Aquaculture Reports 30 (2023) 101574

Contents lists available at ScienceDirect

Aquaculture Reports

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

Effects of dietary Pennyroyal essential oil on growth performance, digestive enzymes' activity, and stress responses of common carp, *Cyprinus carpio*

Morteza Yousefi [a], Hossein Adineh [b,*], Maryam Ghadamkheir [c], Seyed Amir Mahdi Hashemianfar [b], Sevdan Yilmaz [d]

[a] Department of Veterinary Medicine, Peoples' Friendship University of Russia (RUDN University), Miklukho-Maklaya St, Moscow 117198, Russian Federation
[b] Department of Fisheries, Faculty of Agriculture and Natural Resources, Gonbad Kavous University, Gonbad Kavous, Golestan, Iran
[c] Department of Economics and Organization of Agricultural Production, Institute of Land Management, State University of Land Use Planning, Moscow 105064, Russian Federation
[d] Department of Aquaculture, Faculty of Marine Sciences and Technology, Canakkale Onsekiz Mart University, Canakkale 17100, Turkey

ARTICLE INFO

Keywords:
Herbal supplements
Feed
Crowding stress
Health
Biochemistry

ABSTRACT

This study aimed at assessing the effects of dietary Pennyroyal essential oil (PE) supplementation on growth performance, digestive enzymes, and stress, antioxidant, and immunological responses to an acute stress. Four experimental diets containing 0 (C), 100 (100PE), 250 (250PE), and 500 (500PE) mg/kg PE were used in this experiment. The fish were fed with these diets for eight weeks, followed by a 3-h crowding stress (40 kg/m^3) and 24-h recovery. Growth performance, intestinal amylase, lipase, and protease activities, plasma total protein, albumin, and globulin levels were determined before stress, whereas plasma cortisol, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lysozyme, alternative complement (ACH50), total immunoglobulin (Ig), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) were measured before and after stress. Dietary PE supplementation significantly increased growth performance and digestive enzymes of the fish. The highest growth performance was observed in 250PE treatment. Highest amylase, lipase and protease activities were observed in 500PE, 250PE, and 250PE/500PE treatments, respectively. Dietary PE significantly increased plasma total protein, albumin, and globulin and decreased plasma triglyceride and cholesterol levels. Dietary PE supplementation significantly decreased cortisol, glucose, ALT, AST, and MDA before the crowding stress and mitigated the elevations in these parameters after stress. On the other hand, dietary PE significantly increased plasma lysozyme, ACH50, total Ig, SOD, CAT, and GPx before stress and mitigated the alteration in these parameters after stress. In conclusion, dietary PE at 250 mg/kg is recommended for common carp feed, as it improve growth performance, digestive enzymes' activities, and physiological and immunological responses to acute stress in fish.

1. Introduction

Global demand for protein has been increasing along with increase in the world population. Thus, agriculture sectors have shown a rapid growth in quantity to meet the protein demand. Aquaculture is one of the most important sectors in the food production system, which has shown rapid progress in recent decades (Garlock et al., 2020). Fish and shellfish are rich sources of high quality protein, fat, and other micronutrients, which guarantee human health (Vianna et al., 2020). This rapid expansion of aquaculture has arose a need for functional feed to assure fast growth and high health of aquatic animals. Therefore, study of fish nutrition has increased in the recent decades, leading to development of functional feeds (Lee et al., 2015). However, there are many gapes in knowledge that arises the need for further researches.

Stress is common in aquaculture and encompasses of series of internal physiological alterations that help the animal to re-establish homeostasis (Arab-Bafrani et al., 2022). Stress has negative effects in fish including growth retardation, immunosuppression, oxidative stress, and tissue damage (Kumar et al., 2020; Roychowdhury et al., 2020; Ciji and Akhtar, 2021). Increase in aquaculture production has been accompanied by various stresses in aquaculture facilities, for example crowding stress. Increase in stocking density to have higher yield per unit of area

* Corresponding author.
E-mail addresses: adineh.h@gonbad.ac.ir, adineh.h@gmail.com (H. Adineh).

https://doi.org/10.1016/j.aqrep.2023.101574
Received 3 February 2023; Received in revised form 8 April 2023; Accepted 17 April 2023
Available online 21 April 2023

Table 1
Ingredients and chemical composition of the basal diet.

Ingredients	Percentage	Proximate composition	Percentage
Fishmeal[1]	150	Dry matter	86.16
Meat meal[2]	200	Crude protein	37.35
Soybean meal	210	Crude lipid	6.23
Wheat meal	320	Ash	6.24
Fish oil	7	Energy (kcal/kg)	4103.50
Soybean oil	7		
Corn flour	89		
L-Lysine[3]	7		
L-Methionine[3]	5		
Vitamin premix[4]	2.5		
Mineral premix[5]	2.5		

1- Pars kilka Co., Mazandaran, Iran (Kilka powder analysis; Protein: 70–72%, Fat: 8–11%, Ash: 11.6%, Moisture: 7–9%).
2- Makianmehr Co., Golestan, Iran.
3- Morghenojan.Co., Tehran, Iran.
4- Vitamin premix (per kg of diet): vitamin A, 2000 IU; vitamin B1 (thiamin), 5 mg; vitamin B2 (riboflavin), 5 mg; vitamin B6, 5 mg; vitamin B12, 0.025 mg; vitamin D3, 1200 IU; vitamin E, 63 mg; vitamin K3, 2.5 mg; folic acid, 1.3 mg; biotin, 0.05 mg; pantothenic acid calcium, 20 mg; inositol, 60 mg; ascorbic acid (35%), 110 mg; niacinamide, 25 mg.
5- Mineral premix (per kg of diet): MnSO4, 10 mg; MgSO4, 10 mg; KCl, 95 mg; NaCl, 165 mg; ZnSO4, 20 mg; KI, 1 mg; CuSO4, 12.5 mg; FeSO4, 105 mg; Co, 1.5 mg.

and fish crowding stress due to routine aquaculture practices threaten the fish health (Santos et al., 2021). Therefore, it worth seeking for methods to improve fish growth and health and mitigate the negative effects of stress in aquaculture. Medicinal herbs are a class of feed additives with a variety of biological effects. These herbs contain various phytochemicals that affect physiological pathways in fish (Elumalai et al., 2021). Dietary supplementation with medicinal herbs and their bioactive compounds results in improved digestive enzymes' activity, growth promotion, stress mitigation, antioxidant capacity and immune boosting, and disease resistance (Hernández-Contreras and Hernández, 2020; Hoseinifar et al., 2020; Taheri Mirghaed et al., 2020). In this regard, various medicinal plants and their bioactive compounds have been investigated in different fish, but there is a need for further study on new plants.

Pennyroyal, *Mentha pulegium*, is a medicinal plant native to Europe, North Africa, and Middle East (Mahboubi and Haghi, 2008). Leave and flower of this plant have been traditionally used for medicinal purposes, because of the antiseptic, antioxidant, and cytotoxic properties (Mahboubi and Haghi, 2008). The main ingredient of pennyroyal oil is pulegone, which is a known antioxidant (El-Ghorab, 2006), antimicrobial (Flamini et al., 1999), and anxiolytic (Silveira et al., 2014) agent. Despite such benefits of pennyroyal, there is information regarding its application as feed supplement in aquaculture. Therefore, the present study aimed at investigating the effects of dietary pennyroyal essential oil (PE) on growth performance, digestive enzymes, and stress, antioxidant and immunological responses to an acute stress in common carp, *Cyprinus carpio*.

2. Materials and methods

2.1. Experimental diets

Four experimental diets containing 0 (C), 100 (100PE), 250 (250PE), and 500 (500PE) mg/kg PE were used in this experiment. PE was purchased from Dr. Soleimany Giahessence Co. (Gorgan, Iran), which contained 80.5% pulegone (analyzed by GC-MS). The feedstuffs were mixed together and PE was added to this mixture after combining with the dietary oils. The feedstuffs' mixture was moisturized by adding 400 g/kg water and the resultant dough was passed through a mesh to form feed pellets. The pellets were dried against a fan blower overnight. All diets were analyzed for proximate composition according to AOAC (2005). The ingredients and chemical composition of the basic diet were presented in Table 1.

2.1.1. Experimental protocol

Common carp juveniles were purchased from a local farm and transported to our aquaculture laboratory (Gonbad Kavous University). Four hundred fish (~ 13 g) were stocked in one 1000-L tank and fed a commercial diet over a 2-week period to acclimatize to the laboratory conditions. Subsequently, 360 healthy fish of comparable size were divided into four experimental groups with three replications each, and placed into 12 tanks of 60 liters capacity. The experimental groups were fed either C, 100PE, 250PE, or 500PE diet over an 8-week period. The fish were fed based on 3% of biomass per day, divided into three meals. The tanks' biomass was recorded every other week to adjust feed amount. The tanks' water was daily renewed by half and all tanks were continuously aerated. The fish growth parameters including final weight (FW), feed conversion ratio (FCR), weight gain (WG), and specific growth rate (SGR) were calculated at the end of the experiment according to De Silva and Anderson (1998). Then, intestinal and blood samples were collected from all treatments. Fish were starved for 24 h and then exposed to crowding stress for 3 h. Further blood samples were taken from all treatments after the 3-h stress and 24-h recovery. To induce the crowding stress, the water level in the tanks were decreased and the fish density was set at 40 kg/m^3 (Adineh et al., 2021b). The fish were kept at this density for 3 h and allowed to recover by restoring the water volume. Final sampling was conducted after 24 h recovery.

Weight gain (WG, %) = 100 × (final weight – initial weight)/initial weight

Specific growth rate (SGR, % day^{-1}) = 100 × (ln final weight – ln initial weight)/days

Feed conversion ratio (FCR, g g^{-1}) = dry feed intake/(final weight –initial weight)

2.2. Sampling and analysis

Fish were caught from each tank by a dip net and anesthetized in clove oil bath (100 mg/l) (Yousefi et al., 2021). Then, blood sample was collected form the caudal vein by heparinized syringe. The plasma of the blood samples was separated by centrifugation at 4 °C (5000 g; 10 min) and kept at − 80°C, to collect the intestinal samples, the whole intestine was dissected and immediately frozen in liquid nitrogen. After adding cold distilled water, the samples were homogenized using a homogenizer and then extracted through centrifugation at 4 °C (25,000 g; 20 min) (Yilmaz et al., 2018).

The supernatant was used for digestive enzymes' analysis (Najdegerami et al., 2016). The intestinal amylase activity was determined using 0.3% soluble starch as substrate (Langlois et al., 1987). The samples' lipase activity was determined based on hydrolysis of p-nitrophenyl myristate as described before (Iijima et al., 1998). AZO-Casein method was used for the intestinal protease assay, using 1% casein as substrate (Iversen and Jørgensen, 1995).

Plasma cortisol levels were measured based on the competitive ELISA method using a commercial kit provided by IBL Co. (Gesellschaft für Immunchemie und Immunbiologie). Plasma glucose, total protein, albumin, triglyceride, and cholesterol levels were determined colorimetrically, using commercial kits supplied by Pars Azmun Co. (Tehran, Iran). Plasma ALT and AST activities were determined kinetically, using Pars Azmun Co. kits.

Plasma lysozyme activity was determined based on lysis rate of *Micrococcus luteus* according to Ellis (1990). Phosphate buffer (pH 6.2) was used as reaction medium and decrease in absorbance was recorded for 3 min at 450 nm. One unit of lysozyme activity was equal to 0.001

Table 2
Growth performance of common carp fed PE-supplemented diets over eight weeks.

	C	100PE	250PE	500PE
IW (g)	13.66 ± 0.06	13.40 ± 0.07	13.45 ± 0.06	13.32 ± 0.06
FW (g)	24.79 ± 0.10^{a}	26.96 ± 0.08^{b}	29.20 ± 0.06^{d}	27.78 ± 0.07^{c}
WG (%)	81.54 ± 0.65^{a}	101.49 ± 0.82^{b}	117.46 ± 1.08^{d}	108.82 ± 1.03^{c}
SGR (%/d)	1.06 ± 0.006^{a}	1.24 ± 0.006^{b}	1.38 ± 0.009^{d}	1.31 ± 0.009^{c}
FCR	1.89 ± 0.02^{d}	1.57 ± 0.007^{c}	1.36 ± 0.009^{a}	1.46 ± 0.010^{b}
Survival (%)	100	100	100	100

Different letters within a row indicate significant difference among the treatments (n = 3 tank; Duncan). Data are presented as mean ± SEM. Data was analyzed through one-way ANOVA besides Duncan comparisons.
C: control group; 100PE, 250PE, and 500PE mean 100, 250 and 500 mg/kg pennyroyal essential oil.

unit decrease in absorbance per minute. Plasma alternative complement activity (ACH50) was determined based on Yano (1992). Hemolysis rates of the plasma samples were determined against sheep erythrocytes used for ACH50 calculation. Plasma total immunoglobulin (Ig) levels were determined after precipitation with polyethylene glycol according to Siwicki and Anderson (1993).

Plasma catalase (CAT) activity was determined according to Goth (1991) by measuring the rate of hydrogen peroxide decomposition. Plasma superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) were determined using commercial kits provided by ZellBio GmbH Co. (Veltinerweg, Germany). SOD activity was assessed based on the rate of the cytochrome C reduction; whereas, GPx activity was determined based on the oxidation of glutathione. MDA level was determined based on the reaction with thiobarbituric acid at 95 °C.

2.3. Statistical analysis

Growth, digestive enzymes, and plasma total protein, albumin, globulin, triglyceride, and cholesterol levels were analyzed by one way ANOVA and Duncan tests, after confirmation of normality (Shapiro-Wilk's test) and variance homogeneity (Levene's test). Plasma cortisol, glucose, ALT, AST, SOD, CAT, GPx, MDA, lysozyme, and total Ig were analyzed by two way ANOVA (dietary PE × sampling times), after confirmation of normality (Shapiro-Wilk's test) and variance homogeneity (Levene's test). Plasma ACH50 data did not meet the ANOVA assumption, so log-transformed before running two way ANOVA and Duncan tests. All analyses were conducted in SPSS v.22 and the data were presented as mean ± SEM.

3. Results

All PE-treated treatments exhibited significantly higher final weight, weight gain, SGR, and FCR, compared to C treatment. The highest final weight, weight gain, SGR, and lowest FCR were observed in 250PE treatment (Table 2). There was no mortality during the study in any treatments (Table 2).

All PE-treated fish exhibited significantly higher intestinal amylase, lipase, and protease activities, compared to C treatment. The highest amylase and lipase activities were observed in 500PE and 250PE treatments, respectively. The highest protease activity was observed in 250PE and 500PE treatments (Fig. 1).

Plasma total protein, albumin, and globulin levels in 250PE and 500PE were significantly higher than C treatment and the highest values were related to 250PE treatment. All PE-treated fish exhibited

Table 3
Plasma biochemical parameters of common carp fed PE-supplemented diets over eight weeks.

	C	100PE	250PE	500PE	P
Total protein (g/dL)	2.60 ± 0.01^{a}	2.71 ± 0.02^{a}	3.16 ± 0.02^{c}	2.99 ± 0.02^{b}	< 0.001
Albumin (g/dL)	1.14 ± 0.00^{a}	1.21 ± 0.02^{a}	1.39 ± 0.02^{c}	1.30 ± 0.01^{b}	< 0.001
Globulin (g/dL)	1.46 ± 0.01^{a}	1.50 ± 0.02^{a}	1.77 ± 0.00^{c}	1.69 ± 0.01^{b}	< 0.001
Triglyceride (mg/dL)	158 ± 3.07^{c}	105 ± 1.23^{a}	131 ± 1.45^{b}	135 ± 1.14^{b}	< 0.001
Cholesterol (mg/dL)	121 ± 0.64^{d}	109 ± 0.39^{c}	93.1 ± 0.31^{a}	97.0 ± 0.80^{b}	< 0.001

Different letters within a row indicate significant difference among the treatments (n = 6 fish; Duncan). Data are presented as mean ± SEM. Data was analyzed through one-way ANOVA besides Duncan comparisons.
C: control group; 100PE, 250PE, and 500PE mean 100, 250 and 500 mg/kg pennyroyal essential oil.
Figure captions

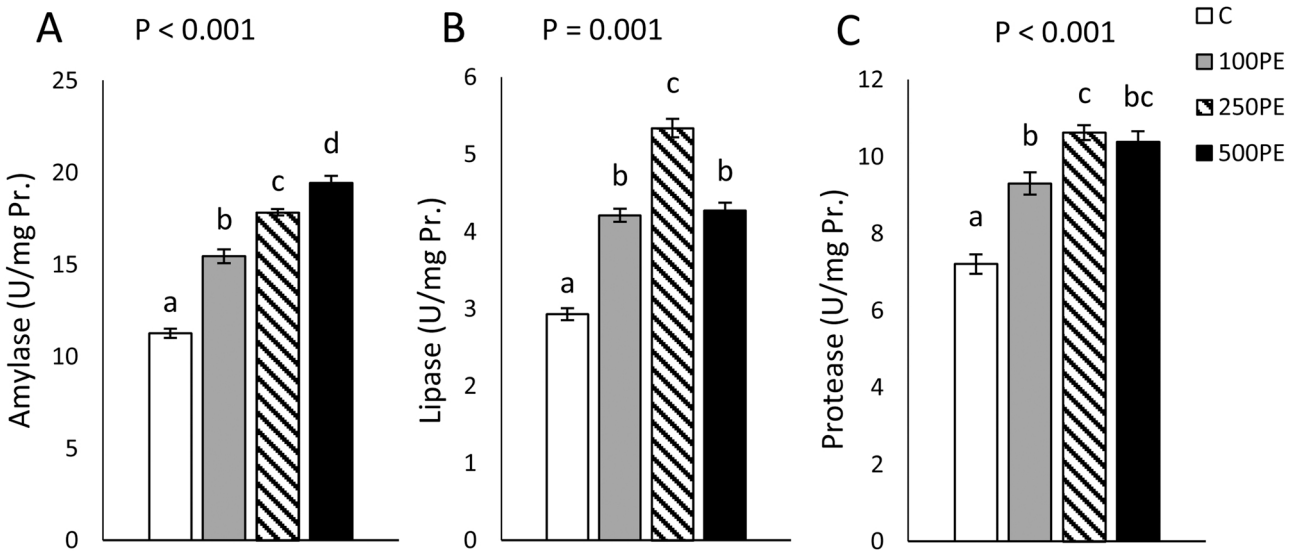

Fig. 1. Intestinal digestive enzymes' activity of common carp fed PE-supplemented diets over eight weeks. Different letters above the bars indicate significant difference among the treatments (n = 6 fish; Duncan). C: control group; 100PE, 250PE, and 500PE mean 100, 250 and 500 mg/kg pennyroyal essential oil.

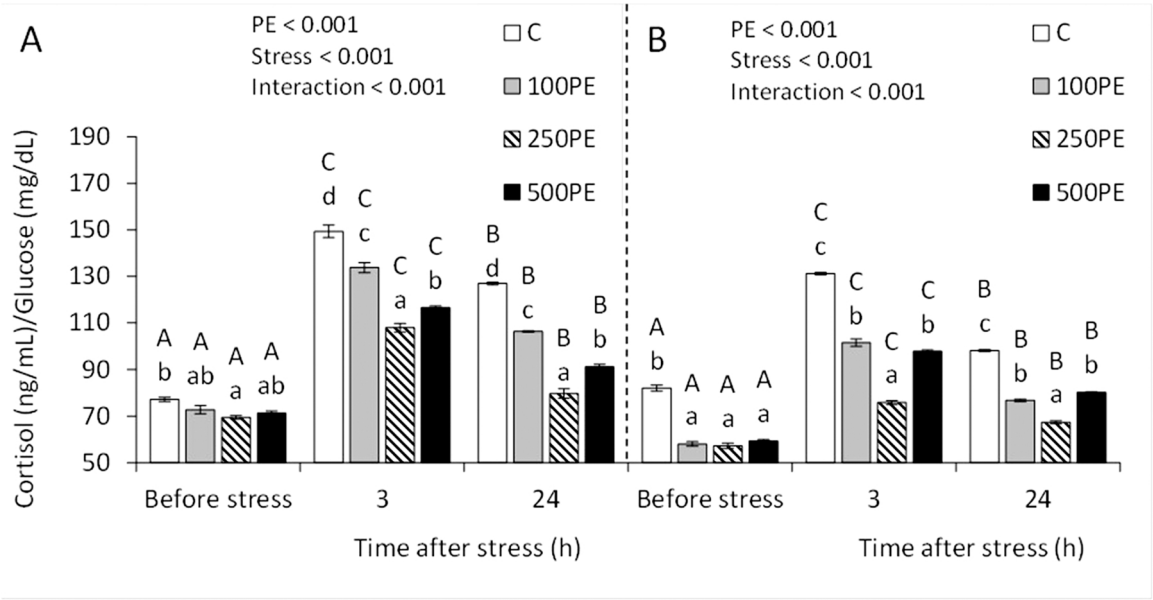

Fig. 2. Plasma cortisol (A) and glucose (B) levels of common carp fed PE-supplemented diets over eight weeks and subjected to a 3-h crowding stress. Different uppercase letters above the bars indicate significant difference among sampling times within each treatment; whereas different lowercase letters above the bars indicate significant difference among the treatments, within each sampling time (n = 6 fish; Duncan). C: control group; 100PE, 250PE, and 500PE mean 100, 250 and 500 mg/kg pennyroyal essential oil.

significantly lower plasma triglyceride and cholesterol levels, compared to C treatment. The lowest triglyceride level was observed in 100PE treatment; whereas, the lowest cholesterol level was observed in 250PE treatment (Table 3).

Plasma cortisol level in 250PE treatment showed a significant decrease, compared to C treatment, before the stress. At this time, all PE-treated fish had similar plasma glucose levels, being significantly lower than C treatment. Stress resulted significant elevation in the plasma cortisol and glucose levels in all treatments; however, the PE-treated fish showed significantly lower plasma cortisol and glucose elevations, compared to C treatment. The lowest cortisol and glucose levels after stress (3 and 24 h) were observed in 250PE treatment (Fig. 2A and B).

All PE-treated fish showed similar plasma lysozyme activity and significantly higher than that of control, before stress. After 3-h crowding stress, there were significant decreases in the plasma lysozyme activities in all treatments; however, the PE-treated fish exhibited lower decreases, compared to C treatment. Plasma lysozyme activity increased in all treatments, 24 h after stress. The lowest and highest plasma lysozyme activities were related to C and 250PE/500PE treatments at this time, respectively (Fig. 3).

Plasma ACH50 in 250PE and 500PE were significantly higher than C treatment before stress. After 3-h crowding stress, there were significant decreases in the plasma ACH50 activities in all treatments; however, 250PE and 500PE treatments exhibited lower decreases, compared to C treatment. Plasma ACH50 activity in 100PE and 250PE treatments returned to pre-stress levels, 24 h after the stress (Fig. 3).

Before and after the crowding stress, the PE-treated fish showed significantly higher plasma total Ig levels, compared to C treatment, and the highest level was related to 250PE treatment. Plasma total Ig levels showed significant decrease after 3-h stress with partial recovery after 24 h in all treatments (Fig. 3).

There was no significant difference in plasma ALT activity among the treatments, before and after 3- stress; however, the stress resulted in significant increase in plasma ALT activity after 3 h. 24 h after stress, plasma ALT activity significantly increased in C and 100PE treatments but decreased 500PE treatment. Both 250PE and 500PE treatments showed significantly lower plasma ALT activities, compared to C treatment, at this time (Fig. 4).

Before and after the crowding stress, the PE-treated fish showed significantly lower plasma AST activities, compared to C treatment, and the lowest activity was related to 250PE treatment. Plasma AST activities showed significant increase after 3-h stress with partial recovery after 24 h in all treatments. 250PE and 500PE treatments exhibited significantly lower plasma AST activities, compared to C treatment 3 and 24 h after stress. At 24-h post stress, the lowest plasma AST activity was related to 250PE treatment (Fig. 4).

Before the crowding stress, the PE-treated fish showed significantly higher plasma SOD activity, compared to C treatment, and the highest level was related to 250PE treatment. After 3-h crowding stress, there were significant decreases in the plasma SOD activities in all treatments, except 500PE. At this time, 250PE and 500PE treatments showed significantly higher plasma SOD activity, compared to C treatment. Plasma SOD activity in C treatment returned to the pre-stress level, whereas there was significant elevation in the enzyme's activity in PE-treated fish, 24 h after stress (Fig. 5A).

Before and after the crowding stress, the PE-treated fish showed significantly higher plasma CAT activity, compared to C treatment. Plasma CAT acidity showed significant elevations in all treatment after 3 and 24 h crowding stress. The highest activity before the stress was observed in 100PE and 250PE treatments. All PE-treated fish exhibited similar plasma CAT activity, 3 h after the crowding stress, whereas the highest activity after 24 h was related to 250PE treatment (Fig. 5B).

Plasma GPx activity in 250PE and 500PE were significantly higher than C treatment before stress. After 3-h crowding stress, there were significant decreases in the plasma GPx activities in all treatments; however, 250PE and 500PE treatments exhibited lower decreases, compared to C treatment. Plasma GPx activity in 250PE treatments returned to pre-stress levels, 24 h after the stress. The PE-treated fish had significantly higher plasma GPx activities than C treatment, both after 3 and 24 h stress (Fig. 5C).

Stress significantly increased the plasma MDA levels in C treatment, after 3 and 24 h; however, 250PE treatment exhibited no change in the plasma MDA after stress. At both sampling time, the lowest plasma MDA level was related to 250PE treatment. The plasma MDA levels in 100PE and 500PE treatments significantly increased 3 or 24 h after stress (Fig. 5D).

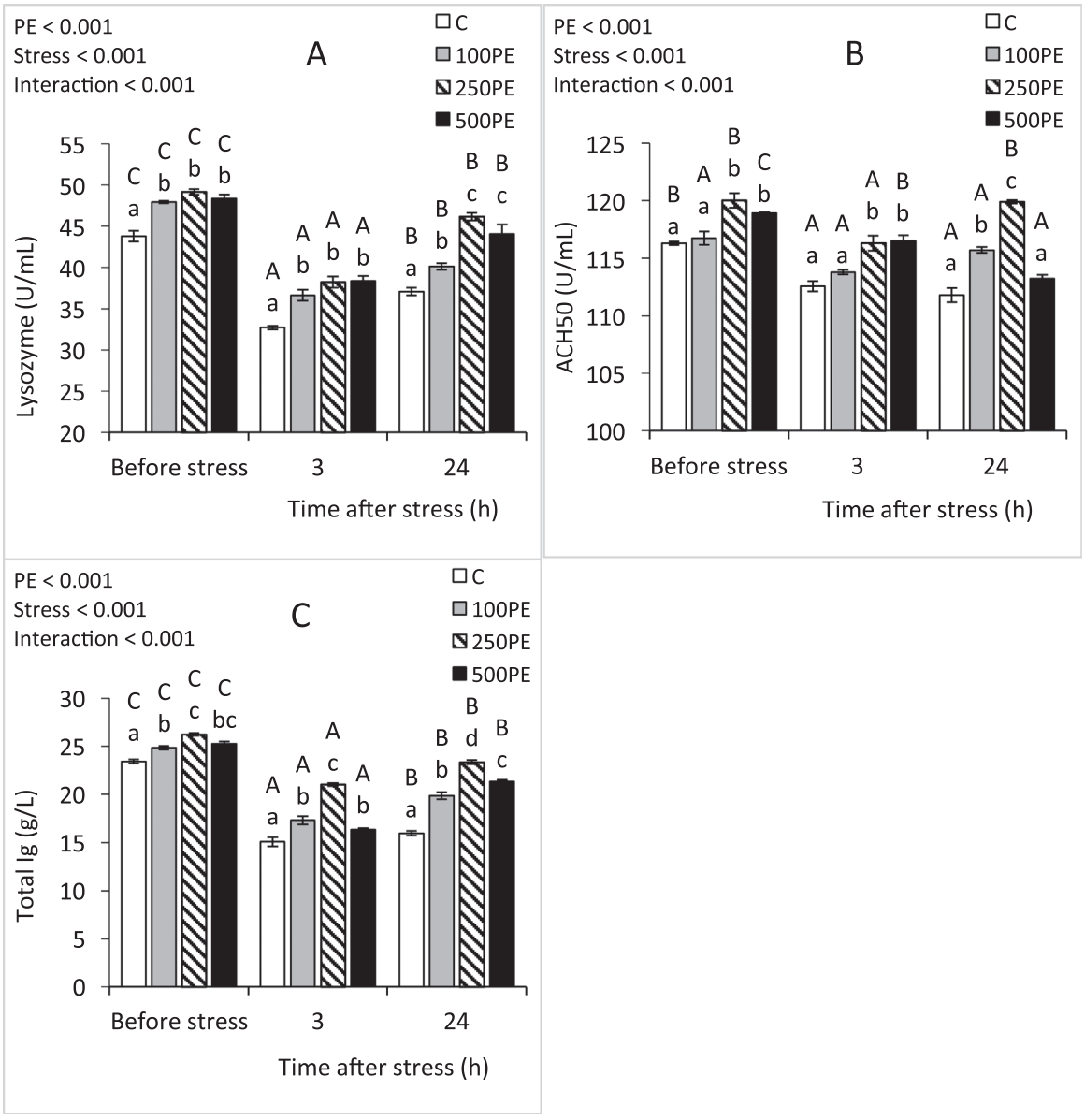

Fig. 3. Plasma lysozyme (A), ACH50 (B), and total Ig levels (C) of common carp fed PE-supplemented diets over eight weeks and subjected to a 3-h crowding stress. Different uppercase letters above the bars indicate significant difference among sampling times within each treatment; whereas different lowercase letters above the bars indicate significant difference among the treatments, within each sampling time (n = 6 fish; Duncan). C: control group; 100PE, 250PE, and 500PE mean 100, 250 and 500 mg/kg pennyroyal essential oil.

4. Discussion

Fish have shown different growth responses to dietary phytobiotic supplementation. In common carp, some phytobiotics have presented growth-promoting effects, such as juniper berry oil (Kesbiç, 2019), *Malva sylvestris* extract (Bilen et al., 2020), and curcumin (Giri et al., 2019); however, there are other phytobiotics without such benefits in this species (Paray et al., 2020; Rajabiesterabadi et al., 2020b; Hoseini et al., 2021a; Hoseini et al., 2021b). The present study have demonstrated that PE can promote growth performance and feed efficiency in common carp, particularly in 250PE treatment. Although the exact mechanisms of such a benefit are not clear, but 250PE treatment exhibited elevations in digestive enzymes' activity and stress suppression. Improve in digestive enzymes' activity may result in higher digestion and nutrient retention and previous studies have shown such a relationship between digestive enzymes' activity and growth performance in fish/shrimp fed phytobiotic supplemented diets (Anand et al., 2013; Adineh et al., 2021a; Wangkahart et al., 2022). Besides, 250PE significantly decreased cortisol and glucose levels, suggesting lower energy expenditure and higher energy available for growth. Such anti-stress and growth promoting effects have been previously reported in common carp, fed phytobiotic-supplemented diets (Yousefi et al., 2021; Hoseini et al., 2022). Additionally, supplementing the diet of juvenile pirarucu with 2.0 mL/kg of sweet basil *Ocimum basilicum* essential oil led to enhanced growth performance, as evidenced by improvements in final weight, weight gain, specific growth rate, condition factor, and feed conversion ratio. Furthermore, this supplementation resulted in decreased plasma urea levels and increased plasma albumin and total protein levels. Nevertheless, it did not have any impact on plasma glucose, cortisol, and acid uric levels (Chung et al., 2020).

Apart from the mentioned cases, the results may be attributed to the high concentration of Pulegone present in the essential oil of pennyroyal supplied for this study, which exceeded 80%. Pulegone is a naturally occurring organic compound found in various plant species, including

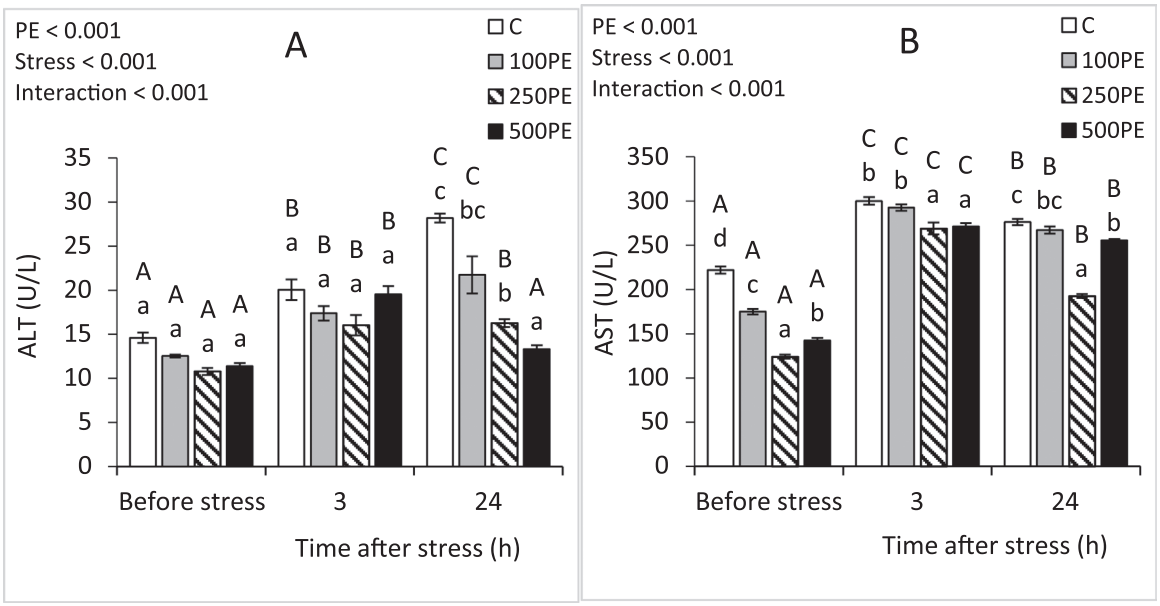

Fig. 4. Plasma alanine aminotransferase (ALT) (A) and aspartate aminotransferase (AST) (B) activity of common carp fed PE-supplemented diets over eight weeks and subjected to a 3-h crowding stress. Different uppercase letters above the bars indicate significant difference among sampling times within each treatment; whereas different lowercase letters above the bars indicate significant difference among the treatments, within each sampling time (n = 6 fish; Duncan). C: control group; 100PE, 250PE, and 500PE mean 100, 250 and 500 mg/kg pennyroyal essential oil.

mint and pennyroyal (El-Ghorab, 2006; Silveira et al., 2014). The mechanism by which pulegone might increase the growth performance of common carp is not yet fully understood, as there is limited research on this topic. However, pulegone has been reported to have antimicrobial properties, which could potentially improve the gut health of fish. A healthy gut microbiota can enhance nutrient absorption and utilization, leading to improved growth performance. Therefore, it is possible that pulegone may have improved the gut health of common carp, leading to increased growth (Ringø et al., 2016; Kaur et al., 2018). Improved digestive enzymes in this study can confirm this hypothesis. Additionally, pulegone has been reported to have anti-inflammatory properties, which could reduce the stress response in fish. Stress can negatively impact fish growth, so reducing stress through the consumption of pulegone may have led to improved growth performance in common carp (Nam et al., 2013).

Plasma proteins are mainly synthetized in the liver (Haschek et al., 2009) and have a variety of physiological roles, including antioxidant, immunological, carrying, etc. functions. Therefore, plasma proteins are used as indicators of welfare and hepatic health in fish. According to the present study, PE is capable to improve the hepatic health in common carp, particularly at 250 mg/kg diet. Supporting this, plasma ALT and AST activities in 250PE treatment were significantly lower than the other treatments. ALT and AST are concentrated in hepatocytes and are leaked into the blood stream, if the cells are damaged (Ghelichpour et al., 2017). Similarly, dietary inclusion of *Nigella sativa* meal (Yousefi et al., 2020b) and *Artemisia absinthium* extract (Yousefi et al., 2021) have increased plasma proteins' level and decreased ALT and AST activities.

Plasma lipid levels depend on many factors, including dietary fat, dietary supplements, hepatic health, and cell health (Haschek et al., 2009). There is a body of evidence suggesting that plasma triglyceride and cholesterol levels increase, when fish are exposed to toxic substance (Hoseini et al., 2014; Mazandarani and Hoseini, 2017; Elbialy et al., 2021) or are reared under unfavorable conditions (Abdel-Tawwab et al., 2021). Therefore, the decreases in triglyceride and cholesterol levels in PE-treated fish suggest that dietary PE supplementation favored the conditions for these fish. On the other hand, phytobiotics are known as lipid-lowering agents and studies on common carp have shown phytobiotic such as licorice (Adineh et al., 2021b) or *Rosmarinus officinalis* extract (Chelemal Dezfoulnejad and Molayemraftar, 2022) decrease plasma cholesterol and triglyceride levels. Studies on non-aquatic animals have shown that pennyroyal administration lower blood cholesterol and/or triglyceride levels (Shamlo et al., 2014; Farid and Eddouks, 2020). Therefore, lower triglyceride and cholesterol levels in PE-treated fish might be due to improved fish health and/or hypolipidemic effects of PE.

Stress is common in aquaculture, which deteriorates the immune strength and induces oxidative stress (Yousefi et al., 2016; Fazelan et al., 2020b). Cortisol is the main stress hormone in fish that secreted from the interrenal cells, following stimulation by adrenocorticotropic hormone. This a primary stress response that occurs within a short time after stress (Barton, 2002). Cortisol stimulates gluconeogenesis in the liver to provide required glucose and energy source to cope with the stress, so hyperglycemia is a secondary responses of fish after stresses (Hoseini et al., 2011). Elevated energy expenditure is accompanied by higher rate of cell respiration and production of pro-oxidants, which increase the risk of oxidative stress. To deal with such conditions, the antioxidant system should work well to prevent oxidative stress. SOD is the main antioxidant enzymes in cell respiration that prevent oxidative stress derived from excess superoxide ion accumulation (Perry et al., 2010). SOD breakdowns superoxide ions to hydrogen peroxide, which is further broken-down to water and oxygen by CAT and GPx (Burk and Hill, 2010; Sepasi Tehrani and Moosavi-Movahedi, 2018). A decrease in MDA, accompanied by increase in the antioxidant enzymes' activities suggest the activation of the antioxidant system that mitigated oxidative stress. Accordingly, the highest antioxidant activity was observed in the 250PE treatment, which also showed lower stress and energy expenditure. Therefore, it is suggested that dietary PE supplementation can suppress stress in common carp, which leads to lower oxidative conditions. Similarly, previous studies have shown that dietary supplementation with *Mentha* sp. such as *Mentha piperita* (Adel et al., 2015; Adel et al., 2016; Ribeiro et al., 2018) and *Mentha longifolia* (Gholamhosseini et al., 2020) improved antioxidant enzymes' activities and decreased oxidative stress in fish.

Cortisol has strong immunosuppressive effects, which increase the risk of disease outbreak (Yousefi et al., 2016). Phytobiotics are known for their anti-stress, antioxidant, and immunostimulant properties,

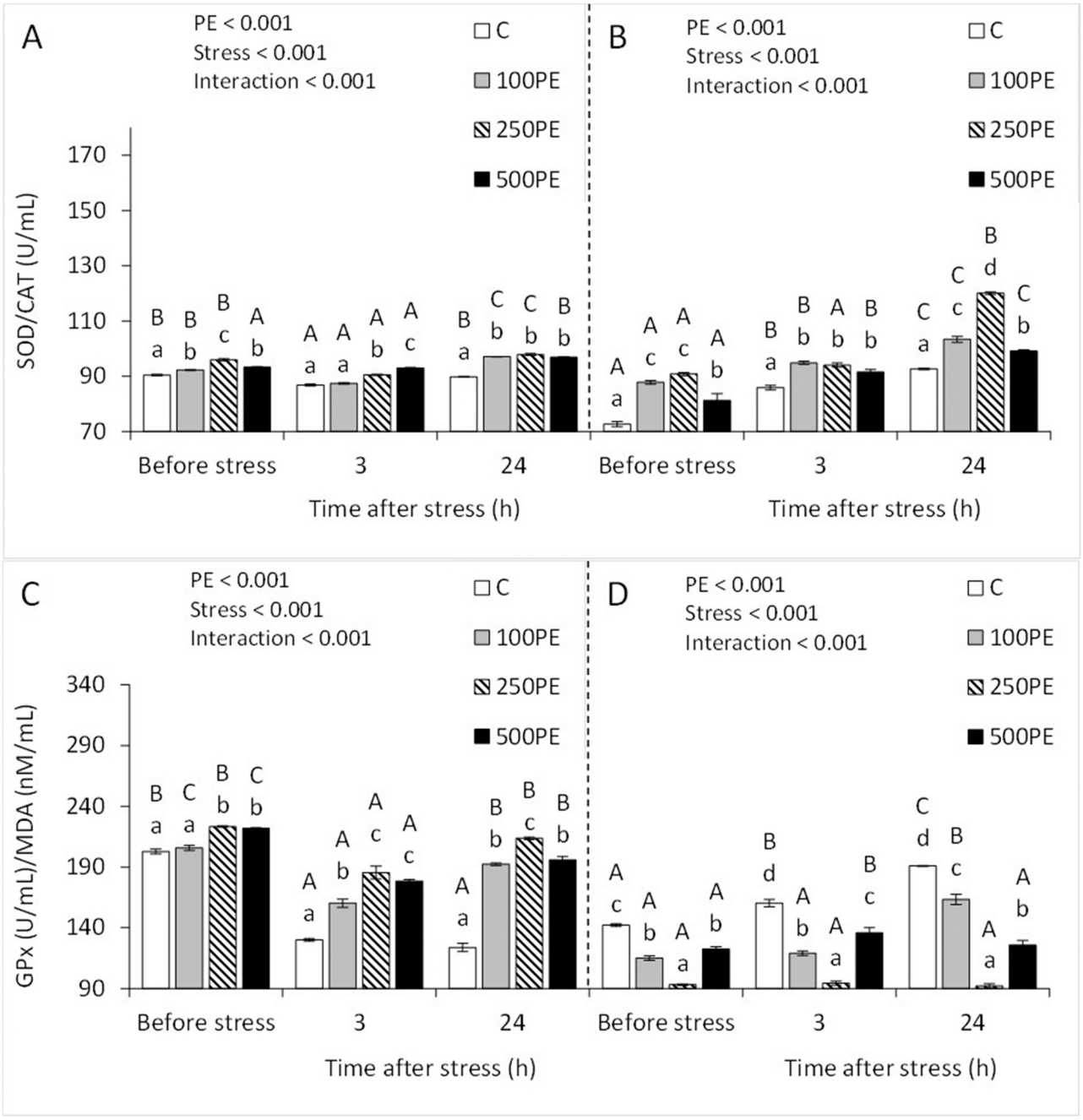

Fig. 5. Plasma superoxide dismutase (SOD) (A), catalase (CAT) (B), glutathione peroxidase (GPx) (C), and malondialdehyde (MDA) (D) levels of common carp fed PE-supplemented diets over eight weeks and subjected to a 3-h crowding stress. Different uppercase letters above the bars indicate significant difference among sampling times within each treatment; whereas different lowercase letters above the bars indicate significant difference among the treatments, within each sampling time (n = 6 fish; Duncan). C: control group; 100PE, 250PE, and 500PE mean 100, 250 and 500 mg/kg pennyroyal essential oil.

which cumulatively favor conditions for the host (Zhu, 2020). The present results demonstrated that dietary PE supplementation can reduce stress, and improves the immune strength in common carp, during an 8-week administration period. According to the results, 250 mg/kg PE maximally reduces cortisol, which is accompanied by increases in humoral immune parameters. These results suggest that 250PE is efficient to reduce stress in common carp, which may be, at least in part, responsible for higher immune strength. Similarly, different essential oils such as *Oregano vulgare* (Zhang et al., 2020), *Ocimum americanum* (Sutili et al., 2016), and thyme (Yousefi et al., 2022) exhibited immunomodulation in fish, when added to diets. Moreover, common carp has shown lower stress and higher antioxidant and immune responses, when fed diets supplemented with different essential oils, including *Lavandula angustifolia* extract (Yousefi et al., 2020a), anthraquinone extract from *Rheum officinale* (Xie et al., 2008), licorice (Adineh et al., 2021b), and turmeric (Rajabiesterabadi et al., 2020a).

Stress prompts consecutive negative effects including immunosuppression (Taheri Mirghaed et al., 2018a; Taheri Mirghaed et al., 2018b; Mirzargar et al., 2022), oxidative stress (Fazelan et al., 2020a; Adineh et al., 2021a), and tissue damage (Kumar et al., 2020; Roychowdhury et al., 2020). The present results showed that a 3-h crowding stress resulted in significant decrease in humoral immune parameters and dietary PE supplementation suppressed such changes, particularly at

250 mg/kg. It has been reported that plasma lysozyme, ACH50, and total Ig levels decrease in common carp under crowding stress. Nevertheless, PE showed immunostimulant properties in common carp, as it improved basal and post stress plasma lysozyme, ACH50, and total Ig levels. There is no study on the effects of dietary PE on fish immune responses; however, the present results are in line with previous studies regarding the use of *Zingiber officinale* (Fazelan et al., 2020a) and *Artemisia annua* (Hoseini et al., 2022) in common carp. As plasma lysozyme, ACH50, and total Ig are good indicators of disease resistance of fish, it is speculated that dietary PE at 250 mg/kg may decrease the risk of disease outbreak after an acute stress in common carp.

It has been reported that high stocking density increases plasma ALT and AST activity in fish (Abdel-Tawwab et al., 2014; Onxayvieng et al., 2021). These enzymes are concentrated in cell cytoplasm, particularly in hepatocytes; therefore, hepatocyte damage leads to leakage of these enzymes into circulation (Taheri Mirghaed et al., 2017). The present results showed that the crowding stress induces oxidative stress and elevates the cortisol and glucose levels in fish, which is in line with Jia et al. (2016), Mahmoud et al. (2021), and Sahin et al. (2014). When fish are exposed to crowding stress, it can cause a decrease in oxygen availability, accumulation of metabolic waste products, and an increase in the levels of stress hormones such as cortisol. These physiological changes can lead to a reduction in liver function, resulting in liver damage and the release of ALT and AST into the bloodstream (Barton, 2002; Ghelichpour et al., 2020). PE and its main compound (pulegone) exhibited antioxidant and hepatoprotective properties, both before and after stress, in fish, which has been previously approved in mammals (Farid et al., 2019; Farid and Eddouks, 2020).

In conclusion, dietary PE potentiates to improve growth performance of common carp, which is believed to be related to improvement in digestive enzymes' activities. Moreover, PE has anti-stress, hypoglycemic, hypolipidemic, antioxidant, and immunostimulant effects in common carp, which improves the fish health after an acute crowding stress. Based on the present study, 250 mg/kg PE is suitable for common carp feed supplementation. Further research is suggested to evaluate the mechanism of action of PE at the molecular level as well as its protective effects in fish exposed to infectious agents.

Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

CRediT authorship contribution statement

Morteza Yousefi: Supervision, Conceptualization, Project administration, Writing – review & editing. **Hossein Adineh:** Conceptualization, Study design, Formal analysis, Resources. **Maryam Ghadamkheir:** Methodology, Formal analysis, Writing – original draft. **Seyed Amir Mahdi Hashemianfar:** Methodology, Formal analysis. **Sevdan Yilmaz:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

This paper has been supported by the RUDN University Strategic Academic Leadership Program dedicated to Morteza Yousefi.

References

Abdel-Tawwab, M., Hagras, A.E., Elbaghdady, H.A.M., Monier, M.N., 2014. Dissolved oxygen level and stocking density effects on growth, feed utilization. Physiol. innate Immun. Nile Tilapia, *Oreochromis niloticus*. J. Appl. Aquac. 26, 340–355.

Abdel-Tawwab, M., Abdulrahman, N.M., Ahmad, V.M., Ramzi, D.O.M., Hassan, B.R., 2021. Effects of dietary oak (*Quercus aegilops* L.) acorn on growth performance, somatic indices, and hemato-biochemical responses of common carp, *Cyprinus carpio* L., at different stocking densities. J. Appl. Aquac. 1–17.

Adel, M., Safari, R., Pourgholam, R., Zorriehzahra, J., Esteban, M.Á., 2015. Dietary peppermint (*Mentha piperita*) extracts promote growth performance and increase the main humoral immune parameters (both at mucosal and systemic level) of Caspian brown trout (*Salmo trutta caspius* Kessler, 1877). Fish. Shellfish Immunol. 47, 623–629.

Adel, M., Pourgholam, R., Zorriehzahra, J., Ghiasi, M., 2016. Hemato–Immunological and biochemical parameters, skin antibacterial activity, and survival in rainbow trout (*Oncorhynchus mykiss*) following the diet supplemented with *Mentha piperita* against *Yersinia ruckeri*. Fish. Shellfish Immunol. 55, 267–273.

Adineh, H., Naderi, M., Nazer, A., Yousefi, M., Ahmadifar, E., 2021a. Interactive effects of stocking density and dietary supplementation with Nano selenium and garlic extract on growth, feed utilization, digestive enzymes, stress responses, and antioxidant capacity of grass carp, *Ctenopharyngodon idella*. J. World Aquac. Soc. 52, 789–804.

Adineh, H., Naderi, M., Yousefi, M., Khademi Hamidi, M., Ahmadifar, E., Hoseini, S.M., 2021b. Dietary licorice (*Glycyrrhiza glabra*) improves growth, lipid metabolism, antioxidant and immune responses, and resistance to crowding stress in common carp, *Cyprinus carpio*. Aquacult Nutr. 27, 417–426.

Anand, P.S.S., Kohli, M.P.S., Roy, S.D., Sundaray, J.K., Kumar, S., Sinha, A., Pailan, G.H., Sukham, Mk, 2013. Effect of dietary supplementation of periphyton on growth performance and digestive enzyme activities in *Penaeus monodon*. Aquaculture 392–395, 59–68.

AOAC, 2005. Official methods of analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Washington, DC, USA.

Arab-Bafrani, Z., Zabihi, E., Hoseini, S.M., Sepehri, H., Khalili, M., 2022. Silver nanoparticles modify the hypothalamic–pituitary–interrenal axis and block cortisol response to an acute stress in zebrafish, *Danio rerio*. Toxicol. Ind. Health 38, 201–209.

Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42, 517–525.

Bilen, S., Filogh, A.M.O., Ali, A.B., Kenanoğlu, O.N., Zoral, M.A., 2020. Effect of common mallow (*Malva sylvestris*) dietary supplementation on growth performance, digestive enzyme activities, haemotological and immune responses of common carp (*Cyprinus carpio*). Aquac. Int. 28, 73–84.

Burk, R.F., Hill, K.E., 2010. 4.13 - Glutathione peroxidases. In: McQueen, C.A. (Ed.), Comprehensive Toxicology, Second ed. Elsevier, Oxford, pp. 229–242.

Chelemal Dezfoulnejad, M., Molayemraftar, T., 2022. Use of dietary rosemary (*Rosmarinus officinalis* L.) extract as a growth promotor and immunostimulant in common carp. Aquac. Res. 53, 1553–1562.

Chung, S., Lemos, C.H.D.P., Teixeira, D.V., Fortes-Silva, R., Copatti, C.E., 2020. Essential oil from *Ocimum basilicum* improves growth performance and does not alter biochemical variables related to stress in pirarucu (*Arapaima gigas*). . Acad. Bras. Cienc. 2020 (92 Suppl 1), e20181374.

Ciji, A., Akhtar, M.S., 2021. Stress management in aquaculture: a review of dietary interventions. Rev. Aquacult 13, 2190–2247.

Elbialy, Z.I., Salah, A.S., Elsheshtawy, A., Rizk, M., Abualreesh, M.H., Abdel-Daim, M.M., Salem, S.M.R., Askary, A.E., Assar, D.H., 2021. Exploring the multimodal role of *Yucca schidigera* extract in protection against chronic ammonia exposure targeting: growth, metabolic, stress and inflammatory responses in Nile tilapia (*Oreochromis niloticus* L.). Animals 11, 2072.

El-Ghorab, A.H., 2006. The chemical composition of the *Mentha pulegium* L. essential oil from Egypt and its antioxidant activity. J. Essent. oil Bear. Plants 9, 183–195.

Ellis, A.E., 1990. Lysozyme assays. In: Stolen, J.S. (Ed.), Techniques in fish immunology. SOS publication, Fair Haven, pp. 101–103.

Elumalai, P., Kurian, A., Lakshmi, S., Faggio, C., Esteban, M.A., Ringø, E., 2021. Herbal immunomodulators in aquaculture. Rev. Fish. Sci. Aquac. 29, 33–57.

Farid, O., Eddouks, M., 2020. Evaluation of the anti-hypercholesterolemic and antioxidant activity of *Mentha pulegium* (L.) aqueous extract in normal and streptozotocin-induced diabetic rats. Nat. Prod. J. 10, 236–243.

Farid, O., Zeggwagh, N.A., Ouadi, F.E., Eddouks, M., 2019. *Mentha pulegium* aqueous extract exhibits antidiabetic and hepatoprotective effects in streptozotocin-induced diabetic rats. Endocr., Metab. Immune Disord. -Drug Targets 19, 292–301.

Fazelan, Z., Vatnikov, Y.A., Kulikov, E.V., Plushikov, V.G., Yousefi, M., 2020a. Effects of dietary ginger (*Zingiber officinale*) administration on growth performance and stress, immunological, and antioxidant responses of common carp (*Cyprinus carpio*) reared under high stocking density. Aquaculture 518, 734833.

Fazelan, Z., Hoseini, S.M., Yousefi, M., Khalili, M., Hoseinifar, S.H., Van Doan, H., 2020b. Effects of dietary eucalyptol administration on antioxidant and inflammatory genes in common carp (*Cyprinus carpio*) exposed to ambient copper. Aquaculture 520, 734988.

Flamini, G., Cioni, P.L., Puleio, R., Morelli, I., Panizzi, L., 1999. Antimicrobial activity of the essential oil of *Calamintha nepeta* and its constituent pulegone against bacteria and fungi. Phytother. Res. 13, 349–351.

Garlock, T., Asche, F., Anderson, J., Bjørndal, T., Kumar, G., Lorenzen, K., Ropicki, A., Smith, M.D., Tveterås, R., 2020. A global blue revolution: aquaculture growth across regions, species, and countries. Rev. Fish. Sci. Aquac. 28, 107–116.

Ghelichpour, M., Taheri Mirghaed, A., Mirzargar, S.S., Joshaghani, H., Ebrahimzadeh Mousavi, H., 2017. Plasma proteins, hepatic enzymes, thyroid hormones and liver histopathology of *Cyprinus carpio* (Linnaeus, 1758) exposed to an oxadiazin pesticide, indoxacarb. Aquac. Res 48, 5666–5676.
Ghelichpour, M., Taheri Mirghaed, A., Hoseini, S.M., Perez Jimenez, A., 2020. Plasma antioxidant and hepatic enzymes activity, thyroid hormones alterations and health status of liver tissue in common carp (*Cyprinus carpio*) exposed to lufenuron. Aquaculture 516, 734634.
Gholamhosseini, A., Adel, M., Dawood, M.A., Banaee, M., 2020. The potential benefits of *Mentha longifolia* on growth performance and innate immunity parameters in Caspian kutum (*Rutilus frisii kutum*). Aquac. Res. 51, 5212–5227.
Giri, S.S., Sukumaran, V., Park, S.C., 2019. Effects of bioactive substance from turmeric on growth, skin mucosal immunity and antioxidant factors in common carp, *Cyprinus carpio*. Fish. Shellfish Immunol. 92, 612–620.
Goth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta 196, 143–151.
Haschek, W.M., Rousseaux, C.G., Wallig, M.A., 2009. Fundamentals of Toxicologic Pathology. Academic Press, San Diego, CA.
Hernández-Contreras, Á., Hernández, M.D., 2020. Chapter 14 - Application of aromatic plants and their extracts in aquaculture. In: Florou-Paneri, P., Christaki, E., Giannenas, I. (Eds.), Feed Additives. Academic Press, pp. 239–259.
Hoseini, S.M., Hosseini, S., Nodeh, A., 2011. Serum biochemical characteristics of Beluga, *Huso huso* (L.), in response to blood sampling after clove powder solution exposure. Fish. Physiol. Biochem. 37, 567–572.
Hoseini, S.M., Hedayati, A., Ghelichpour, M., 2014. Plasma metabolites, ions and thyroid hormones levels, and hepatic enzymes' activity in Caspian roach (*Rutilus rutilus caspicus*) exposed to waterborne manganese. Ecotoxicol. Environ. Saf. 107, 84–89.
Hoseini, S.M., Gharavi, B., Taheri Mirghaed, A., Hoseinifar, S.H., Van Doan, H., 2021a. Effects of dietary phytol supplementation on growth performance, immunological parameters, antioxidant and stress responses to ammonia exposure in common carp, Cyprinus carpio (Linnaeus, 1758). Aquaculture 545, 737151.
Hoseini, S.M., Taheri Mirghaed, A., Iri, Y., Hoseinifar, S.H., Van Doan, H., Reverter, M., 2021b. Effects of dietary Russian olive, *Elaeagnus angustifolia*, leaf extract on growth, hematological, immunological, and antioxidant parameters in common carp, *Cyprinus carpio*. Aquaculture 536, 736461.
Hoseini, S.M., Aydın, B., Hoseinifar, S.H., Moonmanee, T., Van Doan, H., 2022. Dietary Artemisia, *Artemisia annua*, supplementation improves common carp welfare under high stocking density. Aquac. Res. 53, 3494–3503.
Hoseinifar, S.H., Sun, Y.-Z., Zhou, Z., Van Doan, H., Davies, S.J., Harikrishnan, R., 2020. Boosting immune function and disease bio-control through environment-friendly and sustainable approaches in finfish aquaculture: herbal therapy scenarios. Rev. Fish. Sci. Aquac. 28, 1–19.
Jia, R., Liu, B.L., Han, C., Huang, B., Lei, J.L., 2016. Influence of stocking density on growth performance, antioxidant status, and physiological response of juvenile Turbot, *Scophthalmus maximus*, reared in land-based recirculating aquaculture system. J. World Aquac. Soc. 47, 587–599.
Kaur, G., Athar, M., Alam, M.S., 2018. Pulegone: a potential candidate for the development of antimicrobial agents. Biomed. Pharmacother. 107, 372–380.
Kesbiç, O.S., 2019. Effects of juniper berry oil on growth performance and blood parameters in common carp (*Cyprinus carpio*). Aquac. Res. 50, 342–349.
Kumar, N., Chandan, N.K., Wakchaure, G.C., Singh, N.P., 2020. Synergistic effect of zinc nanoparticles and temperature on acute toxicity with response to biochemical markers and histopathological attributes in fish. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 229, 108678.
Lee, C.-S., Lim, C., Webster, C.D., 2015. Dietary Nutrients, Additives, and Fish Health. Wiley-Blackwell, NJ, USA.
Mahboubi, M., Haghi, G., 2008. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. J. Ethnopharmacol. 119, 325–327.
Mahmoud, H.K., Reda, F.M., Alagawany, M., Farag, M.R., 2021. Ameliorating deleterious effects of high stocking density on *Oreochromis niloticus* using natural and biological feed additives. Aquaculture 531, 735900.
Mazandarani, M., Hoseini, S.M., 2017. Anaemia and plasma lipid profile in common carp (*Cyprinus carpio*) exposed to ambient copper sulphate and nano-scale copper oxide. Aquac. Res. 48, 844–852.
Mirzargar, S.S., Taheri Mirghaed, A., Hoseini, S.M., Ghelichpour, M., shahbazi, M., Yousefi, M., 2022. Biochemical responses of common carp, *Cyprinus carpio*, to transportation in plastic bags using thymol as a sedative agent. Aquac. Res 53, 191–198.
Nam, Y.K., Park, I.S., Kim, K.H., Lee, S.G., 2013. Anti-inflammatory effect of pulegone on lipopolysaccharide-induced acute lung injury in mice. Inflammation 36 (5), 1071–1078.
Onxayvieng, K., Piria, M., Fuka, M.M., Gavrilović, A., Liang, X., Liu, L., Tang, R., Li, L., Li, D., 2021. High stocking density alters growth performance, blood biochemical profiles, and hepatic antioxidative capacity in gibel carp (*Carassius gibelio*). Fish. Physiol. Biochem. 47, 203–212.
Paray, B.A., Hoseini, S.M., Hoseinifar, S.H., Van Doan, H., 2020. Effects of dietary oak (*Quercus castaneifolia*) leaf extract on growth, antioxidant, and immune characteristics and responses to crowding stress in common carp (*Cyprinus carpio*. Aquaculture 524, 735276.
Perry, J.J.P., Shin, D.S., Getzoff, E.D., Tainer, J.A., 2010. The structural biochemistry of the superoxide dismutases. Biochim. Et. Biophys. Acta (BBA) - Proteins Proteom. 1804, 245–262.
Rajabiesterabadi, H., Hoseini, S.M., Fazelan, Z., Hoseinifar, S.H., Doan, H.V., 2020a. Effects of dietary turmeric administration on stress, immune, antioxidant and inflammatory responses of common carp (*Cyprinus carpio*) during copper exposure. Aquacult Nutr. 26, 1143–1153.
Rajabiesterabadi, H., Ghelichi, A., Jorjani, S., Hoseini, S.M., Akrami, R., 2020b. Dietary olive (*Olea europaea*) leaf extract suppresses oxidative stress and modulates intestinal expression of antioxidant-and tight junction-related genes in common carp (*Cyprinus carpio*). Aquaculture 520, 734676.
Ribeiro, S.C., Malheiros, D.F., Guilozki, I.C., Majolo, C., Chaves, F.C.M., Chagas, E.C., Silva de Assis, H.C., Tavares-Dias, M., Yoshioka, E.T.O., 2018. Antioxidants effects and resistance against pathogens of *Colossoma macropomum* (Serassalmidae) fed *Mentha piperita* essential oil. Aquaculture 490, 29–34.
Ringø, E., Zhou, Z., Vecino, J.L.G., Wadsworth, S., Romero, J., Krogdahl, Å., Olsen, R.E., Dimitroglou, A., Foey, A., Davies, S., Owen, M., 2016. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? Aquac. Nutr. 22 (2), 219–282.
Roychowdhury, P., Aftabuddin, M., Pati, M.K., 2020. Thermal stress altered growth performance and metabolism and induced anaemia and liver disorder in *Labeo rohita*. Aquac. Res. 51, 1406–1414.
Sahin, K., Yazlak, H., Orhan, C., Tuzcu, M., Akdemir, F., Sahin, N., 2014. The effect of lycopene on antioxidant status in rainbow trout (*Oncorhynchus mykiss*) reared under high stocking density. Aquaculture 418, 132–138.
Santos, F.A.C., Boaventura, T.P., da Costa Julio, G.S., Cortezzi, P.P., Figueiredo, L.G., Favero, G.C., Palheta, G.D.A., de Melo, N.F.A.C., Luz, R.K., 2021. Growth performance and physiological parameters of *Colossoma macropomum* in a recirculating aquaculture system (RAS): Importance of stocking density and classification. Aquaculture 534, 736274.
Sepasi Tehrani, H., Moosavi-Movahedi, A.A., 2018. Catalase and its mysteries. Prog. Biophys. Mol. Biol. 140, 5–12.
Shamlo, R., Nasr, J., Kheiri, F., 2014. Effects of various levels of pennyroyal (*Mentha pulegium* L.) on carcass characteristics and serum cholesterol in broiler. Res. Opin. Anim. Vet. Sci. 4, 453–457.
Silveira, N.Sd, Oliveira-Silva, G.Ld, Lamanes, Bd.F., Prado, L.Cd.S., Bispo-da-Silva, L.B., 2014. The aversive, anxiolytic-like, and verapamil-sensitive psychostimulant effects of pulegone. Biol. Pharm. Bull. 37, 771–778.
Siwicki, A., Anderson, D., 1993. 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. In: Siwicki, A., Anderson, D., Waluga, J. (Eds.), Fish disease diagnosis and prevention methods. Wydawnictwo Instytutu Rybactwa Srodladowego, Olsztyn, Poland, pp. 105–112.
Sutili, F.J., Velasquez, A., Pinheiro, C.G., Heinzmann, B.M., Gatlin, D.M., Baldisserotto, B., 2016. Evaluation of *Ocimum americanum* essential oil as an additive in red drum (*Sciaenops ocellatus*) diets. Fish. Shellfish Immunol. 56, 155–161.
Taheri Mirghaed, A., Ghelichpour, M., Hoseini, S.M., Amini, K., 2017. Hemolysis interference in measuring fish plasma biochemical indicators. Fish. Physiol. Biochem. 34, 1143–1151.
Taheri Mirghaed, A., Fayaz, S., Hoseini, S.M., 2018a. Dietary 1, 8–cinoele affects serum enzymatic activities and immunological characteristics in common carp (*Cyprinus carpio*) exposed to ambient ammonia. Aquac. Res. 50, 146–153.
Taheri Mirghaed, A., Hoseini, S.M., Ghelichpour, M., 2018b. Effects of dietary 1, 8-cineole supplementation on physiological, immunological and antioxidant responses to crowding stress in rainbow trout (*Oncorhynchus mykiss*). Fish. Shellfish Immunol. 81, 182–188.
Taheri Mirghaed, A., Hoseini, S.M., Hoseinifar, S.H., Van Doan, H., 2020. Effects of dietary thyme (Zataria multiflora) extract on antioxidant and immunological responses and immune-related gene expression of rainbow trout (Oncorhynchus mykiss) juveniles. Fish Shellfish Immunol.
Vianna, G.M.S., Zeller, D., Pauly, D., 2020. Fisheries and policy implications for human nutrition. Curr. Environ. Health Rep. 7, 161–169.
Wangkahart, E., Wachiraamonloed, S., Lee, P.-T., Subramani, P.A., Qi, Z., Wang, B., 2022. Impacts of *Aegle marmelos* fruit extract as a medicinal herb on growth performance, antioxidant and immune responses, digestive enzymes, and disease resistance against *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*). Fish. Shellfish Immunol. 120, 402–410.
Xie, J., Liu, B., Zhou, Q., Su, Y., He, Y., Pan, L., Ge, X., Xu, P., 2008. Effects of anthraquinone extract from rhubarb *Rheum officinale* Bail on the crowding stress response and growth of common carp *Cyprinus carpio* var. Jian. Aquaculture 281, 5–11.
Yano, T., 1992. Assays of hemolytic complement activity. In: Stolen, J.S. (Ed.), Techniques in Fish Immunology. SOS publication, Fair haven, pp. 131–141.
Yousefi, M., Paktinat, M., Mahmoudi, N., Pérez-Jiménez, A., Hoseini, S.M., 2016. Serum biochemical and non-specific immune responses of rainbow trout (*Oncorhynchus mykiss*) to dietary nucleotide and chronic stress. Fish. Physiol. Biochem. 42, 1417–1425.
Yousefi, M., Shabunin, S.V., Vatnikov, Y.A., Kulikov, E.V., Adineh, H., Hamidi, M.K., Hoseini, S.M., 2020a. Effects of lavender (*Lavandula angustifolia*) extract inclusion in diet on growth performance, innate immunity, immune-related gene expression, and stress response of common carp, *Cyprinus carpio*. Aquaculture 515, 734588.
Yousefi, M., Adineh, H., Reverter, M., Hamidi, M.K., Vatnikov, Y.A., Kulikov, E.V., Hoseinifar, S.H., Van Doan, H., 2020b. Protective effects of black seed (*Nigella sativa*) diet supplementation in common carp (*Cyprinus carpio*) against immune depression, oxidative stress and metabolism dysfunction induced by glyphosate. Fish. Shellfish Immunol. 109, 12–19.
Yousefi, M., Zahedi, S., Reverter, M., Adineh, H., Hoseini, S.M., Van Doan, H., El-Haroun, E.R., Hoseinifar, S.H., 2021. Enhanced growth performance, oxidative capacity and immune responses of common carp, *Cyprinus carpio* fed with *Artemisia absinthium* extract-supplemented diet. Aquaculture 545, 737167.
Yousefi, M., Ghafarifarsani, H., Hoseini, S.M., Hoseinifar, S.H., Abtahi, B., Vatnikov, Y.A., Kulikov, E.V., Van Doan, H., 2022. Effects of dietary thyme essential oil and

prebiotic administration on rainbow trout (*Oncorhynchus mykiss*) welfare and performance. Fish. Shellfish Immunol. 120, 737–744.
Zhang, R., Wang, X., Liu, L., Cao, Y., Zhu, H., 2020. Dietary oregano essential oil improved the immune response, activity of digestive enzymes, and intestinal microbiota of the koi carp, *Cyprinus carpio*. Aquaculture 518, 734781.
Zhu, F., 2020. A review on the application of herbal medicines in the disease control of aquatic animals. Aquaculture 526, 735422.