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ASSESSING THE IMPACT OF PURSLANE (*PORTULACA OLERACEA* L.) ON GROWTH PERFORMANCE, ANTI-OXIDATIVE, AND IMMUNE ACTIVITIES IN GRASS CARP (*CTENOPHARYNGODON IDELLA*)

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Abstract

In this study, the basal diet was supplemented with ethanolic extract of purslane (*Portulaca oleracea* L.) and the possible effects on growth performance, anti-oxidative, and immune activities of grass carp were evaluated. Fish with initial weight 1.23 ± 0.11 g were randomly divided into four groups (triplicates) and fed purslane extract at 0% (T0), 0.5% (T1), 1% (T2), and 1.5% (T3) for 56 days. At the end of the feeding trial, the results showed that growth parameters were enhanced in T1 groups compared to the control group (P<0.05). Lipase activity in T1 and T2 groups increased, whereas no significant changes were noticed in cases of amylase and protease activities (P>0.05). Catalase and superoxide dismutase activities were enhanced in all groups fed the supplemented diets in comparison with the control group (P<0.05). However, no significant alteration was noticed in the case of glutathione peroxidase activity following the administration of purslane extract (P>0.05). A significant increase in total immunoglobulin level was noted in the T1 group, but lysozyme activity was higher in T1 and T2 groups compared to the control group (P<0.05). In conclusion, supplementation of grass carp diet with the purslane ethanolic extract, especially at 0.5%, can improve growth performance, lipase activity, the antioxidant enzyme activities as well as the immune response of grass carp fingerlings.

Key words: purslane extract, grass carp, growth, immunity, anti-oxidative status

Aquaculture is one of the fastest-growing sectors in the world to produce the animal protein for people consumption (Ahmadifar et al., 2019 a, b, c; Shekarabi et

al., 2019). Thus, this industry represents an essential source of food, healthy protein, and income for millions of people worldwide (Gjedrem et al., 2012). During the past years, there was an increasing interest toward the administration of medicinal plants for treatment and prevention of infectious diseases in aquatic animals (Adel et al., 2017; Ahmadifar et al., 2019 a, b, c; Amin et al., 2019; Cabello, 2006; Van Doan et al., 2019 a, b). It has been demonstrated that the medicinal herbs can be used as an alternative method to antibiotics and chemotherapies (Hoseinifar et al., 2017 a, b; Moustafa et al., 2019; Van Doan et al., 2019 a, b). The natural compounds of herbal medicine can increase the resistance against infectious diseases by improving their immune system. Herbal medicine also plays active roles in nutrient utilization and growth performance (Bjørklund et al., 2018; Hoseinifar et al., 2017 a, b; Van Doan et al., 2019). Using plant-enriched diets is a reasonable practice that can be adopted by both small and large-scale fish farmers and which can offer several benefits from increasing fish growth to increasing immune activity and disease resistance (Shekarabi et al., 2019).

Purslane (*Portulaca oleracea* L.) belongs to the Portulacaceae family, which is found in different areas of the Mediterranean, Asia, and Europe regions (Uddin et al., 2014). This herb is traditionally used for the treatment of headaches, burns, and different diseases associated with the stomach, liver, intestine, cough, and arthritis (Abaza et al., 2010; Abdel-Razek et al., 2019; Naeem and Khan, 2013). It is also useful as an antiseptic and anti-inflammatory agent with positive effects on the nervous system as well as improving cholesterol metabolism (Besong et al., 2011; Movahedian et al., 2007; Naeem and Khan, 2013; Sultana and Rahman, 2013). Previous studies showed that various parts of purslane from the roots to the stem could be applied as herbal medicine (Besong et al., 2011; Movahedian et al., 2007; Naeem and Khan, 2013; Ruiz, 2017; Uddin et al., 2014). In aquaculture, the effects of purslane as a medicinal herb on the growth, immune response, and antioxidant activity of gilthead seabream (*Sparus aurata* L.) and Nile tilapia (*Oreochromis niloticus*) were investigated (Abdel-Razek et al., 2019; Ruiz, 2017).

Therefore, considering the protective effects of purslane reported in the studies as mentioned earlier, this study was designed to evaluate the impact of different doses of the purslane extract on the growth performance, antioxidant responses as well as immune activity in grass carp fingerlings.

Material and methods

Experimental fish and diet

Grass carp with an initial body weight of 1.23 ± 0.11 g were obtained from a fish farm (Rasht, Iran). Fish health status was verified by normal coloration, the absence of cysts, spots or patches over the body and gills, and normal behavioral signs (swimming and feeding reflexes). A total number of 180 fish were randomly distributed into 12 tanks (80 L) and assigned to four treatments in triplicates with 15 fish in each tank. During the 10-day adaptation period, all the fish were adminis-

tered with the basal diet up to apparent satiation. All tanks were supplied with river water under constant aeration using air stone and 50% daily water exchange. The water physicochemical parameters (temperature, dissolved oxygen, and pH) during the feeding trial were as follows: 23.7 ± 2.1°C temperature; 7.6 ± 0.4 mg/L dissolved oxygen; pH 7.8 ± 0.4. Feed ingredients and proximate composition of the basal diet are presented in Table 1.

Table 1. Formulation and proximate composition of the basal diet

Ingredients (g/kg)	
Kilka fish meal[a]	140
Soybean meal[b]	350
Wheat flour	265
Cottonseed meal	150
Zeolite	10
Monocalcium phosphate	20
Antifungal[c]	5
Vitamin mixture[d]	30
Mineral mixture[d]	30
Proximate composition (%)	
Dry matter	86.47
Crude protein	30.77
Crude lipid	5.26
Ash	6.81
Gross energy (kcal/kg)	3912.90

[a]Crude protein: 60.6%.
[b]Crude protein: 44.2.
[c]ToxiBan antifungal (Vet-A-Mix, Shenan-doah, IA).
[d]Mixture detailed by Mousavi et al. (2019).

Purslane leaves were collected from Gorgan city, Iran, and were verified at Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. The collected leaves were sun-dried and milled into a fine powder. The extraction was performed using ethanol 80% and then filtration using a filter paper as described by Mousavi et al. (2019). The ethanol solvent was evaporated at 40°C and reduced pressure. Four experimental diets were formulated with different levels of purslane extract at 0, 0.5, 1, and 1.5%. The levels of purslane were selected based on the recommendations of Abdel-Razek et al. (2019). All the ingredients were well-mixed with purslane extracts and pelleted using an extruder machine, followed by air drying at room temperature (Ahmadifar et al., 2019 a, b, c). The prepared diets were separately kept in sealed plastic bags at 4°C until use.

Growth performance

Initial and final body weight and length of all fish from each tank were determined at the beginning and the end of the 56-day feeding trial. The weight gain (WG), specific growth rate (SGR), and feed conversion rate (FCR) were calculated at the end of the experiment using the following formulae:

WG (g) = final weight (g) – initial weight (g)
SGR = (Ln final weight – Ln initial weight) / days (56) × 100
FCR = feed given (g) / WG (g)

Sampling procedure

At the end of the feeding trial, ten grass carp were euthanized with clove oil (50 µl/L), and the heads and fins were removed. Then, each fish sample was homogenized with a hand homogenizer and suspended in 25 mM Tris-HCl buffer for evaluating the immunological and antioxidant enzymes as well as the digestive enzymes (Ahmadifar et al., 2019). Before the assays, the protein concentration in fish homogenates was determined according to the standard protocol suggested by Bradford (1976) for calculating the enzyme activity.

Digestive enzyme activity

The lipase activity in fish homogenates was determined according to Ahmadifar et al. (2019). Briefly, fish homogenates (20 µl) were added to 1 ml of Tris-HCl buffer (50 mM) and 60 µl of p-nitrophenyl butyrate (p-NPB) (50 mM) in ethanol. After that, absorbance was read at 540 nm for 5 min with 30 s interval.

The protease activity in fish homogenates was determined as described by Erlanger et al. (1961). Briefly, fish homogenates (100 µl) were added to 1.0 ml of phosphate buffer (0.1 M, pH 8). Then, 20 µl of BApNA (0.01 M) was added to start the reaction. Then absorbance was recorded at 410 nm for 4 min with 30 s interval.

The amylase activity in fish homogenates was determined as mentioned by Ahmadifar et al. (2019). Briefly, fish homogenates (50 µl) were added to 250 µl of soluble starch (1%) and incubated at 37°C for 5 min. Then, 0.5 ml of 1% dinitrosalicylic acid was added to this mixture to terminate the reaction. Final absorbance was recorded at 540 nm using a spectrophotometer.

Antioxidant enzyme activities

The glutathione peroxidase (GPX) activity in fish homogenates was determined after Bell et al. (1985). The rate of NADPH oxidation at 340 nm was recorded using the extinction coefficient of 6.22 mM/cm. The protocol suggested by Aebi (1974) was followed to determine the catalase (CAT) activity in fish homogenates. The reduction of hydrogen peroxide (H_2O_2) at 240 nm (ε = 40 M/cm) was recorded using 50 mM H_2O_2 as a substrate. Also, the superoxide dismutase (SOD) activity in fish homogenates was determined according to Panchenko et al. (1975). The inhibition of the epinephrine oxidation to adenochrome was recorded at 480 nm using xanthine oxidase plus xanthine.

Immune parameters

Fish homogenates (20 µl) were added to 1 ml of alkaline buffer solution and then incubated at 37°C for 5 min. NaOH solution (0.05 N) was added to this mixture to finish the reaction. Final absorbance was recorded at 410 nm using a spectrophotometer (Ahmadifar et al., 2019).

The total immunoglobulin (Ig) level in fish homogenates was determined, according to Siwicki and Anderson (1993). Before and after the addition of 12% polyethylene glycol (PEG; Sigma), the total protein content in fish homogenates was determined by following Bradford (1976).

To determine the lysozyme activity, fish homogenates (25 µl) were added to 175 µl of *Micrococcus lysodeikticus* suspension (75 µg/ml) prepared in phosphate citrate buffer (0.1 M, pH 5.8); after that absorbance was read at 410 nm for 5 min with 30 s interval (Sheikhzadeh et al., 2012).

Statistical analysis

Data were shown as mean ± standard deviation (SD). After assessing the data normality with the Kolmogorov–Smirnov test, one-way analysis of variance (ANOVA) was performed to compare the means of different treatment groups followed by the Tukey HSD test. SPSS software version 23.0 (IBM Corp., USA) was used for the analysis.

Results

Growth performance

No significant differences were noticed when the initial weight or length of treated groups was compared with those of the control group. However, at the end of the feeding trial, final weight, WG, SGR, and FCR of fish in the T1 group were significantly higher than the control group ($P<0.05$). No significant differences in survival rate were also observed between the three treatment groups and the control group ($P>0.05$) (Table 2).

Table 2. Effects of purslane extract on growth performance and survival rate in grass carp

Parameters	T0	T1	T2	T3
Initial weight (g)	1.22±0.12	1.21±0.12	1.25±0.08	1.20±0.14
Initial length (cm)	4.66±0.11	4.59±0.10	4.54±0.16	4.61±0.19
Final weight (g)	2.37±0.09 a	2.68±0.16 b	2.48±0.15 ab	2.50±0.16 ab
Final length (cm)	5.66±0.14	5.96±0.23	5.75±0.14	5.87± 0.20
WG (g)	1.14±0.05 a	1.47±0.10 b	1.23±0.12 a	1.30±0.07 ab
SGR	1.18±0.05 a	1.43±0.08 b	1.23±0.06 a	1.31±0.10 ab
FCR	2.18±0.09 b	1.70±0.12 a	2.04±0.19 b	1.92±0.10 ab
SR (%)	100	100	100	100

Different letters indicate differences between groups ($P<0.05$).
WG – weight gain; SGR – specific growth rate; FCR – feed conversion ratio; SR – survival rate.

Digestive enzyme activity

Lipase activity in T1 and T2 treatment groups was higher than that of the control group and the highest level was observed in the T1 group ($P<0.05$). However, feeding on purslane supplemented diets caused no significant changes in amylase and protease activities ($P>0.05$) (Figure 1).

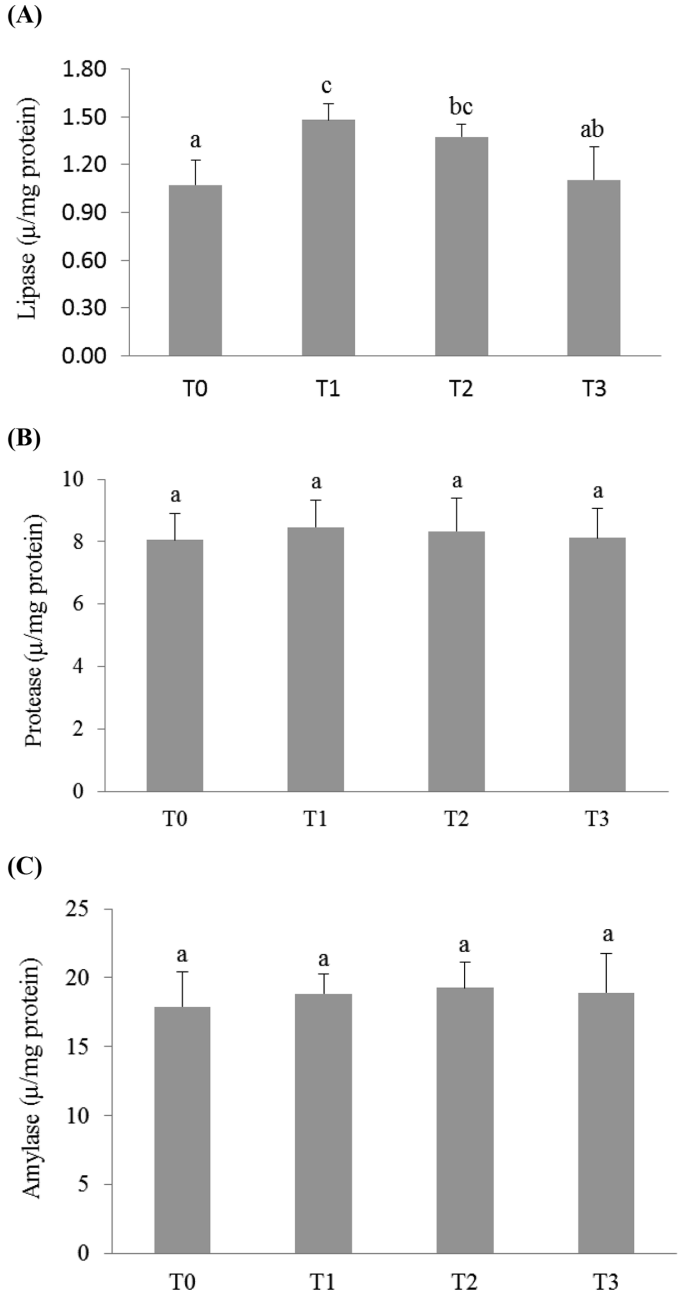

Figure 1. Effects of purslane extract on digestive enzyme activity, including lipase (A), protease (B), and amylase (C) in grass carp homogenates. Data are expressed as means ± SD (n=30). Different letters indicate differences between groups ($P<0.05$)

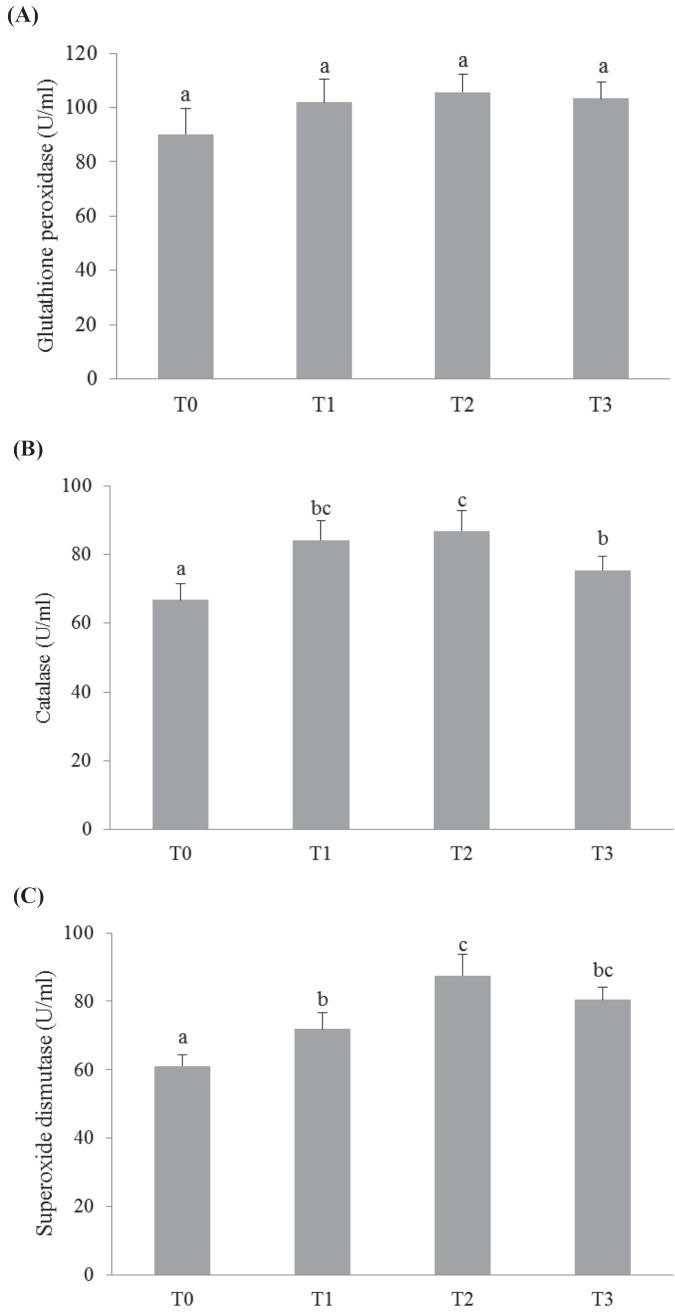

Figure 2. Effects of purslane extract on antioxidant enzyme activities, including glutathione peroxidase (A), catalase (B), and superoxide dismutase (C) in grass carp homogenates. Data are expressed as means ± SD (n=30). Different letters indicate differences between groups ($P<0.05$)

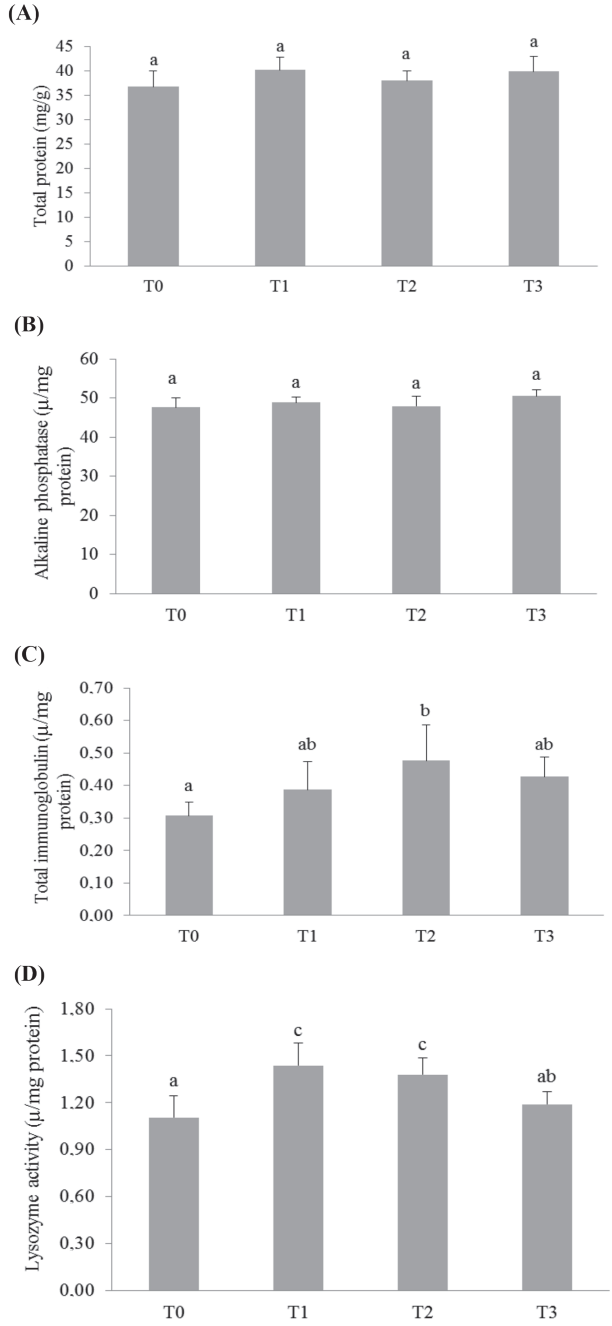

Figure 3. Effects of purslane extract on immunological parameters including total protein (A), alkaline phosphatase (B), total immunoglobulin (C), and lysozyme activity (D) in grass carp homogenates. Data are expressed as means ± SD (n=30). Different letters indicate differences between groups ($P<0.05$)

Antioxidant enzyme activities

Among antioxidant enzymes, GPX enzyme was not altered with dietary purslane extract. In contrast, CAT and SOD activities in three treatment groups were higher than those of the control group ($P<0.05$). The highest CAT and SOD activities were also shown in the T2 group compared to other groups (Figure 2).

Immunological parameters

There were no significant differences in the total protein and alkaline phosphatase activity among the treated groups and the control group. Total Ig level in the T1 group was higher than that of the control group ($P<0.05$). Lysozyme also showed higher activity in T1 and T2 groups compared to the control group with the highest activity noted in the T1 group ($P<0.05$) (Figure 3).

Discussion

In the intensive aquaculture system, feeding strategies are known as the most important factors that can affect production (Dawood et al., 2020 a, b, c; Zaki et al., 2020). Therefore, using functional feed additives is a practical regime which can improve the growth rate and welfare of farmed fish (Hoseinifar et al., 2017 a, b). The present results reported that dietary supplementations of purslane at 0.5% (T1) improved the growth performance of grass carp fingerlings, which can be attributed to increased palatability and feed efficiency (Güllü et al., 2016; Sarhadi et al., 2020). Conversely, purslane at levels higher than 1% had adverse effects on the growth parameters of Nile tilapia and gilthead seabream (Abdel-Razek et al., 2019; Ruiz, 2017). It seems that anti-nutritional factors, including phytate and oxalate in this herb, can negatively affect the feed utilization and growth performance when administered at high levels. Besides, the different fish species, feeding duration, and doses of feed additives can result in the discrepancy between the results of growth performance among fish species (Zemheri-Navruz et al., 2019). Different factors, including the intestinal flora and structure, as well as the digestive enzyme activity, can affect the fish growth performance of fish (Dawood et al., 2020 a, b, c; Dawood et al., 2019 a, b). Many plants can act as animal growth promoters and can increase feeding efficiency (Dawood et al., 2018; Zemheri-Navruz et al., 2019). However, some studies have shown that some plants can also contain anti-nutritional molecules that can result in decreased growth and lower feed digestibility (Awad and Awaad, 2017). In this study, we observed a significant difference in growth between control and treated fish (T1), which suggests that purslane did not display any adverse effect on the fish appetite and feed utilization in the gastrointestinal tract (GIT).

The digestive enzymes induce a vital role in the digestion process with the final impact on fish wellbeing (Ahmadifar et al., 2019 a, b, c; Dawood et al., 2019 a, b). Our findings revealed that dietary inclusion of purslane improved the lipase activity of grass carp fingerlings, although no remarkable differences were noted for amylase and protease activities. In parallel, hypolipidaemic and hypocholesterolemic effects

of purslane were attributed to the functional compounds in purslane, including flavonoids, omega-3 fatty acids, and polysaccharides (El-Sayed, 2011; Hussein, 2010). It has also been suggested that the bio-active compounds in herbs can act as a tasty appetizer for aquaculture organisms. Indeed, they can improve the feed utilization through stimulation of digestive enzymes secretions in the fish GIT (Citarasu, 2010).

The use of medicinal plants as feed supplements has shown to have many benefits for aquatic animals such as improved growth rate and feed efficiency, immune response, and enhanced antioxidative activity (Adel et al., 2019; Güllü et al., 2016; Srichaiyo et al., 2020). Our study has shown the potential of purslane in enhancing some serum immune parameters (lysozyme activity and total immunoglobulin) and increasing the activity of the antioxidant enzymes (SOD and CAT) in grass carp.

The antioxidant enzymes such as CAT, GPX, and glutathione-S-transferase are the main enzymes that act against free oxygen radicals production in cells (Dawood et al., 2019 a, b; Mugesh and Singh, 2000; Sharma et al., 2007). Therefore, higher activity of the antioxidant enzymes could maintain an efficient pro-oxidant and antioxidant balance, which results in the better health status of animals (Abdel-Daim et al., 2020). In this study, purslane could increase the CAT and SOD activities in the treated groups. The antioxidant activity of purslane has been previously studied in humans and different animals (El-Sayed, 2011; Ghorbani et al., 2013; Sadeghi et al., 2016; Yue et al., 2015). However, limited information is available about the antioxidant effects of purslane in fish species. Previously, the administration of purslane dried powder resulted in higher SOD, CAT, and GPX activities accompanied by lower malondialdehyde levels in Nile tilapia (Abdel-Razek et al., 2019). This herb has phenolic compounds, including flavonoids, phenolic acids, and alkaloids, with potent antioxidant capacity. Other biological components such as saponins, ascorbic acid, vitamin E, carotene, and glutathione may also contribute to the antioxidant capacity of this plant (Erkan, 2012; Iranshahy et al., 2017; Lim and Quah, 2007). In this study, we have observed that purslane extract enriched diets significantly boosted the activities of CAT and SOD, confirming that the known antioxidant properties of this plant are not lost when administered orally in grass carp.

The innate immune system of teleost fish is composed of both cellular and humoral components (Dawood et al., 2020 a, b, c). Antibodies are the main component of the humoral immune system that plays a significant role in neutralizing and destroying the invading pathogens in fish (Magnadottir, 2010). In particular, IgM participates in the opsonization of pathogens by facilitating their phagocytosis. In this study, a higher Ig level was shown in fish that received purslane supplemented diet. Similarly, feeding purslane to gilthead seabream could increase the Ig level in the fish skin (Ruiz, 2017).

Lysozyme is also a significant defense molecule of the innate immunity that mediates protection against bacterial agents (Saurabh and Sahoo, 2008). Lysozyme also acts as an opsonin and therefore activates both phagocytes and the complement system. In this study, higher lysozyme activity was shown after the administration of purslane extract. In agreement with this result, the active role of dietary purslane leaves powder on lysozyme activity of Nile tilapia has been reported by Abdel-Razek et al. (2019). Activation of the immune system can result in higher

protection against the infection of pathogenic bacteria. Similarly, purslane supplementation could improve the survival rate after infection with *Aeromonas hydrophila* in Nile tilapia (Abdel-Razek et al., 2019). In parallel, the characteristics of biological activities of different medicinal plants have increased the global interest in their use as immunostimulants in aquaculture (Awad and Awaad, 2017; Baba et al., 2016; Zemheri-Navruz et al., 2019).

Conclusion

In conclusion, the inclusion of purslane ethanolic extract as a feed supplement for grass carp, especially at 0.5%, improved some immune parameters of fish as well as antioxidant enzyme activity, which indicates that it could be a potential useful feed supplement to improve the grass carp immune status. Furthermore, the growth rates were enhanced, meaning that including purslane in fish diets does not negatively affect fish growth and feed utilization. However, further investigations are still demanded to define the optimum dose and duration of administering this herb in different fish species.

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