finally, after centrifugation, the supernatant was stored at -80 °C for relevant analysis [16].

Measurement of blood parameters

White blood cells (WBCs) and red blood cells (RBCs) were counted using a Neubauer hemocytometer slide after being diluted with Dacie's solution. The hematocrit (Hct) was measured utilizing microhematocrit tubes. Hemoglobin (Hb) concentrations were evaluated with a commercial kit from ZiestChem Diagnostics (Tehran, Iran), which employs the cyano-meth-hemoglobin method [17]. Blood parameters, including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were calculated using a standard equation.

$$\text{MCV (fL)} = \frac{\text{Hct (\%)}}{\text{RBC}}$$

$$\text{MCH (pg)} = \frac{[\text{Hb (g/dL)} \times 10]}{\text{RBC}}$$

$$\text{MCHC (g/dL)} = \frac{[\text{Hb (g/dL)} \times 100]}{\text{Hct (\%)}}$$

Mucus and serum immune assays

Lysozyme assay

Mucus and serum lysozyme activity was measured using D Subramanian, Y-H Jang, D-H Kim, B-J Kang and M-S Heo [18] method with minor modifications [19]. First, *Micrococcus luteus* (0.2 mg mL^{-1}) was dissolved in NaH_2PO_4 (Sigma-Aldrich, USA, cat no 7558-80-7) buffer (pH = 7.0, 0.04 M). Then, two µL of the sample (from both mucus and serum) were mixed with 1 µL of a bacterial suspension dissolved in buffer. In the final stage, the absorption after ten minutes (at room temperature) was read by a spectrophotometer at 450 nm wavelength. The decrease of 0.001 unit per minute was considered a unit of lysozyme activity.

Mucus and serum total Immunoglobulin (Ig) assay

AK Siwicki and DP Anderson [19] (1993) method was used with some modifications to measure the amount of total Ig [20]. Briefly, 1 mL of sample (serum and mucus separately) was mixed with 0.1 mL of 12% polyethylene glycol (Sigma-Aldrich, USA, cat no 25322-68-3) (v:v) solution. Then, the solution was placed in an incubator at room temperature for two hours under continuous shaking to mix well. In the next step, the samples were centrifuged (5000 × g, 4 °C for 10 min), and then the precipitated protein concentration was measured. The amount of total Ig was calculated by subtracting the protein concentration in the initial sample and the protein concentration after adding polyethylene glycol. The absorption number at the wavelength of 546 nm was calculated.

Serum ACH50 assay

Serum alternative complement activity ACH50 was determined using the method described by Tort et al. (2003). Briefly, Rabbit red blood cells (RaRBC) were subjected to a washing process involving three cycles in a phenol red-free Hank's buffer that included magnesium ions (Mg^{2+}) and GVB-EGTA. Following this, the cells were diluted to a concentration of 1×10^8 cells per mL in the same buffer. Different volumes of serum, ranging from 0.1 to 0.25 mL, were added to test tubes, with the total volume adjusted to 0.25 mL using barbitone buffer containing EGTA and Mg^{2+}. Subsequently, 0.1 mL of RaRBC was introduced into each tube. The samples were incubated at 21 °C for 2 h. After incubation, 3.15 mL of 0.9% NaCl was added to each sample, followed by centrifugation at 836 g for 10 min at 4 °C to eliminate non-lysed erythrocytes. The optical density (OD) of the supernatant was measured at a wavelength of 414 nm. To establish baseline values for maximum (100%) and minimum (spontaneous) hemolysis, distilled water or Hank's Balanced Salt Solution (HBSS) was added to separate RaRBC samples, respectively. The volume obtained from the hemolysis of 50% of rabbit RBCs was determined and used to calculate the samples' ACH50 activity L Tort, J Balasch and S Mackenzie [20].

Mucus and serum agglutination titer

The agglutination titer of mucus and serum was determined based on the research of AC Barnes and AE Ellis [21] (2004) with some modifications [22]. First, serum and mucus were heated for 30 min at 45 °C to inactivate complement proteins. Serum and mucus were incubated in PBS (50 µL per well) in a 96-well plate with 50 µL of *Aeromonas hydrophila* (*A. hydrophila*, PTCC No: 1890) dissolved in sterile PBS at 22 °C for 24 h. After incubation, the agglutination was recorded by the bacteria depositing on the bottom of the wells with the unaided eye [22].

Serum bactericidal activity (against A. hydrophila)

To measure the bactericidal activity of serum, *A. hydrophila* (kindly provided by IROST, PTCC No: 1890) $n = 9$ fish per group was cultured in a blood-agar medium (Sigma-Aldrich, USA, 70133) and then mixed with PBS. The dilution of this suspension was adjusted to show 0.5 absorbance at 564 nm wavelength. Then, this suspension was serially diluted five times (1:10). 450 µL of the suspension was mixed with 50 µL of blood serum and incubated for one hour at room temperature. Then, 100 µL of this mixture was cultured on nutrient agar plates, and after one day, the grown colonies were counted [23].

Mucus protease activity

The protease activity of skin mucus was assessed using the azocasein hydrolysis method. Briefly, 100 µL of skin mucus was mixed with 100 µL of 0.7% azocasein (Sigma-Aldrich, USA, cat. no. A2765) solution and then incubated at 30 °C with mechanical shaking for one day. Then 4.5% trichloroacetic acid (TCA, Sigma-Aldrich, USA, cat. no. 76-03-9) was added to stop the reaction, and the supernatant was obtained by centrifugation at 15,000 × rpm for 5 min. Supernatants were pipetted into a 96-well plate previously filled with 100 µl of sodium hydroxide (NaOH, 1 N; Sigma-Aldrich, USA, cat. no. 1310-73-2) per well. Optical density was recorded at a wavelength of 450 nm [24].

The activity of serum antioxidant enzymes

Employing a commercial kit, the serum activity of catalase (CAT) (ZellBio GmbH, Germany, E-BC-K031-S), superoxide dismutase (SOD), (ZellBio GmbH, Germany, ZX-44108-96) and glutathione peroxidase (GPx) (ZellBio GmbH, Germany, E-BC-K096-M) was measured according to the manufacturer's protocol [25]. SOD activity was assessed by measuring the formation of a chromogenic product, which was quantified colorimetrically at

Table 2 Effect of ALE with different levels on growth parameters of *C. carpio*

Parameters	CTRL	ALE 0.5 g/Kg	ALE 1 g/Kg	ALE 2 g/Kg
IW (g)	10.02 ± 0.014^{a}	10.05 ± 0.014^{a}	10.08 ± 0.049^{a}	10.07 ± 0.014^{a}
FW (g)	26.85 ± 1.76^{a}	29.25 ± 0.07^{a}	29.05 ± 0.49^{a}	31.45 ± 2.19^{a}
WG (%)	167.98 ± 18.02^{a}	191.04 ± 1.11^{a}	188.07 ± 6.32^{a}	212.33 ± 22.2^{a}
FCR (g /g)	1.47 ± 0.02^{a}	1.27 ± 0.06^{a}	1.24 ± 0.05^{a}	1.20 ± 0.08^{a}
SGR (% day $^{-1}$)	1.75 ± 0.120^{a}	1.90 ± 0.007^{a}	1.89 ± 0.042^{a}	2.03 ± 0.127^{a}
SR (%)	100	100	100	100

IW: initial weight; FW: final weight; WG: weight gain; FCR: food conversion ratio; SGR: specific growth rate; SR: survival rate; CTRL: control; ALE: artichoke leaf extract. ($n=60$ fish per treatment). Similar lowercase Latin letters within each row denote non-significant differences $P>0.05$

a wavelength of 420 nm. In this assay, one unit of SOD activity is defined as the quantity of enzyme required to catalyze the conversion of 1 micromole of superoxide (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) within one minute. The results of the enzyme activity were reported in units per millilitre (U/mL). The activity of CAT was measured colorimetrically at 405 nm using an ELISA reader. In this assay, one unit of CAT activity is defined as the amount of enzyme that catalyzes the breakdown of 1 micromole of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) within one minute, with results expressed in U/mL. This method provides a sensitivity of 0.5 U/mL for detecting CAT activity.

Statistical analysis

The study design was completely randomized designs, and the experimental unit was each tank. The study included a control group and three treatments, each of them repeated in triplicates. After data collection, first, the normality of the data was checked with Kolmogorov-Smirnov and the homogeneity of the variances was checked using Levene's test. Following approving the normality of data and homogeneity of variances, data analysis was done using a one-way analysis of variance (ANOVA) followed by a Post Hoc test (Tukey's test). Data analysis was performed using GraphPad PRISM software (version 10). Data were presented as mean ± SD. The mean values were considered significantly different at a significance level of $P<0.05$.

Results

Growth performance

Table 2 represents the growth performance of common carp after eight weeks of feeding on different levels of ALE. At the end of the experiment, the results of this study showed that final weight, weight gain, and SGR were notably increased in carps fed with ALE supplemented diet. The highest increase was noticed in those fish fed 2 g/kg ALE. However, these increases were not statistically significant compared to the control group ($P>0.05$). Also, feeding with ALE decreased the feed conversion ratio, with the lowest FCR recorded in the 2 g/kg ALE group. However, when compared with the FCR rate of the control group, such improvement was not statistically significant ($P>0.05$).

Table 3 The effect of different levels of dietary ALE on the haematological parameters of *C. carpio* ($n=9$ fish per group)

Parameters	CTRL	ALE 0.5 g/Kg	ALE 1 g/Kg	ALE 2 g/Kg
RBC (10^6 / μl^{-1})	2.05 ± 0.01^{d}	2.19 ± 0.04^{c}	2.75 ± 0.04^{a}	2.44 ± 0.02^{b}
WBC (10^3 / μl^{-1})	20.53 ± 0.30^{c}	30.53 ± 0.11^{b}	36.86 ± 0.41^{b}	38.8 ± 0.87^{a}
Hb (g/dL)	6.59 ± 0.05^{d}	9.21 ± 0.07^{b}	8.72 ± 0.14^{c}	9.73 ± 0.22^{a}
HCT (%)	35.05 ± 0.50^{c}	38.53 ± 0.76^{a}	38.00 ± 0.00^{b}	39.33 ± 0.28^{a}
MCV (FL)	173.16 ± 1.38^{a}	176.57 ± 2.38^{a}	139.85 ± 2.28^{c}	161.20 ± 1.78^{b}
MCH (pg)	32.29 ± 0.09^{b}	42.08 ± 1.22^{a}	31.68 ± 1.05^{b}	39.92 ± 1.15^{a}
MCHE (g/ dL)	18.59 ± 0.19^{c}	23.83 ± 0.64^{ab}	22.65 ± 0.38^{b}	24.76 ± 0.72^{a}

RBC: red blood cells, WBC: white blood cell, Hb: Hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, and MCHC: mean corpuscular hemoglobin concentration, CTRL: control; ALE: artichoke leaf extract. Different lowercase Latin letters within each row denote significant differences ($P<0.05$)

Blood parameters

The results of ALE effects on the haematological parameters of common carp are shown in Table 3. The results revealed that feeding on ALE supplemented diet significantly elevated blood parameters such as RBCs, HB, HCT, MCV, MCH and MCHC compared to the CTRL group (Table 3, $P<0.05$). Also, WBC was significantly elevated in ALE-fed carp, and the highest count was recorded in carp fed 2 g/kg (Table 3, $P<0.05$).

Serum and skin mucus lysozyme

The results of ALE effects on serum and mucus lysozyme activity of common carp are shown in Figs. 1 and 2, respectively. The findings of this study demonstrated a notable rise in serum lysozyme activity in the groups treated with ALE in comparison to the CTRL group (Fig. 1, $P<0.05$), regardless of inclusion level. In the case of skin mucus lysozyme activity, the highest level was noticed in carps fed 2 g/kg (Fig. 2, $P=0.0440$). However, lower inclusion levels (0.5 and 1 g/kg) caused no significant difference compared to the CTRL group (Fig. 2, $P>0.05$).

Serum and skin mucus total Ig

Figures 3 and 4 represent the effects of dietary ALE on the serum and mucus total Ig activity in common carp. The results of this research showed a significant increase in the serum total Ig activity in carps fed ALE compared

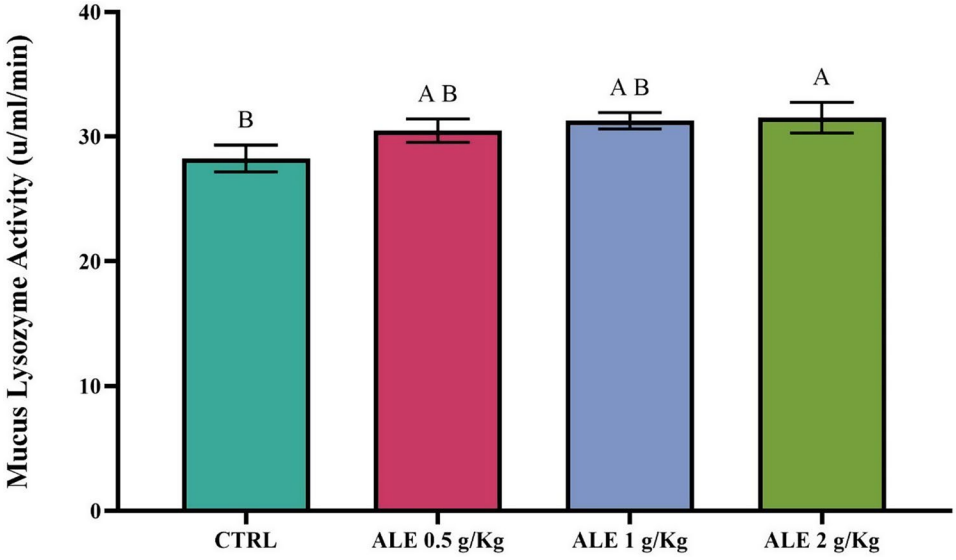

Fig. 2 Effect of different levels of ALE on skin mucus lysozyme activity in *C. carpio* ($n=9$ fish per group). CTRL: control; ALE: artichoke leaf extract. Identical Latin letters denote no significant difference, whereas different letters represent a significant difference. Significance levels were considered to be $P<0.05$

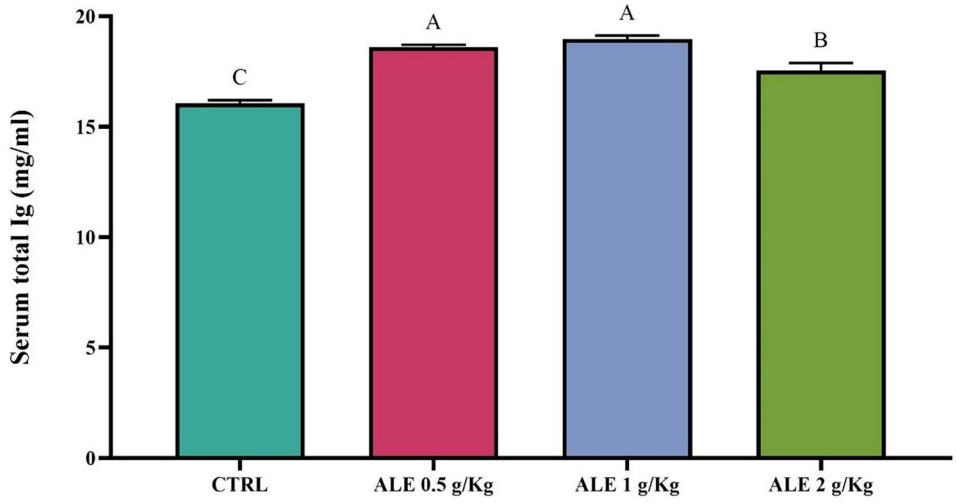

Fig. 3 Effect of different levels of ALE on serum total Ig in *C. carpio* ($n=9$ fish per group). CTRL: control; ALE: artichoke leaf extract. Identical Latin letters denote no significant difference, whereas different letters represent a significant difference. Significance levels were considered to be $P<0.05$

to the CTRL group (Fig. 3, $P<0.05$). A significant difference was observed between the groups 0.5 with 2 g/kg and 1 with 2 g/kg (Fig. 3, $P<0.05$). Similarly, the mucus total Ig level in the groups treated with 0.5, 1 and 2 g/kg ALE showed a significant increase compared to the CTRL group (Fig. 4, $P<0.05$). The highest skin mucus total Ig level was noticed in fish fed 2 g/kg ALE (Fig. 4, $P<0.05$).

Serum and skin mucus agglutination titer

The results related to serum and mucus agglutination titer against *A. hydrophila* in groups fed with different levels of ALE after eight weeks of feeding are shown in Figs. 5 and 6, respectively. The results showed a significant increase in serum and mucus agglutination titer against *A. hydrophila* in the groups containing 1 and 2 g/kg ALE compared to the CTRL group (Figs. 5 and 6, $P<0.05$). Such increase was not dose-dependent, and no significant differences were observed among the ALE-treated groups regarding serum and mucus agglutination titer (Figs. 5 and 6, $P>0.05$).

Serum alternative complement activity (ACH50)

Figure 7 shows the impact of varying levels of ALE on the Serum alternative complement activity (ACH50) of common carp. In the groups containing 0.5, 1 and 2 g/kg of ALE, a significant increase was observed compared to the CTRL group (Fig. 7, $P<0.05$). Also, a significant

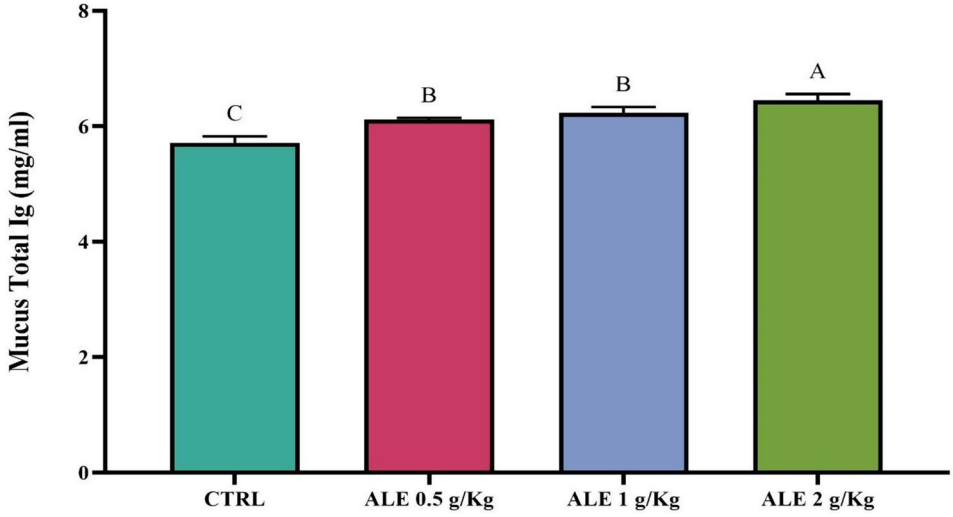

Fig. 4 Effect of different levels of ALE on skin mucus total Ig in *C. carpio* ($n=9$ fish per group). CTRL: control; ALE: artichoke leaf extract. Identical Latin letters denote no significant difference, whereas different letters represent a significant difference. Significance levels were considered to be $P<0.05$

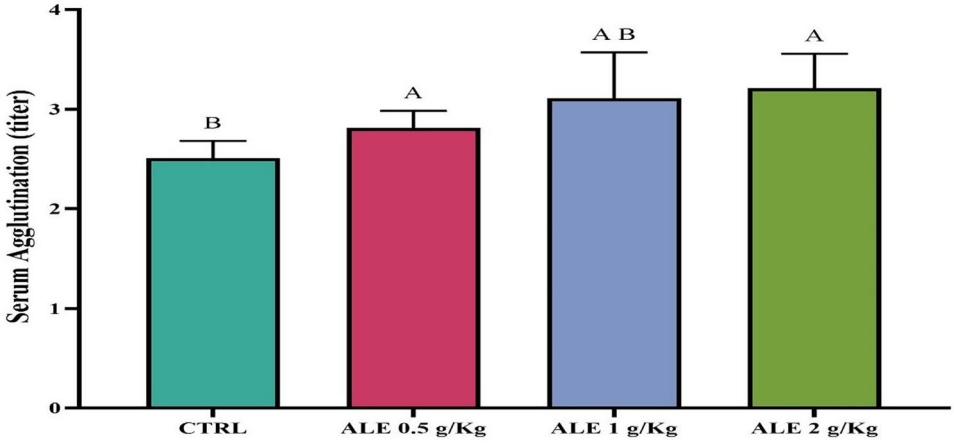

Fig. 5 Effect of different levels of ALE on serum agglutination titer against *A. hydrophila C. carpio* ($n=9$ fish per group). CTRL: control; ALE: artichoke leaf extract. Identical Latin letters denote no significant difference, whereas different letters represent a significant difference. Significance levels were considered to be $P<0.05$

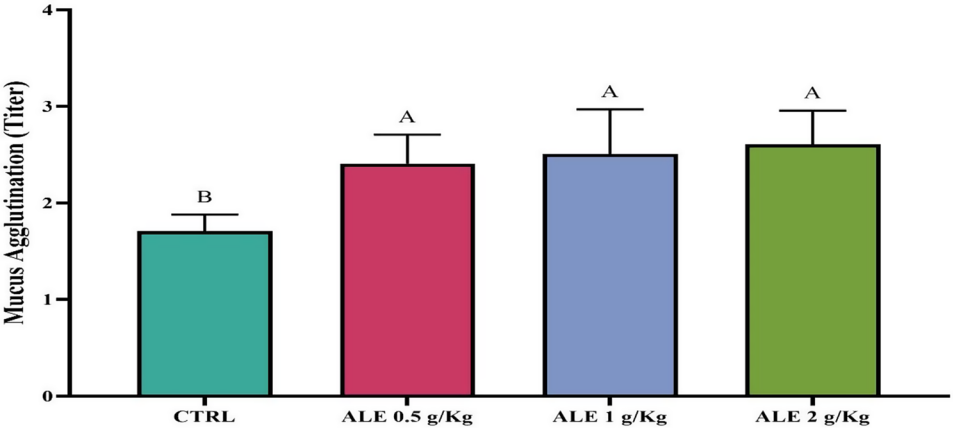

Fig. 6 Effect of different levels of ALE on skin mucus agglutination titer against *A. hydrophila* in *C. carpio* ($n=9$ fish per group). CTRL: control; ALE: artichoke leaf extract. Identical Latin letters denote no significant difference, whereas different letters represent a significant difference. Significance levels were considered to be $P<0.05$

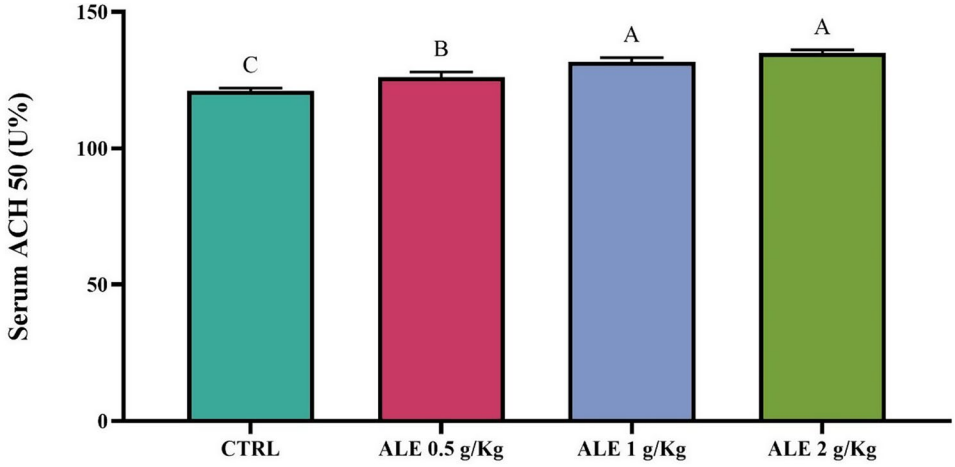

Fig. 7 Effect of different levels of ALE on serum ACH50 in *C. carpio* ($n=9$ fish per group). CTRL: control; ALE: artichoke leaf extract. Identical Latin letters denote no significant difference, whereas different letters represent a significant difference. Significance levels were considered to be $P<0.05$

Table 4 Effect of different levels of ALE on serum antioxidant enzymes activity of *C. Carpio*

Parameters	CTRL	ALE 0.5 g/Kg	ALE 1 g/Kg	ALE 2 g/Kg
SOD (mg/protein)	63.33 ± 1.90^{c}	69.23 ± 0.49^{b}	73.73 ± 1.15^{a}	75.87 ± 0.95^{a}
CAT (min/mg/protein)	83.66 ± 0.80^{c}	87.56 ± 1.10^{b}	85.03 ± 0.70^{b}	91.26 ± 1.46^{a}
GPx (min/mg/protein)	192.33 ± 7.02^{c}	203 ± 2.00^{b}	232.66 ± 8.50^{a}	257 ± 16.09^{a}

CTRL: control; ALE: artichoke leaf extract. Different lowercase Latin letters within each row denote significant differences. ($n=9$ fish per group). Significance levels were considered to be $P<0.05$

difference was observed between 0.5 and 1 g/kg as well as 0.5 and 2 g/kg ALE (Fig. 7, $P<0.05$).

Serum antioxidant parameters

The results related to ALE effects on serum antioxidant enzyme activity of common carp are presented in Table 4. Dietary ALE caused a significant increase in serum SOD enzyme activity compared to the CTRL group ($P<0.05$). Also, a significant difference (increase) in the activity of serum SOD was observed between the groups 0.5 and 1 g/kg as well as 0.5 and 2 g/kg ($P<0.05$). Also, serum CAT enzyme activity in 0.5 and 2 g/kg ALE-fed carps significantly increased in comparison to the CTRL group ($P<0.05$). Similarly, dietary ALE (1 and 2 g/kg) caused a significant increase in the serum GPx enzyme activity compared to the CTRL group ($P<0.05$).

Skin mucus protease activity

At the end of the 8th week, the activity of the mucus protease enzyme in the 0.5, 1 and 2 g/kg ALE groups exhibited a significant increase in comparison to the CTRL group (Fig. 8, $P<0.05$). However, there was no significant difference between the ALE-treated groups on the level of mucus protease activity.

Serum bactericidal activity

The bactericidal activity of serum against *A. hydrophila* in the groups containing 0.5, 1 and 2 g/kg of ALE, in the dilutions of 1/2 and 1/4, showed no significant difference compared to the CTRL group (Fig. 9A; $P>0.05$). A significant difference in the bactericidal activity of serum against *A. hydrophila* was observed in the groups containing 0.5, 1 and 2 g/kg of ALE in the dilutions of 1/8, 1/16 and 1/32 compared to the CTRL group (Fig. 9B, $P<0.05$). Also, a significant difference in the bactericidal activity of serum against *A. hydrophila* was observed in 0.5 with 2 and 1 with 2 g/kg groups in the dilutions of 1/8, 1/16 and 1/32 (Fig. 9B, $P<0.05$).

Discussion

Experimental evidence clearly indicates that the plant extracts main potential is due to the presence of different phytochemicals of particular relevance to polyphenols, powerful natural antioxidants, anti-inflammatories and immunostimulants [26, 27]. The use of feed enriched by natural extracts rich in polyphenols is an increasingly widespread practice in terrestrial animal farming as well as in aquaculture to stimulate immune defenses and reduce the risk of diseases [28].

Growth performance is of critical importance for farmers due to the high costs of diet. The positive effect of polyphenols on growth parameters has been reported in literature [8, 9, 29–31]. However, there needs to be more reports about ALE's effects on growth performance. In a previous study of our research group, we noticed that dietary artichoke extract significantly improved growth performance in goldfish (*Carassius auratus*) [14]. The results of the present study contrast with some previous

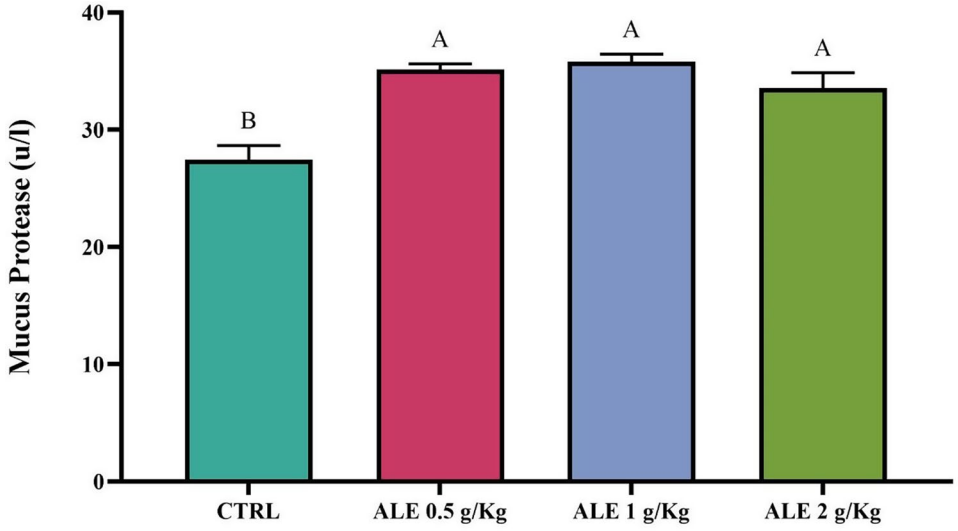

Fig. 8 Effect of different levels of ALE on mucus protease in *C. carpio* ($n = 9$ fish per group). CTRL: control; ALE: artichoke leaf extract. Identical Latin letters denote no significant difference, whereas different letters represent a significant difference. Significance levels were considered to be $P < 0.05$

studies that reported positive effects of artichoke extracts on fish growth performance. For instance, dietary tea polyphenols improved the growth performance of Wuchang bream (*Megalobrama amblycephala*) [32, 33]. These contradictory results underscore the fact that species, inclusion level, polyphenol type, and life stage can influence the effects of polyphenols on fish growth. In the case of ALE, it's worth noting that the presence of chlorogenic acid and caffeic acid has been shown to reduce body weight in obese mice [34, 35]. This highlights the need for further research to optimize the use of artichoke in carp diets, with a focus on the various aspects of polyphenols on fish growth. This research is of utmost importance and should be a priority for the aquaculture industry.

The study of haematological parameters is very important both in diagnostic as well as economic aspects. They are helpful in identifying diseases, the type of nutrition, and determining the health status of fish [36]. The results revealed that dietary ALE showed a significant effect on all blood parameters like RBCs, WBCs, HB, HCT, MCV, MCH and MCHC compared to the CTRL group. To the best of our knowledge, there is no report on the possible effects of ALE on the haematological parameters of fish. However, in line with the results of this research, the use of chestnut as a food supplement in carp diet showed a significant increase in haematological parameters [8]. Also, in another study, polyphenols obtained from canola significantly increased blood parameters in carp fingerlings [37]. However, it was reported that chestnut wood tannin extract had no significant effect on the blood parameters of beluga sturgeon and significantly increased WBCs [9]. WBCs are the main components of the non-specific immune response that regulate the function of the immune system in bony fish and serve a vital function in dealing with pathogens [20]. Likewise, we noticed a significant increase in WBCs of ALE-fed carp. This can be attributed to the beneficial effects of polyphenols on fish immune responses [14].

The innate immune system of fish consists of three main components, including physical barriers, humoral factors and cellular elements [38]. The physical barriers include scales, epithelial cells, and mucus layers that cover the digestive tract, gills, and skin, which play a crucial role in preventing infection [39]. The skin, in particular, serves as the first line of defense against pathogens due to its constant exposure to potential pathogens in the aquatic environment [39].

The results of this study showed that the use of different levels of ALE in the diet of common carp brought about a significant increase in serum and mucus immune parameters such as lysozyme, immunoglobulin, bactericidal activity, agglutination titer and ACH50. Lysozyme is an enzyme that destroys the cell walls of certain bacteria [20]. The use of different levels of ALE significantly increased serum and mucus lysozyme activity compared to the control group. The result agrees with previous findings on the immunomodulatory effects of artichokes in various fish species. Most recently, another study by S Mousavi, S Mohammadzadeh, S Mood, E Ahmadifar, N Sheikhzadeh, N Kalhor, M Moghadam, S Yilmaz, S Hoseinifar and M Paolucci [14] on goldfish (*Carassius auratus*) showed a significant increase in lysozyme activity of ALE-treated goldfish. Similar results in cases using other polyphenol-rich plant extracts, such olive polyphenols on serum lysozyme in Asian sea bass [30] and Chestnut polyphenols on serum and mucus lysozyme) in Nile tilapia [31] and common carp [8]. No clear evidence on the mode of action is available. It will be postulated,

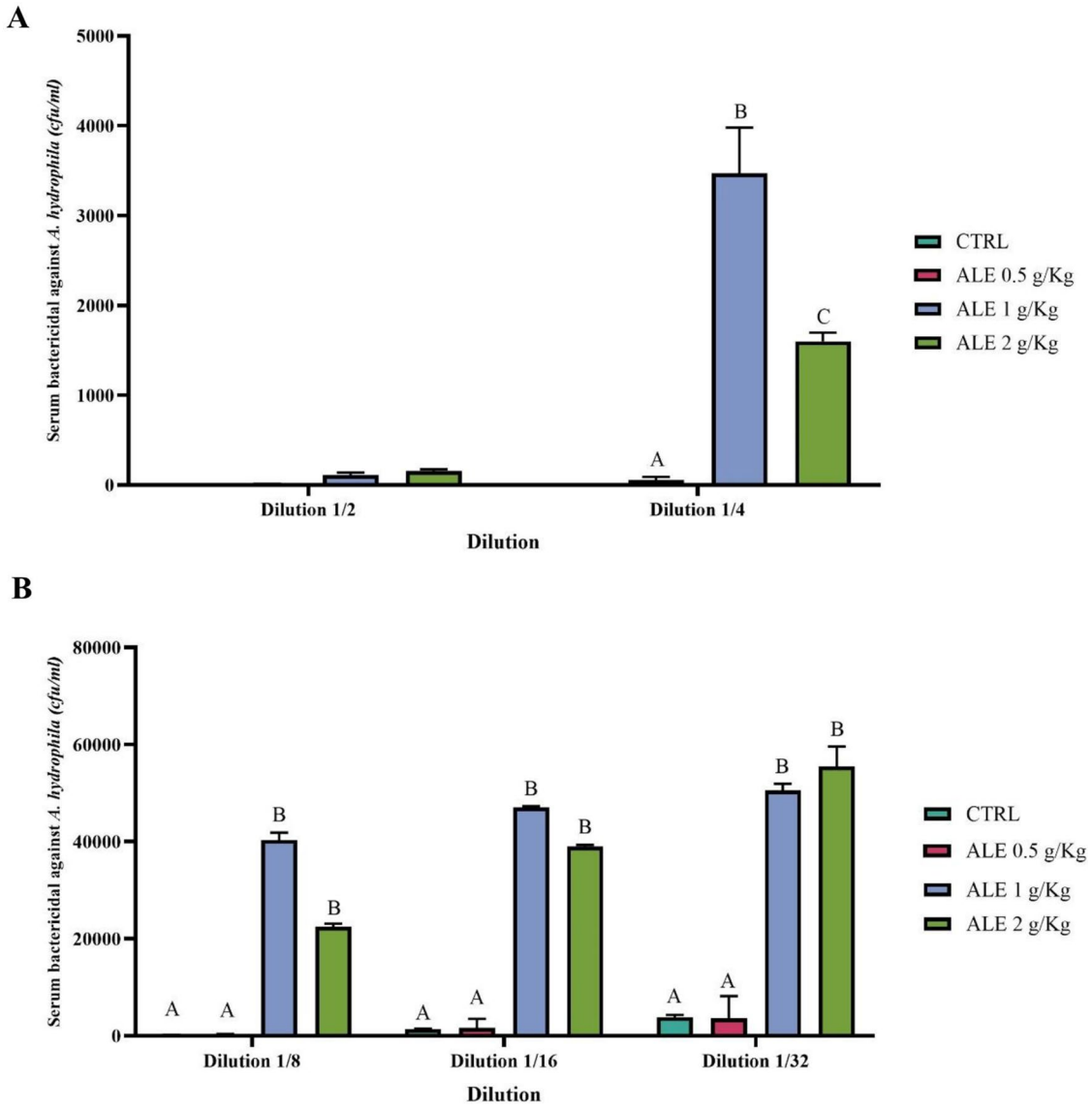

Fig. 9 A-B. Effect of different levels of ALE on serum bactericidal activity against *A. hydrophila* in *C. carpio* ($n=9$ fish per group). CTRL: control; ALE: artichoke leaf extract. Identical Latin letters denote no significant difference, whereas different letters represent a significant difference. Significance levels were considered to be $P<0.05$

however, that the artichoke's bioactive compounds will activate immune cells such as macrophages and lymphocytes responsible for producing lysozyme and other immune factors [7]. On the other hand, artichokes may also help in maintaining a healthy gut microbiota, which is important in the optimal absorption of nutrients and also in immunity [11]. A balanced gut microbiota would, therefore, support an overall healthy fish, indirectly implicating improved immune responses.

Immunoglobulins play a pivotal role in the immune response to combat pathogens [20]. Different levels of ALE significantly increased serum and mucus total immunoglobulin levels. Few studies have been conducted on the effect of polyphenols on serum or mucus total Ig levels. In accordance with the present findings, feeding goldfish with ALE significantly increased serum total Ig levels [14]. Similarly, carps treated with polyphenols extracted from chestnut and olive mill wastewater showed notable increases in serum and mucus total immunoglobulin levels [8]. The same results have been reported following feeding fish with tea or chestnut polyphenols [29, 33]. This increase can be attributed to the activation of immune cells, such as B lymphocytes, which causes an increase in immunoglobulin production [7].

There is no evidence about the effect of polyphenols on agglutination titer in aquatic animals, but there are few studies on ACH50. In this current study, the impact of ALE increased serum and mucus agglutination titer as

well as ACH50 levels in common carp. Likewise, a previous study showed that ALE can significantly increase the serum ACH50 activity of goldfish [14]. These findings are in accordance with other studies on other types of polyphenols, such as chestnut, Oak or olive waste polyphenols [13, 31, 40]. Enhancing the immune response of densely reared fish is a valuable approach to disease control, infection prevention, and mortality reduction. Research indicates the immunomodulatory effects of polyphenols [7]. Polyphenols are taken up by the intestine and engage with the intestinal immune system, triggering protective responses in the host [41]. Different varieties of immune cells express polyphenol receptors on their cell membrane, allowing the recognition and uptake of polyphenols, which subsequently initiate immune responses by activating signalling pathways [42].

Artichoke extract, being rich in polyphenols, contains many hydroxyl groups with high antioxidant potential [43]. The findings of this study demonstrated that the use of different levels of ALE in the diet of common carp has a significant effect on serum antioxidant enzymes such as SOD, CAT and GPx. Also, ALE had a significant increase in mucus protease enzyme. Several studies have shown the positive and significant effect of polyphenols on the antioxidant system of fish [7]. Parallel to our results, using ALE in a goldfish diet significantly increased antioxidant enzyme activity [14]. Such beneficial effects have been reported in cases of other polyphenols [8, 30, 31, 33, 40, 44]. It is now widely recognized that polyphenols possess antioxidant properties and can prevent the production of oxygen anions and neutralize free radicals [6]. Polyphenols' antioxidant characteristics are linked to the presence and quantity of phenolic rings. These rings act by capturing electrons and neutralizing free radicals, including anionic superoxide radicals and hydroxyl radicals. Polyphenols also possess the capacity to enhance the activity of enzymes involved in combating oxidative stress [6].

Conclusions

The findings of this study indicate that utilizing ALE in the diet of common carp increases and improves the non-specific immune parameters of serum and mucus. Also, dietary ALE could notably increase carp's antioxidant defense. Based on these results, ALE can be considered a natural bioactive substance, immune stimulant and oxidative stress reducer in carp culture. Although this article reports valuable points about ALE, there are limitations in this research that should be noted. To gain a clearer insight into the impact of ALE on fish physiology and immune function, conducting histopathological examinations and analyzing the expression of immune and antioxidant genes would be highly beneficial. We recommend that future research focuses on these aspects. Additionally, evaluating the effects of ALE on other cultured fish species could provide deeper insights into its mechanisms of action in aquatic organisms.

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Author contributions
E. Kia:. Fish culture and sampling, writing an original draft, data analysis and manuscript drafting. SH. Hoseinifar: Supervisor, conceptualization and study design, formal analysis, manuscript drafting, critical review, interpretation of results. M. Mazandarani: Supervisor, immunological parameters analysis, writing an original draft. R. Safari: Advisor, writing an manuscript drafting. V. jafari: Advisor. M. Paolucci: Advisor, preparation of artichoke polyphenolic extract, manuscript reviewing. All authors reviewed the manuscript.

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Data availability
All data produced or examined during this study are incorporated in this published article.

Declarations

Ethical approval
All experiments were performed following the protocol approved by the ethics committee of the faculty of sciences of the University of Tehran (357; 8 November 2000). All Iranian universities follow the mentioned protocol and the corresponding author is a graduate of Tehran University. The study was conducted in accordance with the local legislation and institutional requirements.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Vijayaram S, Ringø E, Ghafarifarsani H, Hoseinifar SH, Ahani S, Chou C-C. Use of algae in aquaculture: A review. Fishes. 2024;9(2):63.
2. Khanzadeh M, Beikzadeh B, Hoseinifar SH. The Effects of Laurencia caspica Algae Extract on Hemato-Immunological Parameters, Antioxidant Defense, and Resistance against Streptococcus agalactiae in Nile tilapia (Oreochromis niloticus). Aquaculture Nutrition. 2023;2023.
3. Llewellyn MS, Boutin S, Hoseinifar SH, Derome N. Teleost microbiomes: the state of the Art in their characterization, manipulation and importance in aquaculture and fisheries. Front Microbiol. 2014;5:207. https://doi.org/10.3389/fmicb.2014.00207.
4. Erfanmanesh A, Beikzadeh B, Khanzadeh M. Efficacy of polyvalent vaccine on immune response and disease resistance against streptococcosis/lactococcosis and yersiniosis in rainbow trout (Oncorhynchus mykiss). Vet Res Commun. 2023;47(3):1347–55.
5. Van Doan H, Sumon MAA, Tran HQ, Le CX, Mohammady EY, El-Haroun ER, et al. Role of β-glucan on finfish and shellfish health and well-being: A systematic review and meta-analysis. Reviews in Aquaculture; 2024.
6. Bešlo D, Golubić N, Rastija V, Agić D, Karnaš M, Šubarić D, et al. Antioxidant activity, metabolism, and bioavailability of polyphenols in the diet of animals. Antioxidants. 2023;12(6):1141.
7. Ahmadifar E, Yousefi M, Karimi M, Fadaei Raieni R, Dadar M, Yilmaz S, et al. Benefits of dietary polyphenols and polyphenol-rich additives to

aquatic animal health: an overview. Reviews Fisheries Sci Aquaculture. 2021;29(4):478–511.
8. Jahazi MA, Hoseinifar SH, Jafari V, Hajimoradloo A, Van Doan H, Paolucci M. Dietary supplementation of polyphenols positively affects the innate immune response, oxidative status, and growth performance of common carp, Cyprinus carpio L. Aquaculture. 2020;517:734709.
9. Safari R, Hoseinifar SH, Imanpour MR, Mazandarani M, Sanchouli H, Paolucci M. Effects of dietary polyphenols on mucosal and humoral immune responses, antioxidant defense and growth gene expression in beluga sturgeon (Huso huso). Aquaculture. 2020;528:735494.
10. Ben Salem M, Affes H, Athmouni K, Ksouda K, Dhouibi R, Sahnoun Z, et al. Chemicals compositions, antioxidant and anti-inflammatory activity of cynara scolymus leaves extracts, and analysis of major bioactive polyphenols by HPLC. Evidence-Based Complement Altern Med. 2017;2017(1):4951937.
11. Wittemer S, Ploch M, Windeck T, Müller S, Drewelow B, Derendorf H, et al. Bioavailability and pharmacokinetics of caffeoylquinic acids and flavonoids after oral administration of artichoke leaf extracts in humans. Phytomedicine. 2005;12(1–2):28–38.
12. Rodríguez-Daza MC, Pulido-Mateos EC, Lupien-Meilleur J, Guyonnet D, Desjardins Y, Roy D. Polyphenol-mediated gut microbiota modulation: toward prebiotics and further. Front Nutr. 2021;8:689456.
13. Hoseinifar SH, Shakouri M, Yousefi S, Van Doan H, Shafiei S, Yousefi M, et al. Humoral and skin mucosal immune parameters, intestinal immune related genes expression and antioxidant defense in rainbow trout (Oncorhynchus mykiss) fed Olive (Olea Europea L.) waste. Fish Shellfish Immunol. 2020;100:171–8.
14. Mousavi S, Mohammadzadeh S, Mood S, Ahmadifar E, Sheikhzadeh N, Kalhor N, et al. Dietray artichoke (Cynara scolymus) extract ameliorated the growth performance, humoral immune parameters and resistance against Aeromonas hydrophila in goldfish (Carassius auratus). Annals of Animal Science; 2024.
15. Khanzadeh M, Hoseinifar SH, Zargari A, Tabibi H, Van Doan H, Rabetimarghezar N. Fucoidan derived from Sargassum ilicifolium affects growth and hemato-immunological parameters and antioxidant defense in oscar (Astronotus ocellatus). Front Mar Sci. 2024;11:1370871.
16. Subramanian S, MacKinnon SL, Ross NW. A comparative study on innate immune parameters in the epidermal mucus of various fish species. Comp Biochem Physiol B: Biochem Mol Biol. 2007;148(3):256–63.
17. Grant KR. Fish hematology and associated disorders. Veterinary Clinics: Exotic Anim Pract. 2015;18(1):83–103.
18. Subramanian D, Jang Y-H, Kim D-H, Kang B-J, Heo M-S. Dietary effect of Rubus Coreanus ethanolic extract on immune gene expression in white leg shrimp, *Penaeus vannamei*. Fish Shellfish Immunol. 2013;35(3):808–14.
19. Siwicki AK, Anderson DP. Nonspecific defense mechanisms assay in fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total Immunoglobulin level in serum. Poland: Fish Disease Diagnosis and Prevention Methods Olsztyn; 1993. pp. 105–12.
20. Tort L, Balasch J, Mackenzie S. Fish immune system. A crossroads between innate and adaptive responses. Inmunología. 2003;22(3):277–86.
21. Barnes AC, Ellis AE. Role of capsule in serotypic differences and complement fixation by Lactococcus garvieae. Fish Shellfish Immunol. 2004;16(2):207–14.
22. Khalil SMI, Bulfon C, Galeotti M, Acutis PL, Altinok I, Kotzamanidis C, et al. Immune profiling of rainbow trout (Oncorhynchus mykiss) exposed to Lactococcus garvieae: evidence in asymptomatic versus symptomatic or vaccinated fish. J Fish Dis. 2023;46(7):731–41.
23. Rao YV, Das B, Jyotyrmayee P, Chakrabarti R. Effect of Achyranthes aspera on the immunity and survival of Labeo rohita infected with Aeromonas hydrophila. Fish Shellfish Immunol. 2006;20(3):263–73.
24. Zoheiri F, Hoseinifar SH, Mozanzadeh MT, Ahangarzadeh M, Lieke T, Van Doan H. Dietary fulvic acid increased growth, stress tolerance and disease resistance against Vibrio harveyi in Asian Seabass (Lates calcarifer) juvenile. Aquaculture Rep. 2023;32:101738.
25. Yazici M, Zavvar F, Hoseinifar SH, Nedaei S, Doan HV. Administration of red macroalgae (Galaxaura oblongata) in the diet of the rainbow trout (Oncorhynchus mykiss) improved immunity and hepatic gene expression. Fishes. 2024;9(2):48.
26. Vijayaram S, Razafindralambo H, Ghafarifarsani H, Sun Y-Z, Hoseinifar SH, Van Doan H. Synergetic response on herbal and probiotic applications: a review. Fish Physiol Biochem. 2024:1–15.
27. Raissy M, Ghafarifarsani H, Hoseinifar SH, El-Haroun ER, Naserabad SS, Van Doan H. The effect of dietary combined herbs extracts (oak Acorn, coriander, and common mallow) on growth, digestive enzymes, antioxidant and immune response, and resistance against Aeromonas hydrophila infection in common carp, Cyprinus carpio. Aquaculture. 2022;546:737287.
28. Hoseinifar SH, Sun Y-Z, Zhou Z, Van Doan H, Davies SJ, Harikrishnan R. Boosting immune function and disease Bio-Control through Environment-Friendly and sustainable approaches in finfish aquaculture: herbal therapy scenarios. Reviews Fisheries Sci Aquaculture. 2020:1–19.
29. Hoseinifar SH, Jahazi A, Nikdehghan N, Van Doan H, Volpe MG, Paolucci M. Effects of dietary polyphenols from agricultural by-products on mucosal and humoral immune and antioxidant responses of convict cichlid (Amatitlania nigrofasciata). Aquaculture. 2019:734790.
30. Ahmadi A, Bagheri D, Hoseinifar SH, Morshedi V, Paolucci M. Beneficial role of polyphenols as feed additives on growth performances, immune response and antioxidant status of Lates Calcarifer (Bloch, 1790) juveniles. Aquaculture. 2022;552:737955.
31. Van Doan H, Hoseinifar SH, Hung TQ, Lumsangkul C, Jaturasitha S, El-Haroun E, et al. 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. 2020;105:319–26.
32. Long M, Lin W, Hou J, Guo H, Li L, Li D, et al. Dietary supplementation with selenium yeast and tea polyphenols improve growth performance and nitrite tolerance of Wuchang Bream (Megalobrama amblycephala). Fish Shellfish Immunol. 2017;68:74–83.
33. Pan S, Yan X, Li T, Suo X, Liu H, Tan B, et al. Impacts of tea polyphenols on growth, antioxidant capacity and immunity in juvenile hybrid grouper (Epinephelus fuscoguttatus♀× E. lanceolatus♂) fed high-lipid diets. Fish Shellfish Immunol. 2022;128:348–59.
34. Cho A-S, Jeon S-M, Kim M-J, Yeo J, Seo K-I, Choi M-S, et al. Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. Food Chem Toxicol. 2010;48(3):937–43.
35. Xu J, Ge J, He X, Sheng Y, Zheng S, Zhang C, et al. Caffeic acid reduces body weight by regulating gut microbiota in diet-induced-obese mice. J Funct Foods. 2020;74:104061.
36. Rufchaei R, Nedaei S, Hoseinifar SH, Hassanpour S, Golshan M, Sayad Bourani M. Improved growth performance, serum and mucosal immunity, haematology and antioxidant capacity in pikeperch (Sander lucioperca) using dietary water hyacinth (Eichhornia crassipes) leaf powder. Aquac Res. 2021;52(5):2194–204.
37. Hussain SM, Gohar H, Asrar M, Shahzad MM, Rasul A, Hussain M, et al. Effects of polyphenols supplemented Canola meal based diet on proximate composition, minerals absorption and hematology of Cyprinus carpio fingerlings. Pakistan J Zool. 2020;54(3):1071–9.
38. Mokhtar DM, Zaccone G, Alesci A, Kuciel M, Hussein MT, Sayed RK. Main components of fish immunity: an overview of the fish immune system. Fishes. 2023;8(2):93.
39. Esteban MÁ. A review of soluble factors and receptors involved in fish skin immunity: the tip of the iceberg. Fish Shellfish Immunol. 2023:109311.
40. Zhao Z, Zhao F, Cairang Z, Zhou Z, Du Q, Wang J, et al. Role of dietary tea polyphenols on growth performance and gut health benefits in juvenile hybrid sturgeon (Acipenser baerii♀× A. schrenckii♂). Fish Shellfish Immunol. 2023;139:108911.
41. Sobhani M, Farzaei MH, Kiani S, Khodarahmi R. Immunomodulatory; anti-inflammatory/antioxidant effects of polyphenols: a comparative review on the parental compounds and their metabolites. Food Rev Int. 2021;37(8):759–811.
42. Ding S, Jiang H, Fang J. Regulation of immune function by polyphenols. J Immunol Res. 2018;2018(1):1264074.
43. Garcia-Castello EM, Mayor L, Calvo-Ramirez A, Ruiz-Melero R, Rodriguez-Lopez AD. Response surface optimization of inulin and polyphenol extraction from artichoke (Cynara scolymus (L.)) solid wastes. Appl Sci. 2022;12(16):7957.
44. Imperatore R, Orso G, Facchiano S, Scarano P, Hoseinifar SH, Ashouri G, et al. Anti-inflammatory and immunostimulant effect of different timing-related administration of dietary polyphenols on intestinal inflammation in zebrafish, Danio rerio. Aquaculture. 2023;563:738878.

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