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Original article

Effects of dietary administration of *Echinacea purpurea* on growth indices and haematological indices in common carp (*Cyprinus carpio*)

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Abstract

This study was conducted to determine the effects of *Echinacea purpurea* (EP) root on the growth performance and some haematological parameters of juvenile common carp (*Cyprinus carpio*). Echinacea meal was incorporated into the diets as follows: no Echinacea meal (E-0), 5 g Echinacea meal kg^{-1} diet (E-5), 10 g Echinacea meal kg^{-1} diet (E-10) and 20 g Echinacea meal kg^{-1} diet (E-20). Triplicate groups of fish averaging 9.6 ± 0.5 g were hand-fed twice daily for 60 days. At the end of the feeding trial, weight gain (WG), feed conversion ratio (FCR), and specific growth rate (SGR) were significantly higher in fish fed the Echinacea-supplemented diets than in those fed the control diet. Red blood cell count (RBC) and white blood cell count (WBC) were significantly higher in the E-10 and E-20 groups than in the E-0 group. Fish fed the E-10 diet had the highest haematocrit (Hct), and haemoglobin (Hb) among all treatments. The nitroblue tetrazolium (NBT) assay showed a significant increase in the E-10 and E-20 groups compared to the E-0 and E-5 groups.

After a 14-day challenge with *Aeromonas hydrophila*, Kaplan-Meier analysis revealed significant differences in survival between treatments. Survival of carp following the challenge was significantly higher in fish fed the E-10 and E-20 diets than in the E-0 group, whereas the E-5 group showed no significant difference compared to the E-0 group.

In conclusion, our findings revealed that supplementing diets with EP root (10 g EP kg^{-1} and 20 g EP kg^{-1} diet) can enhance growth performance, haematological parameters, and resistance to *A. hydrophila* in juvenile common carp.

Keywords: carp, diet, *Echinacea purpurea*, fish, hematology

Introduction

Echinacea purpurea (EP), belonging to the Asteraceae (Compositae) family, is one of the most important and wellknown medicinal plants in the world. Its biological properties have been attributed to several classes of bioactive compounds (Manayi et al. 2015). The majority of studies indicate that EP exerts immunomodulatory effects through the action of both innate and adaptive immune cells (Zhai et al. 2007). The plant has shown antibacterial, antiviral, antifungal, and antioxidant properties and is used in humans to treat common colds, flu, and respiratory and urinary diseases (Oniszczuk et al. 2019, Raissy et al. 2021). It has markedly ameliorated altered immunological, haematological, and biochemical features without any side effects (Bany et al. 2003, Manayi et al. 2015).

Several groups of bioactive compounds with pharmacological activity have been isolated from EP. The most important components of EP are alkamides, polysaccharides, glycoproteins, flavonoids, and phenolic compounds (Burlou-Nagy et al. 2022). These bioactive compounds can boost the immune response and have been proposed as alternatives to antibiotics for controlling fish diseases (Aly et al. 2008, Aly and Mohamed 2010, Guz et al. 2011, Caruana et al. 2012, Guz et al. 2014, Rohani et al. 2016, Fadeifard et al 2018, Rahman et al. 2018, Alinezhad 2019, Oniszczuk et al. 2019, Burlou-Nagy et al. 2022). Ethanolic extract of EP has shown antibacterial activity against *Streptococcus pyogenes*, *Haemophilus influenza*, and *Legionella pneumophila*, whereas *Acinetobacter baumannii*, *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were relatively resistant (Sharma et al. 2008, 2010).

Diet supplementation with EP has been shown to effectively enhance the response of zebrafish (*Danio rerio*) to a *Flavobacterium columnare* vaccine (Guz et al. 2014) and the response of Nile tilapia (*Oreochromis niloticus*) to an *Aeromonas hydrophila* vaccine (Aly et al. 2016). Moreover, EP has been reported to promote growth in *O. niloticus* (El-Sayed et al. 2014), *Oncorhynchus mykiss* (Gabor et al. 2011), *Pterophyllum scalare* (Kasiri et al. 2011), *Poecilia reticulata* (Guz et al. 2011), *Huso huso* (Nazerian et al. 2016), *Cyprinus carpio* (Alishahi et al. 2012), *Mugil cephalus* (Akbary and Kakoolaki 2019), and *Acipenser baerii* (Khajehpour and Javadian 2020).

In this context, EP, one of the most clinically studied herbal medicines, has been suggested to be an important and useful antibacterial agent. Consequently, this study was designed to evaluate the effect of dietary EP on growth performance, haematology, and disease resistance in juvenile common carp (*C. carpio*).

Materials and Methods

Diets

Four diets were formulated to contain different levels of EP root. The formulation and proximate composition of the experimental diets are shown in Table 1. Echinacea meal was incorporated into the diets as follows: no Echinacea meal (control), 5 g Echinacea meal kg^{-1} diet (group E-5), 10 g Echinacea meal kg^{-1} diet (group E-10), and 20 g Echinacea meal kg^{-1} diet (group E-20). The Echinacea plant meal used in this study was provided by Herbal Farm – Waldemar Lupa, Kolonia Dobryniów, Poland. Extruded feeds were produced at the Department of Food Process Engineering, University of Life Sciences in Lublin, Poland, as described in a previous study by Guz et al. (2011).

Fish and experimental conditions

The carp (*C. carpio* L.) were obtained from a commercial fish breeder (Samoklęski Fish Farm, Poland). The fish were determined to be pathogen-free using standard microbiological techniques. Fourteen days prior to the start of the trial, the fish were held in a 1000-L tank. Fish were fed a commercial feed (Aller Master, containing 35% protein and 9% fat, Aller Aqua Poland) prior to the start of the trial. After the acclimation period, the average weight of the fish was 9.6±0.5 g. The fish were randomly distributed to a flow-through system with 12 experimental 100-L aquariums, with four treatments, three replicates, and 13 fish per replicate. The fish were kept for eight weeks at 22±1°C, pH 7.5±0.2, and dissolved oxygen 6.5±0.5 mg/L, with a natural photoperiod. The experimental diets were hand-fed to triplicate aquariums twice daily (at 09.00 and 15.00 h). The uneaten feed was removed 30 min after feeding. The fish were weighed every two weeks from the start of the experiment. Before weighing, the fish were starved for 24 h to empty the gut. During handling, the fish were anaesthetized using a solution of tricaine methanesulfonate (MS-222, Sandoz LTD, Basle, Switzerland) at a concentration of 0.1 g L^{-1}. The study was carried out under experimental protocols approved by the Local Ethics Committee for Experiments on Animals (license no. 69/2011).

Growth performance

After the eight-week feeding trial, fish selected from each tank were weighed. The initial weight (Wi), final weight (Wf), specific growth rate (SGR), weight gain (WG), and feed conversion ratio (FCR) were calculated with the following equations: WG = Wf – Wi, FCR = feed intake / weight gain, SGR = 100 x [(ln Wf – ln Wi)

Table 1. Formulation (g kg^{-1}), chemical composition (g kg^{-1}) and metabolic energy (MJ kg^{-1}) of the tests diets.

Ingrediens (g kg^{-1} diet)	Groups			
	E-0	E-5	E-10	E-20
Wheat sharps[1]	208	209	209	199
Yellow lupine[1]	100	100	100	100
Soybean sharps[1]	325	319	314	314
Fodder yeast[1]	45	45	45	45
Soybean oil[1]	89	89	89	89
II-calcium phosphate[1]	9	9	9	9
Chalk fodder[1]	12	12	12	12
Vitamin C[3]	1	1	1	1
Fish meal[1,4]	200	200	200	200
Vit/min mix[2,5]	11	11	11	11
Echinacea root	-	5	10	20
Proximate composition				
Crude protein	386.4	388.2	384.4	379.9
Crude fibre	66.9	62.7	62.8	72.1
Crude fat	95.4	96.1	95.6	94.9
Total Ca	14.8	13.7	13.8	14.0
Total P	7.2	7.3	7.1	7.2
Estimated metabolic energy (MJ kg^{-1})[6]	15.15	15.44	15.34	14.82

[1] The source is Animex Grupa Drobiarska SA, Zamość, Poland.
[2] The source is BASF Polska, Kutno, Poland.
[3] The source is POCH, Gliwice, Poland.
[4] Crude protein, 720 g kg^{-1}.
[5] Vit/Min mix (IU or g kg^{-1} diet): vitamin A – 4400.00 IU, vitamin D_3 – 680.00 IU, vitamin E – 0.006 g, thiamin – 0.0006 g, riboflavin – 0.0012 g, pyridoxine – 0.0008 g, vitamin B_{12} – 0.006 g, folic acid – 0.00016 g, biotin – 0.00004 g, niacin – 0.02 g, Ca – 0.004 g, Mn – 0.016 g, Zn – 0.02 g.
[6] The estimated dietary metabolic energy was calculated in accordance with the Polish Committee for Standarization, PKN, PN-A-79011-6 (1998).

/ days], where Wf is the mean final weight and Wi is the mean initial weight.

Blood collection and analysis

For blood sampling, fish were anaesthetized with tricaine methanesulfonate (MS-222, Sigma Chemical Co., MO, USA). At the end of the feeding experiment, blood was drawn from the caudal vein (three fish per aquarium diet group) into tubes with an anticoagulant (10 IU/mL of heparin). Red blood cells (RBC) and white blood cells (WBC) were counted with Bürker chambers, at 100 times dilution with Hayem's diluting solution. Haematocrit (Hct) was determined by spinning blood samples in heparinized capillary tubes in a micro-haematocrit centrifuge and expressed as the percentage of total blood volume. Haemoglobin concentration (Hb) was measured by the spectrophotometric cyanmethemoglobin method at a 540 nm wavelength in blood mixed 1:10 with Drabkin reagent (Sigma-Aldrich, Poznan, Poland). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were established by standard formulas from the haematocrit, blood haemoglobin, and red cell count values: MCV (fL) = Hct x 10 / RBC (10^6 µL^{-1}); MCH (pg) = [Hb (g dL^{-1}) x 10] / RBC (10^6 µL^{-1}); MCHC (%) = [Hb (g dL^{-1}) x 100] / Hct. Differential counts of leukocytes (monocytes, lymphocytes, neutrophils, eosinophils, and basophils) were determined using the May-Grunwald and Giemsa staining method, and blood smears were identified under a light microscope (Jenaval, Carl Zeiss Jena, Germany).

Nitroblue tetrazolium assay

Production of oxygen radicals from phagocytes in the blood was measured using nitroblue tetrazolium (NBT) dye as described by Anderson and Siwicki (1995), with minor modifications. Briefly, blood and 0.2% NBT were mixed in equal proportions (1:1) and incubated for 30 min at 25°C, and then a 5 µL sample was taken out and dispensed in tubes. For solubilization of the reduced formazan product, 100 µL

Table 2. Effect of *Echinacea purpurea* on growth performance of *Cyprinus carpio* in the 8 weeks feeding trial. All data were expressed as mean ± SEM (n=30). Mean values within the same row with different superscripts are significantly different ($p<0.05$).

Parameters	Groups			
	E-0	E-5	E-10	E-20
Initial weight	9.60 ± 0.07^{a}	9.56 ± 0.06^{a}	9.65 ± 0.06^{a}	9.63 ± 0.06^{a}
2 weeks weight	11.31 ± 0.05^{a}	11.62 ± 0.04^{b}	11.81 ± 0.08^{cbd}	12.08 ± 0.04^{d}
4 weeks weight	16.97 ± 0.21^{a}	18.49 ± 0.30^{b}	19.64 ± 0.18^{cd}	19.82 ± 0.22^{d}
6 weeks weight	23.00 ± 0.12^{a}	24.52 ± 0.32^{b}	25.67 ± 0.18^{cd}	25.69 ± 0.18^{d}
Final weight (8 weeks)	31.59 ± 0.35^{a}	32.74 ± 0.39^{a}	34.54 ± 0.26^{b}	34.56 ± 0.28^{b}
Mean WG	21.98 ± 0.46^{a}	23.18 ± 0.25^{a}	24.89 ± 0.30^{b}	24.93 ± 0.20^{b}
FCR	1.74 ± 0.04^{a}	1.66 ± 0.03^{ab}	1.55 ± 0.03^{b}	1.53 ± 0.01^{b}
SGR	2.12 ± 0.03^{a}	2.19 ± 0.02^{ab}	2.27 ± 0.03^{b}	2.28 ± 0.03^{b}
Survival rate	100	100	100	100

WG – weight gain, FCR – feed conversion rate, SGR – specific growth rate.

of N,N-dimethylformamide (Sigma-Aldrich, Saint Louis, USA) was added, followed by centrifuging at 3000x g for 5 min. Finally, the absorbance was read at 540 nm using a spectrophotometer. N,N-dimethylformamide was used as the blank.

Preparation of bacteria and challenge test

The test strain of pathogenic *Aeromonas hydrophila* used for challenge was originally isolated from diseased common carp. The isolated *A. hydrophila* were kept frozen in 20% glycerol, 80% Tryptic Soy Broth (TSB) in aliquots at -70°C until use. The *A. hydrophila* were identified by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS) (Bruker Daltonics, Germany) at the Department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Poland. Mass spectra were processed using Flex Analysis (Bruker Daltonics, Germany) and Biotyper 3.0 software (Bruker Daltonics, Germany). Biotyper log(score) values of 2.300-3.000 indicated highly probable species identification, which was further confirmed using API-20E microbiological tests (bioMerieux, France).

The challenge was performed eight weeks after initial diet administration with 10 fish per tank. Fish from each group were inoculated intraperitoneally with 0.1 ml of a 24-h culture (4.3 x 10^7 CFU/mL) of pathogenic *A. hydrophila*. The challenged fish were monitored twice a day for 14 days to document clinical signs and mortality. The protective efficacy of the Echinacea diet was evaluated by calculating the cumulative percent mortality (CPM) of each treatment and relative percent survival (RPS) of the experimental groups for 14 days after the challenge using the following formulas: CPM = (number of dead fish / total number of fish) x 100, RPS = 1 − (% mortality in test group / % mortality in control group) x 100. At the end of the challenge experiment, all surviving fish were counted, euthanized with 200 mg/L MS-222, and properly disposed of.

Statistical analysis

The data were subjected to one-way ANOVA to test the effect of the dietary treatments on fish. Additionally, the Kolmogorov–Smirnov test was used to determine the normality of the data, and Levene's test was used to test the homogeneity of variance before conducting ANOVA. When a significant difference was detected in one-way ANOVA, Scheffe's test was used to rank the groups. In the survival experiments, Kaplan-Meier cumulative survival curves were analysed for statistical significance with the log-rank test, and significant differences are presented if the p-value was less than $p \leq 0.05$. Statistical analyses were performed using PQStat (version 1.6.8.408) and Statistica 8.0 (StatSoft, Kraków, Poland) with a significance level of $p \leq 0.5$. The data are presented as mean ± standard error of the mean (SEM).

Results

Data on the growth performance of *C. carpio* are shown in Table 2. Over eight weeks of feeding, the body weight of the fish increased to 31.59±0.35, 32.74±0.39, 34.54±0.26, and 34.56±0.28 g, respectively, in the E-0, E-5, E-10, and E-20 groups. Final body weight, mean weight gain, and SGR significantly increased as the dietary Echinacea level increased ($p<0.05$). The Echinacea diet also influenced the FCR. The E-10 and E-20 groups showed significantly better FCRs than the control group ($p<0.05$). The survival rate was not affected by dietary Echinacea.

Table 3. Haematological and immunological parameters of *C. carpio* fed dietary *Echinacea purpurea* for 8 weeks. All data were expressed as mean ± SEM (n=9). Mean values with different superscripts in the same row are significantly different from each other ($p<0.05$).

Blood parameters	Groups			
	E-0	E-5	E-10	E-20
Hct (%)	25.78 ± 0.52^{a}	27.11 ± 0.42^{ab}	27.66 ± 0.37^{b}	27.44 ± 0.44^{ab}
Hb (g dL^{-1})	7.30 ± 0.08^{a}	7.43 ± 0.09^{ab}	7.70 ± 0.11^{b}	7.66 ± 0.10^{ab}
RBC (10^{6} µL^{-1})	1.31 ± 0.05^{a}	1.35 ± 0.04^{ab}	1.51 ± 0.05^{b}	1.52 ± 0.04^{b}
WBC (10^{3} µL^{-1})	35.11 ± 0.67^{a}	35.88 ± 0.53^{ab}	37.55 ± 0.47^{b}	37.44 ± 0.47^{b}
MCV (fL)	199.02 ± 8.34^{a}	201.89 ± 7.70^{a}	183.58 ± 5.47^{a}	180.77 ± 5.97^{a}
MCH (pg)	56.31 ± 1.96^{a}	55.27 ± 1.76^{a}	51.14 ± 1.69^{a}	50.46 ± 1.46^{a}
MCHC (%)	28.41 ± 0.68^{a}	27.46 ± 0.51^{a}	27.84 ± 0.28^{a}	27.99 ± 0.63^{a}
Lymphocyte (%)	92.59 ± 0.62^{a}	92.78 ± 0.43^{a}	94.06 ± 0.48^{a}	94.07 ± 0.42^{a}
Monocyte (%)	1.13 ± 0.04^{a}	1.22 ± 0.03^{a}	1.27 ± 0.03^{a}	1.24 ± 1.03^{a}
Neutrophil (%)	3.69 ± 0.20^{a}	4.00 ± 0.21^{ab}	4.55 ± 0.21^{b}	4.12 ± 0.19^{ab}
Eosinophil (%)	0.25 ± 0.09^{a}	0.21 ± 0.06^{a}	0.31 ± 0.09^{a}	0.30 ± 0.10^{a}
Basophil (%)	0.35 ± 0.19^{a}	0.27 ± 0.11^{a}	0.21 ± 0.09^{a}	0.24 ± 0.08^{a}
NBT	0.34 ± 0.03^{a}	0.38 ± 0.02^{a}	0.48 ± 0.03^{b}	0.51 ± 0.03^{bc}

Hct – haematocrit, Hb – haemoglobin, RBC – red blood cell, WBC – white blood cells, MCV – mean erythrocyte volume, MCH – mean erythrocyte haemoglobin, MCHC – mean erythrocyte haemoglobin concentration, NBT – nitroblue tetrazolium.

The ranges for haematological parameters MCV, MCH, MCHC, lymphocytes, monocytes, eosinophils, and basophils in fish were 180.77-201.89 fL, 50.46-56.31 pg, 27.46-28.41%, 92.59-94.07%, 1.13-1.27%, 0.21-0.31%, and 0.21-0.35%, respectively, with no significant differences between treatments (Table 3). RBC and WBC counts were significantly higher in groups E-10 and E-20 than in group E-0, whereas neutrophils were significantly higher in group E-10 than in group E-0 (Table 3). Fish in the E-10 group had the highest Hct (27.66%) and Hb (7.70 g dL^{-1}); these values were significantly higher than in the control group (E-0), which had Hct of 25.78% and Hb of 7.30 g dL^{-1} (Table 3). The results of the NBT assay in fish ranged from 0.34 to 0.51 and were significantly increased in groups E-10 and E-20 compared to E-0 and E-5 ($p<0.05$) (Table 3).

Survival of fish fed the experimental diets containing various additives for eight weeks and then infected with *A. hydrophila* (MALDI-TOF-MS Biotyper log(score) 2.309 and API-20E code no. 7246125) during the 14-day post observation period is shown in Fig. 1 and Table 4. The cause of death of all fish that died in the challenge trials was *A. hydrophila*, as determined by bacterial isolation from the head kidney. Kaplan-Meier analysis revealed significant differences in survival between treatments (log-rank; $\chi^2 = 12.49$, df = 3, p = 0.006). Survival of carp following challenge was significantly higher in fish fed the E-10 and E-20 diets compared to the control ($\chi^2_{E1.0} = 6.32$, df = 1, p = 0.012 and $\chi^2_{E2.0} = 9.04$, df = 1, p = 0.003), whereas dietary group E-5 did not differ significantly from the control ($\chi^2_{E0.5} = 2.66$, df = 1, p = 0.103) group (Fig. 1).

Discussion

Owing to their beneficial properties, many medicinal plants and their bioactive compounds have been used in fish as an alternative to antibiotics and as immunoprophylactic agents for several years (Manayi et al. 2015, Stratev et al. 2018, Raissy et al. 2021, Effendi et al. 2022).

Kesbiç et al. (2022) demonstrated the effect of extract generated from tomato paste by-products on the growth and blood parameters of carp. Acar et al. (2021) showed the impact of essential oil from bitter orange (*Citrus aurantium*) on growth efficiency, liver and intestine histological status, and the increased expression of genes (growth hormone and insulin-like growth factor) related to muscle growth, as well as TNF-α, IL-8, and IL-1β genes associated with the immune status of common carp. Kesbiç et al. (2020) revealed the influence of essential oil from cypress leaves (*Cupressus macrocarpa*) on growth, hematological, and biochemical parameters in carp. Zemheri-Navruz et al. (2020) observed the effect of olive leaf extract on growth, digestive enzyme activity, and gene expression related to growth in carp. Moreover, there are numerous studies regarding the immunostimulatory effects of EP on humans and animals (Aly et al. 2008, Guz et al. 2011, 2014, Oniszczuk et al. 2019, Raisy et al. 2021). It has been shown that extracts from EP

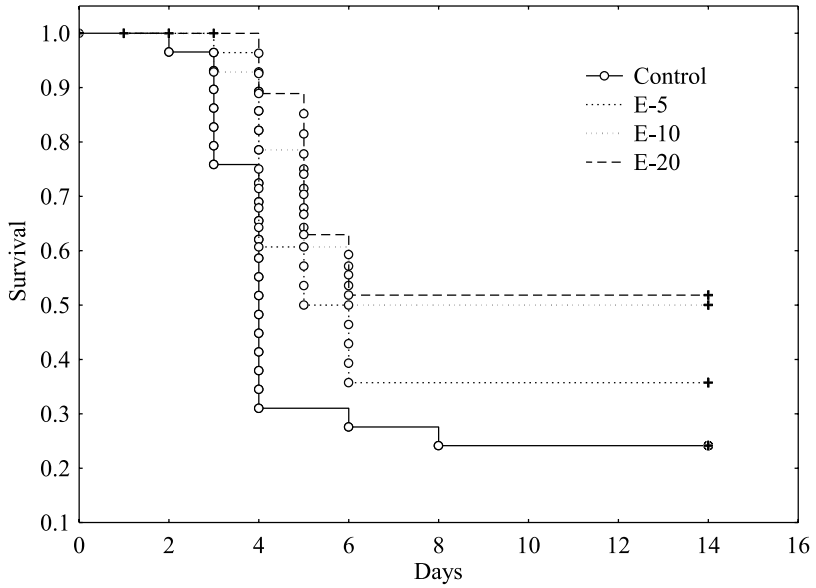

Fig. 1. Kaplan-Meier survival curve analysis (log-rank) of pooled replicates for each feed groups.

Table 4. Means in the column with different superscripts are significantly different ($p<0.05$).

Group	Challenge dose (CFU/mL)	Mortality in replicate groups	CPM (% ± SEM)	RPS (%)
Control	4.3 x 10^7	7/10, 7/10, 8/10	73.33 ± 0.33^a	-
E-5	4.3 x 10^7	6/10, 6/10, 6/10	60.00 ± 0.00^{ab}	18
E-10	4.3 x 10^7	4/10, 5/10, 5/10	46.67 ± 0.33^b	36
E-20	4.3 x 10^7	3/10, 5/10, 5/10	43.33 ± 0.67^b	41

CPM – cumulative percent mortality, RPS – relative percent survival, CFU – colony forming units.

enhance the innate immune response and support the immune system in combating viral and bacterial diseases. The secondary metabolites of EP that exhibit this effect are mainly alkamides, polysaccharides, and derivatives of caffeic acid. Alkamides, by acting on the cannabinoid receptor type 2 (CB2), likely increase the level of cyclic adenosine monophosphate, activating p38/MAPK and JNK protein kinases, as well as NF-κB and ATF-2/CREB-1 factors (Manayi et al. 2015). It has also been shown that they induce an increase in IL-10 levels and a decrease in IL-6, IL-8, and TNFα. Another bioactive compound of EP is polysaccharides. By acting on macrophages, they enhance the production of IL-1, IL-6, and TNFα and stimulate phagocytosis (Vieira et al. 2022). Among the derivatives of caffeic acid, the most important compound found in large quantities in the root of EP is chicoric acid. It is a strong antioxidant, exhibits antibacterial properties, and supports the functioning of the immune system (Burlou-Nagy et al. 2022).

EP is one of the most important medical herbs, with a significant role in activating the immune system. As a feed additive, it has been shown to have good immunoregulatory, anti-inflammatory, and antioxidant capacity (Aly and Mohamed 2010, Alishahi et al. 2017, Aly et al. 2018, Rahman et al. 2018, Alinezhad 2019, Raissy et al. 2021). Along with the immunostimulatory effects, weight gain has also been observed in various studies (Aly et al. 2008, Guz et al. 2011, Alishahi et al. 2012, Oskoii et al. 2012, El-Sayed et al. 2014, Nazerian et al. 2016, Akbary and Kakolaki 2019, Khajehpour and Javadian 2020). In this study, a diet supplemented with EP improved the weight gain, SGR and FCR of juvenile *C. carpio* in the E-10 and E-20 groups compared to the control group (Table 2). These findings are consistent with those of other researchers, who reported that EP improved body weight gain in fish. Adding EP to the diet of *A. baerii* (Khajehpour and Javadian 2020), *C. carpio* (Alishahi et al. 2012), *H. huso* (Nazerian et al. 2016), *M. cephalus* (Akbary et al. 2016, 2019, Kakolaki et al. 2017), *O. mykiss* (Oskoii et al. 2012), *O. niloticus* (Aly et al. 2008, El-Sayed et al. 2014), *P. reticulata* (Guz et al. 2011), and *P. scalare* (Kasiri et al. 2011) improved growth indices. Moreover, beluga (*H. huso*) fed a diet containing *Echinacea angustifolia* extract showed significantly higher final body weight and feed efficiency compared to other experimental diets (Dadras et al. 2019). In contrast, Alinezhad (2019) reported no significant increase in body weight gain or SGR in juvenile *C. carpio* fed an

EP diet. Similarly, Najafpour-Moghaddam et al. (2017) and Eslami and Bahrekazemi (2019) found no significant positive effect of EP on the growth parameters of *Acipenser ruthenus* and *H. huso*, respectively. Mesalhy et al. (2007) observed that the condition factor and SGR of *O. niloticus* fed a diet supplemented with EP was significantly increased during summer, but not after winter. Studies by other authors have shown that medicinal plants support digestive functions (Przybilla and Weib 1998), leading to greater protein deposition in tissues (Nasir 2009). Previous studies suggest that the enhanced growth performance of fish fed dietary EP might be due to improved health status and stimulation of the secretion of digestive enzymes, resulting in better nutrient digestion and absorption (Woelkart et al. 2005, Esmaeili 2021).

Blood is an indicator of the physiological health condition of animals' internal environment. Therefore, this experiment investigated the haematological parameters of juvenile *C. carpio* supplemented with EP. The haematological profile, including MCV, MCH, MCHC, and percentages of lymphocytes, monocytes, eosinophils and basophils, was not significantly different between the groups (Table 3). The findings are consistent with those of Najafpour-Moghaddam et al. (2017), who observed no significant differences in parameters related to RBC, including Hb, Hct, MCV, MCH, and MCHC, in *Acipenser ruthenus* fed a diet supplemented with EP compared to control fish. Some studies have demonstrated significant effects of EP on haematological indices of *O. niloticus* (Aly and Mohamed 2010), *O. mykiss* (Oskoii et al. 2012), *M. cephalus* (Akbary and Kakoolaki 2019), *H. huso* (Dadras et al. 2019), and *A. baerii* (Khajehpour and Javadian 2020).

Erythrocytes and haemoglobin play an important role in the transport of oxygen and carbon dioxide. In this experiment, EP treatment caused significant changes in the RBC count, Hb and Ht. These findings are consistent with those of other researchers, who have reported that EP improved RBC, Ht, and Hb in *M. cephalus* (Akbary et al. 2016, Kakoolaki et al. 2017, Akbary et al. 2019), *O. mykiss* (Oskoii et al. 2012), *A. baerii* (Khajehpour and Javadian 2020), and *O. niloticus* (Khalil and ElHady 2015). Moreover, an EP-supplemented diet significantly increased the Ht of *O. niloticus* (Aly et al. 2008) and the Ht and Hb of *H. huso* (Dadras et al. 2019). These findings are in contrast with those of Najafpour-Moghaddam et al. (2017) in *A. ruthenus*, Alishahi et al. (2017) in *Ctenopharyngodon idella*, and Eslami and Bahrekazemi (2019) in *H. huso*, who reported no changes in RBC count, Hb content, or Ht values in fish fed a diet supplemented with EP.

As in other vertebrates, fish WBCs are involved in defence mechanisms and provide protection against infection. In this experiment, the WBC count increased in the E-10 group compared to the control, which was linked to the increase in neutrophils (Table 3). These findings are consistent with those of other researchers, who have reported that EP improved WBCs in *H. huso* (Nazerian et al 2016, Dadras et al. 2019), *A. ruthenus* (Najafpour-Moghaddam et al. 2017), *O. niloticus* (Aly et al. 2008, El-Sayed et al. 2014, Khalil and El-Hady 2015), *M. cephalus* (Akbary et al. 2016, Kakoolaki et al. 2017, Akbary et al. 2019), *A. baerii* (Khajehpour and Javadian 2020), and *O. mykiss* (Oskoii et al. 2012, Pourgholam et al. 2013 and Rohani et al. 2016). These findings are in contrast with those of Eslami and Bahrekazemi (2019), who did not observe alterations in WBC count in *H. huso* fed a diet supplemented with EP.

Neutrophil assays in studies on the effect of Echinacea on fish have shown an increase in immune cells (Raissy et al. 2021). In this study, the highest percentage of neutrophils was found in the E-10 group (Table 3). This is consistent with the findings of other researchers, who have reported that EP improved the neutrophil count in *O. mykiss* (Oskoii et al. 2012, Pourgholam et al. 2013, Rohani et al. 2016) and *H. huso* (Dadras et al. 2019, Eslami and Bahrekazemi 2019). Some studies have reported no significant effects of EP on the percentage of neutrophils in *O. niloticus* (Aly et al. 2008), *A. ruthens* (Najafpour-Moghaddam et al. 2017), and *H. huso* (Eslami and Bahrekazemi 2019).

In this work, the effect of EP was investigated by quantifying the respiratory burst activity of juvenile *C. carpio* leukocytes by means of an assay which uses the reduction of nitroblue tetrazolium to formazan as a measure of superoxide anion production (Anderson and Siwicki 1995). Respiratory burst activity increased significantly in the E-10 and E-20 groups compared with the E-0 (control) and E-5 groups (Table 2). These findings are consistent with those of researchers reporting that EP significantly improved respiratory burst activity in *O. mykiss* (Terzioğlu and Diler 2016), *M. cephalus* (Akbary et al. 2019), *A. baerii* (Khajehpour and Javadian 2020), *Ctenopharyngodon idella* (Alishahi et al. 2017), and *O. niloticus* (El-Sayed et al. 2014). In contrast, Aly et al. (2008) found no effect of dietary Echinacea on the NBT reduction value of *O. niloticus*. The mechanism by which EP may have reduced superoxide generation is not clear. In an *in vitro* study, an extract from *Echinacea* containing cichoric acid was found to be capable of scavenging hydroxyl radicals (Sloley et al. 2001).

A. hydrophila is regarded as the predominant fish pathogen among mesophilic aeromonads. A bacterial challenge test is primarily used after a feeding trial

as a final measure of fish health status. To date, there have been no studies on the effect of EP root supplementation on *A. hydrophila* bacteria in juvenile *C. carpio*. In the present study, the results of the 14-day challenge trial with *A. hydrophila* showed that dietary EP may have a positive effect in protecting common carp against this pathogenic bacterium (Fig. 1). The lowest CPM caused by *A. hydrophila* was recorded for the E-20 group, at 43.33%, while the highest CPM was recorded for the control group, at 73.33% (Table 4). The results of the challenge test are consistent with other studies, in which an EP-supplemented diet enhanced 1) resistance of *O. niloticus* to *A. hydrophila* (Mesalhy et al. 2007, El-Sayed et al. 2014), *A. sobria* (Khalil and El-Hady 2015), *Pseudomonas* sp. (John et al. 2007), *Pseudomonas fluorescens* (Aly et al. 2008) and *A. hydrophila* (Aly and Mohamed 2010); 2) resistance of *P. reticulata* to *Aeromonas bestiarum* (Guz et al. 2011); 3) resistance of *O. mykiss* to *Streptococcus iniae* (Pourgholam et al. 2013, Rohani et al. 2016) and *Vibrio anguillarum* (Terzioğlu and Diler 2016); 4) resistance of *M. cephalus* to *Photobacterium damselae* (Akbary and Kakoolaki 2019), and 5) resistance of *Ctenopharyngodon idella* to *A. hydrophila* (Alishahi et al. 2017). In contrast, Aly et al. (2008) reported that mortality rates of *O. niloticus* following challenge infection with *P. fluorescens* were non-significantly lower in the group supplemented with EP (0.25 ppt) than in the control group. Tang et al. (2023) revealed that *E. purpurea* affected immune activity through-mRNA regulated pathways, with specific mRNA families involved in both innate and adaptive immune responses.

Conclusions

Dietary supplementation with 10 g EP kg^{-1} diet and 20 g EP kg^{-1} diet in juvenile common carp significantly enhances growth parameters and increases protection against *A. hydrophila* infection. Therefore, the inclusion of EP at these levels in the diet of juvenile common carp in aquaculture is recommended.

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