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Effects of dietary oak (*Quercus castaneifolia*) leaf extract on growth, antioxidant, and immune characteristics and responses to crowding stress in common carp (*Cyprinus carpio*)

Bilal Ahmad Paray[a], Seyyed Morteza Hoseini[b], Seyed Hossein Hoseinifar[c], Hien Van Doan[d,e,*]

[a] Department of Zoology, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia
[b] Inland Waters Aquatics Resources Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization, Gorgan, Iran
[c] Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[d] Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[e] Science and Technology Research Institute, Chiang Mai University, 239 Huay Keaw Rd., Suthep, Muang, Chiang Mai 50200, Thailand

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ABSTRACT

The aim of the present study was to assess the effects of dietary oak (*Quercus castaneifolia*) leaf extract supplementation on growth performance, antioxidant, immune and stress responses in common carp (*Cyprinus carpio*). Ethanolic extract of the oak leaf were prepared and tested for *in vitro* radical scavenging and antibacterial properties. The results showed that the extract has radical scavenging property below that of butylated hydroxytoluene (BHT) at 12.5–75 μg mL^{-1}, but there was no significant difference between the two materials at 100 and 200 μg mL^{-1}. Moreover, the extract had bactericidal effects against *Aeromonas hydrophila*, which was near 5-fold lower than tetracycline. Then, the extract was added to the fish diet at 0 (CTL), 0.5 (0.5E), 1 (1E) and 2 (2E) g kg^{-1} diet. The fish were fed with aforementioned diets for 60 days before subjecting to a 6-h crowding stress. The results showed that the extract had no significant effects on the fish growth performance ($P > .05$). Dietary extract supplementation (1E and 2E) significantly increased plasma superoxide dismutase ($P = .003$), catalase ($P = .015$), glutathione peroxidase ($P < .001$), reduced glutathione ($P < .001$), lysozyme ($P < .001$), complement ($P = .001$), and bactericidal activity ($P = .020$) and decreased malondialdehyde ($P < .001$) levels. Dietary oak leaf extract supplementation had no significant effects on basal cortisol and glucose levels, but significantly ($P < .001$) mitigated post-stress levels of these parameters, compared to the CTL fish. In conclusion, oak leaf extract stimulates antioxidant and immune system of common carp, without affecting the fish growth performance. Moreover, the extract was partially beneficial to reduce stress in the fish. Dietary levels of 1–2 g kg^{-1} oak leaf extract are recommended for common carp feed formulation.

1. Introduction

It is interesting for fish farmers to find out methods for increasing fish growth and health, because of economic reasons as well as constant threat of diseases outbreak in the fish farms. An efficient way to reach these goals is use of feed supplements, which may stimulate the fish growth performance and boost antioxidant and non-specific immune systems (Abdel-Tawwab, 2016).

Aquaculture causes constant threat of free radicals to fish due to various reasons such as high fish stocking density and handling; thus fish must have strong antioxidant defense to counteract these negative effects (Abdel-Tawwab et al., 2018b). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are important antioxidant enzymes in fish that protect cells against superoxide and hydrogen peroxide molecules (Yousefi et al., 2019). Oxidative stress is a condition under which fish antioxidant system fails to counteract pro oxidants. Oxidative stress leads to fatty acid oxidation and formation of malondialdehyde (MDA). Therefore, a strong antioxidant system protects the fish fatty acids against oxidation and guarantees the fish welfare. Evidence show dietary additives may effectively increase the fish antioxidant system and suppress oxidative stress (Giannenas et al., 2012; Pérez Jiménez et al., 2012; Ahmadifar et al., 2019; Yılmaz et al., 2019).

Non-specific immune system is important in fish immunity, because

* Corresponding author at: Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.
E-mail address: hien.d@cmu.ac.th (H. Van Doan).

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it is the fast responding immune system and acts against a wide range of pathogens (Lee et al., 2015). Lysozyme and complement molecules are two well-known components of the fish immune system, which can be stimulated by dietary additives (Taheri Mirghaed and Ghelichpour, 2018). Immunoglobulins (Ig) are components of adaptive immune system of fish, but fish produce basal levels of Ig under normal condition, too (Fazelan et al., 2020). Fish plasma Ig levels increase in response to dietary additive supplementation according to previous studies (Akrami et al., 2015; Safari et al., 2019). Improve in these parameters helps fish to counteract pathogens.

Many aquaculture practices are stressful for fish, triggering hypothalamus-pituitary-interrenal axis and cortisol release (Barton, 2002). Cortisol has been known to increase fish energy expenditure and suppress immune system (Tort, 2011). Moreover, fish face higher levels of free radicals under stressful conditions (Yarahmadi et al., 2016). Therefore, finding practical approaches for stress relieve is important in aquaculture. Nutritional manipulation has been demonstrated as a useful method that can mitigate fish stress (Xie et al., 2008; Sahin et al., 2014; Abdel-Tawwab et al., 2018a).

Among the feed additives, herbal materials have gained great attention in aquaculture industry, because of their natural origins (Lee et al., 2015). Several studies have shown that herbal intact materials/extracts increase fish performance and well-being (Chakraborty and Hancz, 2011; Chakraborty et al., 2014; Baba et al., 2016; Baba et al., 2018; Zemheri-Navruz et al., 2019). Oaks (*Quercus* sp.) are groups of diverse plant species with worldwide distribution (Almeida et al., 2008b). They are used as human food supply and medicinal plant in the world, because they have beneficial properties such as antioxidant and antibacterial activities (Almeida et al., 2008b; Nourafcan et al., 2013). *Quercus castaneifolia* is abundant in hyrcanian forests and studies have shown that its acorn extract has antibacterial activity (Bahador and Baserisalehi, 2011; Sefidgar et al., 2015). However, there is no study on its leaf extracts properties, despite the presence of evidence of antioxidant and antibacterial activity of leaf extract of other oak species (Almeida et al., 2008b; Nourafcan et al., 2013).

As an important aquaculture species, common carp (*Cyprinus carpio*) global production was higher than 4.1 million tonnes in 2017 (FAO, 2019). Accordingly the present study aimed to assess effects of dietary *Q. castaneifolia* leaf extract on growth performance, antioxidant, immune, hemolysis, and stress responses of common carp (*Cyprinus carpio*).

2. Materials and methods

2.1. Oak leaf extract

Ethanol extract of oak (*Q. castaneifolia*) leaf was prepared according to Sadeghinezhad et al. (2010). The fresh oak leaves were collected in June from Gorgan, Iran. They were washed with distilled water three times and allowed to be dried against a fan blow at 25 °C for 48 h. Then the dried leaves were pulverized and 50 g of the powder were added 500 mL of 80% ethanol. The mixture was left at room temperature for 3 days and then it was filtered through a 500 μm mesh. The resultant aqueous solution was concentrated in oven (40 °C) for 48 h and after ethanol evaporation, the solution transferred to a freeze-dryer (Beta LDpluse, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) for 72 h (−50 °C). The dried materials were collected and used for diet preparation.

2.2. Fish rearing and induction of crowding stress

A control diet (Table 1) was prepared by mixing the feedstuffs with 0.0 (CTL), 0.5 (0.5E), 1 (1E) and 2 (2E) g kg^{-1} diet for 30 min. Then, 0.3 L water was added to each kg of the mixture to form dough. The dough was passed through a meat grinder mesh (3 mm) and the resultant sticks were dried against a fan below at 25 °C for 24 h. To prepare the other experimental diets, 0.5, 1 and 2 g of the oak leaf extract were mixed with the dietary oils before mixing with the other feedstuffs.

Table 1
Composition of the control diet.

Feedstuffs	Amount g kg^{-1}
Fishmeal[1]	160
Soybean meal[2]	200
Poultry by-product[3]	230
Wheat meal[4]	373
Fish oil[5]	10
Sunflower oil[6]	10
Lysine[7]	8
Methionine[8]	4
Vitamin mix[9]	2.5
Mineral mix[10]	2.5
Dry matter	90.6
Crude protein	39.9
Crude fat	79.0
Crude ash	81.2

[1] Peygir Co., Gorgan, Iran (crude protein 65.8%).
[2] Soyabean Co., Gorgan, Iran (crude protein 46.2%).
[3] Peygir Co., Gorgan, Iran (crude protein 58.0%).
[4] Zahedi wheat meal Co., Gorgan, Iran.
[5] Peygir Co., Gorgan, Iran (Kilka fish oil).
[6] Khorasan oil seed Co., Mashhad, Iran.
[7] Madtiur Co., Tehran, Iran.
[8] Kimiyazarrin Co., Tehran, Iran.
[9] The premix provided following amounts per kg of diet: Mg: 350 mg; Fe: 13 mg; Co: 2.5 mg; Cu: 3 mg; Zn: 60 mg; NaCl: 3 g; dicalcium phosphate: 10 g.
[10] The premix provided following amounts per kg of feed: A: 1000 IU; D3: 5000 IU; E: 20 mg; B5: 100 mg; B2: 20 mg; B6: 20 mg; B1: 20 mg; H: 1 mg; B9: 6 mg; B12: 1 mg; B4: 600 mg; C: 50 mg.

About 240 common carp juveniles (~20 g) were randomly stocked in 12 tanks (180 L) at a density of 20 fish per each tank, equipped with aeration. The fish were fed the control diet for 10 days to acclimate with the experimental conditions. Then, the tanks assigned to four groups receiving the control (CTL) diet or control diet supplemented with 0.5 (0.5E), 1 (1E) and 2 (2E) g oak leaf extract per kg diet in triplicates. The fish were fed one the tested diets twice a day until apparent satiation for 60 days and the tanks' water was renewed by half every day using well-aerated tap water. The tanks' water temperature, pH, dissolved oxygen, unionized ammonia nitrogen and nitrite nitrogen levels were monitored weekly, and they were 22.3 ± 1.11 °C, 8.11 ± 0.55, 6.21 ± 1.06 mg L^{-1}, 0.06 ± 0.01 mg L^{-1}, and 0.11 ± 0.09 mg L^{-1}. At the end of the feeding period, the fish feed intake (FI), weight gain percentage (WG), feed conversion ratio (FCR), and specific growth rate (SGR) were calculated according to Abtahi et al. (2013):

$$WG\ (\%) = 100 \times [(FW - IW)/IW]$$

$$FCR = FI/(FW - IW)$$

$$SGR\ (\%d^{-1}) = 100 \times [(\ln\ FW - \ln\ IW)/d]$$

where, WG was weight gain percentage, IW and FW were fish initial and final weights in gram, and d was days of rearing.

Two fish were taken from each tank (*i.e.* six fish per treatment) and anesthetized using 100 mg L^{-1} eugenol (Yousefi et al., 2019) for blood sampling (using heparinized syringes). After the blood sampling, 80% of the tanks' water was drained to induce stress in the fish. After the 6-h crowding stress, the fish were blood-sampled again (6 h), before returning the tank water to the original level. 24 h after returning the tank water to the original levels, the fish were sampled again (recovery).

2.3. In vitro antioxidant and bactericidal activity of the oak leaf extract

Antioxidant capacity of the oak leaf extract was determined based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as suggested by Kamkar et al., 2010. Briefly, serial dilutions (12.5–200 μg mL^{-1}) of the extract and reference material (Butylated hydroxytoluene (BHT); Sigma-Aldrich Co., Saint Louis, USA) were prepared in methanol. One mL of DPPH (1 M) was mixed with three mL of the dilutions, mixed vigorously and kept at dark for 30 min. Optical density of the samples were read at 517 nm. Bactericidal activity of the oak leaf extract was determined against *Aeromonas hydrophila* using broth dilution method. Concentrations of 1, 3, 6 and 10 mg L^{-1} of the extract and Mueller Hinton Broth medium containing the bacterium (1.2×10^3) were prepared. Equal volume of the extract and medium was mixed in test tube and incubated at 37 °C for 24 h. Concentrations of 1, 3, 6 and 10 mg L^{-1} of tetracycline was used as control. After the incubation, the colony forming units of each test tube were counted (Harikrishnan and Balasundaram, 2005).

2.4. Plasma analyses

Blood plasma was separated after centrifugation (4 °C; 1500 *g*) for 10 min and kept at −70 °C until biochemical analysis. Plasma lysozyme, alternative complement (ACH50), total immunoglobulin (Ig), bactericidal activity, SOD, CAT, GPx, GSH, MDA, AST and ALP were measured after the feeding period (60 d). The plasma cortisol and glucose levels were measure after the 60 d feeding period (before stress), 6 h after stress and 24 h after stress termination (recovery). A commercial kit (Pars Azmun Co., Tehran Iran) was used for measurement of plasma glucose levels based on glucose oxidase method (Taheri Mirghaed et al., 2018). Plasma SOD (cytochrome C reduction), CAT (decomposition of hydrogen peroxide), GPx (glutathione oxidation) activities, and levels of GSH (glutathione oxidation) and MDA (reaction with thiobarbituric acid) were determined using commercial kits provided by Zellbio Co. (ZellBio, GmbH, Veltinerweg, Germany) as suggested by Fazelan et al. (2020). Plasma cortisol levels were measured by ELISA method using a commercial kit (competitive ELISA kit; IBL, Gesellschaft für Immunchemieund Immunbiologie, Germany) (Taheri Mirghaed et al., 2018).

Micrococcus luteus was used as the target for plasma lysozyme determination in phosphate buffered saline pH 6.2, as described by Ellis (1990). Hemolytic activity used to measure alternative complement system (ACH50) using sheep RBC and magnesium-enriched veronal buffer (pH = 7). Calculation of ACH50 activity was performed according to Yano (1992). Total Ig levels of the plasma samples were measured after precipitation with polyethylene glycol according to Siwicki and Anderson (1993). Bactericidal activity of the plasma samples were assayed based on the ability to kill *A. hydrophila* using nutrient broth and nutrient agar media as described by Fazelan et al. (2020).

2.5. Statistical analysis

Comparison of antioxidant capacity of the oak leaf extract and BHT was performed using *t*-test. The other data were normally distributed according to Shapiro-Wilk test, except the cortisol data, which were log-transformed before ANOVA analysis. The growth, plasma lysozyme, ACH50, total Ig, bactericidal activity, SOD, CAT, GPx, GSH, MDA, AST and ALP data were analyzed by one-way ANOVA to find significant effects of dietary oak leaf extract. Plasma cortisol and glucose were subjected to two-way ANOVA to find interaction effects of sampling time and dietary oak leaf extract. Plasma cortisol data were subjected to one-way ANIVA because of the interaction effects of sampling time and dietary oak leaf extract. Duncan test determined significant differences among the treatments. Significant differences ($P < .05$) among the treatments were checked by Duncan test. The data were analyzed using SPSS v.22 and present as mean ± SE.

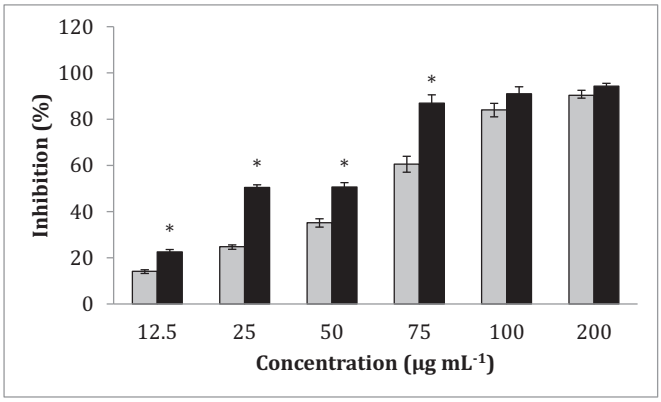

Fig. 1. Inhibition of free radicals (%) by different concentrations of oak leaf extract and BHT. Asterisks show significant differences between the oak leaf extract and BHT at each concentration.

3. Results

The results showed that antioxidant capacity of the oak leaf extract was lower than that of the BHT at the concentrations of 12.5–75 μg mL^{-1}; however, there was no significant difference in antioxidant capacity between the oak leaf extract and BHT at the concentrations of 100 and 200 μg mL^{-1} (Fig. 1). Moreover, the extract bactericidal activity was 3.8–5 folds lower than that of the tetracycline (Fig. 2).

According to Table 2, there were no significant difference in fish growth performance and survival after 60 days feeding of the different experimental diets ($P > .05$).

Plasma lysozyme activity of the 0.5E, 1E and 2E treatments were significantly ($P < .001$) higher than the CTL treatment. The CTL, 0.5E and 1E treatment had similar plasma ACH50 activity, which was significantly lower than the 2E treatment ($P = .001$) lower than the values observed in the 2E treatment. Plasma total Ig levels were statistically similar among the treatments ($P = .720$). The 1E and 2E treatments had significantly ($P = .020$) higher plasma bactericidal activities compared to the CTL treatment; however, there was no significant difference between the 0.5E and CTL treatments (Fig. 3).

Plasma antioxidant parameters showed significant variations among the treatments (Fig. 4). There were significant increases in plasma SOD activities ($P = .003$) and GSH levels ($P < .001$) of the 0.5E, 1E and 2E treatments compared to the CTL. Statistically similar plasma CAT and GPx activities were observed in the 0.5E and CTl treatments. But

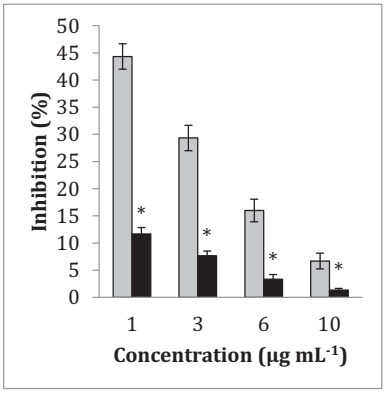

Fig. 2. Bactericidal activity (cfu) of different concentrations of oak leaf extract and tetracycline. Asterisks show significant differences between the oak leaf extract and BHT at each concentration.

Table 2
Effects of dietary oak leaf extract on growth performance and survival of common carp after 60 days.

	CTL	0.5E	1E	2E	*P*-value
Initial weight (g)	20.2 ± 0.43	20.4 ± 0.34	20.0 ± 0.59	20.1 ± 0.44	.954
Final weight (g)	41.0 ± 1.00	42.7 ± 1.20	43.0 ± 1.15	45.3 ± 2.40	.330
Weight gain (%)	102 ± 1.98	110 ± 6.38	115 ± 12.2	126 ± 8.38	.306
SGR (% d^{-1})	1.18 ± 0.02	1.18 ± 0.05	1.27 ± 0.09	1.35 ± 0.06	.304
Feed intake (g)	603 ± 12.0	597 ± 8.82	587 ± 20.3	637 ± 34.7	.434
FCR	1.45 ± 0.04	1.34 ± 0.07	1.28 ± 0.05	1.27 ± 0.08	.227
Survival (%)	100	100	100	100	

activity of the enzymes was significantly higher in the 1E and 2E treatments, compared to the CTL. Feeding the fish with 0.5E, 1E and 2E diets significantly ($P < .001$) lowered plasma MDA levels compared to the CTL treatments; the lowest value was observed in the 1E and 2E treatments.

Dietary oak leaf extract and sampling time had interaction effects on plasma cortisol levels ($P = .001$). Plasma cortisol levels of all dietary treatment were statistically similar, before and after stress; feeding with the extract-supplemented diets significantly decreased cortisol levels during recovery ($P < .001$). Such an effect was more pronounced in the 1E and 2E treatments. The lowest plasma glucose was related to the before stress sampling time, which showed peak levels after the stress, followed a partial recovery at the recovery time. Among the extract-treated fish, the 1E and 2E treatments had the lowest plasma glucose levels (Table 3).

4. Discussion

The present study showed that the extract of *Q. castaneifolia* was a suitable antioxidant and antibacterial agent, which reduced free radical formation and suppressed growth of an important fish pathogenic bacterium. The results are in line with previous studies on acorn extract of *Q. castaneifolia*, showing that the extract had anti-bacterial effects on *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae* and *Yersinia enterocolitica* (Bahador and Baserisalehi, 2011; Sefidgar et al., 2015). Moreover, extract of other oak leaves such as *Quercus ruber* had radical scavenging activity *in vitro* (Almeida et al., 2008b; Almeida et al., 2008a).

The oak leaf extract had no growth promoting effects in the present study, suggesting that the extract may not stimulate digestive enzymes and/or gut absorption. Moreover, leaf extract may have negative effects on fish growth performance, as reported in hybrid grouper (*Epinephelus lanceolatus*♂ × *Epinephelus fuscoguttatus*♀) and Nile tilapia (*Oreochromis niloticus*) fed diets supplemented with *Ginkgo biloba* and *Moringa oleifera* leaf extract (Dongmeza et al., 2006; Tan et al., 2018). Such negative effects of the leaf extract on growth performance of the fish fed the supplemented diets were related to lower feed consumption. This may explain no significant effects of dietary oak leaf extract on the fish growth rate in the present study. Supporting this hypothesis,

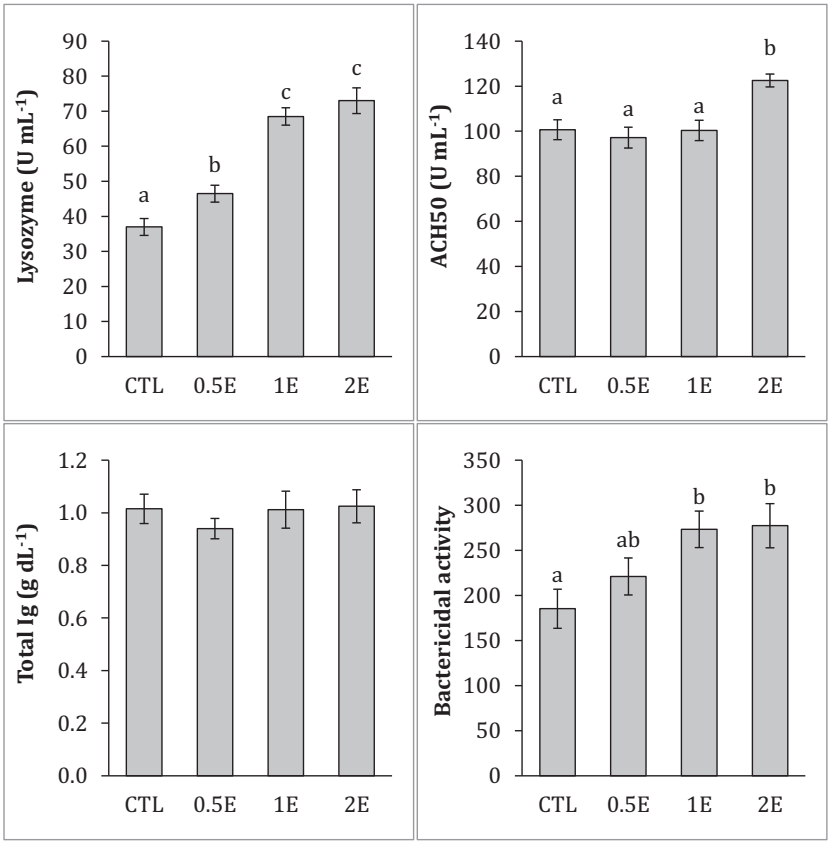

Fig. 3. Effects of dietary oak leaf extract on plasma lysozyme, ACH50, total Ig and bactericidal activity in common carp after 60 days. Different letters above the bars mean significant differences among the dietary treatments.

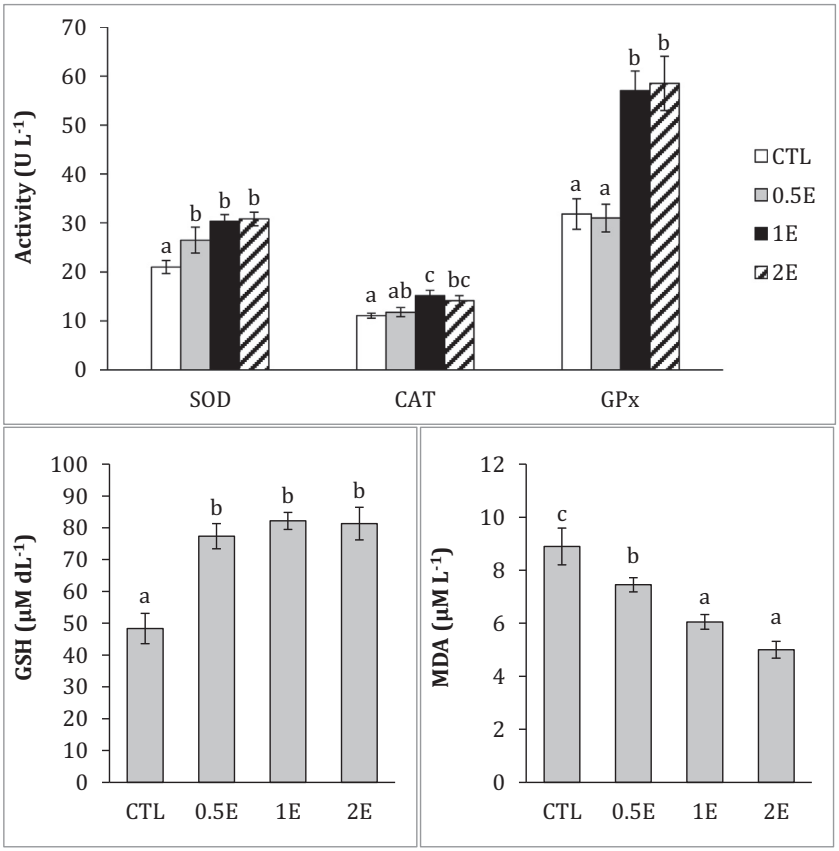

Fig. 4. Effects of dietary oak leaf extract supplementation on antioxidant enzymes, GSH and MDA levels in common carp. Different letters above the bars show significant differences among the treatments (*P*-value: SOD, .003; CAT, .015; GPx, < .001; GSH, < .001; MDA, < .001).

Table 3
Effects of dietary oak leaf extract and a 6-h crowding stress on plasma cortisol and glucose levels in common carp after 60 days.

Time	Treatments	Cortisol (ng mL^{-1})	Glucose (mg dL^{-1})
Before stress	CTL	43.2 ± 4.81a	46.3 ± 1.97
	0.5E	39.5 ± 3.44a	44.2 ± 2.30
	1E	41.7 ± 3.00a	38.7 ± 1.93
	2E	41.7 ± 3.16a	38.2 ± 1.95
After stress	CTL	116 ± 9.75d	118 ± 10.4
	0.5E	123 ± 9.46d	105 ± 11.9
	1E	110 ± 5.18d	91.8 ± 6.85
	2E	114 ± 5.44d	80.8 ± 7.17
Recovery	CTL	104 ± 6.14d	92.5 ± 3.28
	0.5E	71.2 ± 3.45c	89.5 ± 3.79
	1E	57.5 ± 5.00b	79.0 ± 5.23
	2E	56.8 ± 4.78b	76.7 ± 3.74
2-Way ANOVA			
Time		0.003	< 0.001 (Before stress[a]; After stress[c]; Recovery[b])
OE		< 0.001	< 0.001 (CTL[c]; 0.5E[bc]; 1E[ab]; 2E[a])
Time × OE		0.001	0.388

Different letters in a column mean significant differences among the dietary treatments.

Nelumbo nucifera and *Psidium guajava* leaf extracts improved growth rate of Mozambiqu tilapia (*Oreochromis mossambicus*) and grass carp (*Ctenopharyngodon idella*) by augmenting feed intake (Gobi et al., 2016; Zhu et al., 2019).

The *in vivo* results approved *in vitro* antioxidant activity of the oak leaf extract in the present study. Therefore, the leaf extract potentiates to both scavenging free radicals and stimulating antioxidant enzymes. Such elevations in SOD, CAT and GPx activities protect fish against oxidative conditions. The higher GSH levels in the fish fed oak leaf extract-supplemented diets might be attributed to radical scavenging effects of the extract and reducing the conversion of GSH to glutathione disulfide. Altogether, stimulation of the antioxidant enzymes and scavenging free radicals by dietary oak leaf extract suppressed oxidative stress in the fish, characterized by lower plasma MDA levels. There are no data about the use of oak leaf extract in fish diets and its effects on antioxidant system. However, other plant leaf extracts such as *Ocimum gratissimum*, *N. nucifera*, *P. guajava* and *Ginkgo biloba* significantly increased activity and/or gene expression of the antioxidant enzymes and suppressed oxidative stress in different fish species (Gobi et al., 2016; Abdel-Tawwab et al., 2018b; Tan et al., 2018; Adeshina et al., 2019; Zhu et al., 2019).

Lysozyme and complement proteins are important immune components that act non-selectively against bacteria and antigens. Thus, stimulating these immune components may increase fish resistance against bacterial diseases. The increase in plasma lysozyme activity in the fish treated with oak leaf extract might be due to increase in blood neutrophil count and/or lysozyme production (Hoseinifar et al., 2019). Whereas, increase in complement activity might be due to stimulation of the molecules production in the host liver (Ghelichpour et al., 2017). The present results are in line with the previous studies showing the different plant leaf extracts increased lysozyme and complement activities and resistance to bacterial diseases (Gobi et al., 2016; Abdel-Tawwab et al., 2018b; Adeshina et al., 2019; Zhu et al., 2019). Although the *in vitro* results showed that bactericidal activity of the oak leaf extract was about 3-fold lower than tetracycline, the *in vivo* results showed that the extract potentiated to increase the fish plasma bactericidal activity against the same bacterium. This might be due to direct effects of the extract bactericidal compound in the fish circulation and/or due to stimulating other anti-microbial mechanisms. The results

are similar to previous studies showing that dietary *Azadirachta indica* and *Myrtus communis* leaf extracts significantly increased plasma/mucus bactericidal effects against *Vibrio harveyi*, *Yersinia ruckeri* and *A. hydrophila* (Talpur and Ikhwanuddin, 2013; Taee et al., 2017).

It is very important to suppress stress in aquaculture practices because stress causes adverse effects on fish health. Cortisol elevation due to stress triggers hyperglycemia and weakens immune system; consequently, stress led to lower growth rate and disease susceptibility. It is important to suppress cortisol levels and shorten the duration of staying elevated of cortisol. The present results showed that the oak leaf extract failed to inhibit the stress-induced cortisol elevation, but mitigated that. The fish treated with the extract exhibited tendency to faster recovery from stress compared to the CTL fish and this may increase fish welfare during stress and recovery. There are no studies about the benefits of oak leaf extract on stress responses in fish. However, *Rheum officinale* extract significantly mitigated plasma cortisol and glucose levels due to crowding stress in common carp (Xie et al., 2008). Likewise, green tea extract significantly mitigated anesthesia-induced stress black rockfish (*Sebastes schlegeli*) (Hwang et al., 2013) and *Urtica dioica* extract significantly suppressed basal cortisol and glucose levels in Victoria Labeo (*Labeo victorianus*) (Ngugi et al., 2015).

In conclusion, the oak leaf extract has antioxidant and antibacterial properties and improves antioxidant and immune system and mitigate stress in common carp, when administered *via* dietary route. Administering this extract has no negative effects on the fish growth rate and levels of 10–20 g kg^{-1} diet are recommended for common carp dietary inclusion.

Author statement

The study was designed by all authors. The data were collected by S.M. Hoseini and S.H. Hoseinifar. H. Van Doan analyzed the data and B.A. Paray drafted the manuscript. Revisions were done by the full contribution of all authors.

Declaration of competing interest

The authors declare there is no conflict of interest about this article.

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