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Original Article

Effect of *Hyssopus officinalis* extract dietary supplementation on serological parameters, growth indices, and survival of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila*

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Abstract: The hyssop (*Hyssopus officinalis*) is known as a medicinal plant with antimicrobial and antioxidant properties. Despite a long history of medicinal use of *H. officinalis*, very few studies have been reported on the effect of *H. officinalis* on fish. Thus, the present study tried to investigate the effects of *H. officinalis* extract as a food supplement on serological parameters, growth indices, and survival of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila.* For this purpose, the fish were divided into four treatments and fed with concentrations of 0, 250, 500, and 750 mg/kg H. officinalis extract in the diet for 60 days. The results revealed that the growth indices, including weight gain, length gain, specific growth rate (SGR), and food conversion ratio (FCR) in the treatments supplemented with *H. officinalis* extract, have no significant difference compared to the control treatment. Serological analysis showed that total protein increased in the treatments supplemented with *H. officinalis* extract, which was significantly different in 500 and 750 mg/kg *H. officinalis* extract compared to the other treatments. Immunoglobulin significantly increased in 750 mg/kg *H. officinalis* extract compared to the other treatments. There was no significant difference in the amounts of albumin, glucose, cortisol, cholesterol, triglyceride, creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lipase, and amylase of all treatments (P>0.05). Total antioxidant capacity (TAC) significantly increased in the treatment containing *H. officinalis* extract. Serum malondialdehyde (MDA) decreased in the treatments supplemented with *H. officinalis* extract. The lowest MDA was observed in 750 mg/kg *H. officinalis* extract, which was significantly different from other treatments. Relative survival percentage (RSP) against *A. hydrophila* increased in the treatments fed with *H. officinalis* extract. The highest RSP was observed in 750 mg/kg *H. officinalis* extract treatment. It can be concluded that dietary *H. officinalis* extract had a proper effect on immune system stimulation, antioxidant capacity, and resistance against *A. hydrophila* in the studied fish.

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Introduction

the aquaculture industry, fish health management is essential (Oliva-Teles, 2012). In fish farms, inadequate food rations cannot provide the proper growth conditions for fish, causing problems such as debility of the immune system and decreased resistance to various infections. Thus, suitable nutritional supplements in the diet can enhance the growth performance of the fish and increase their immune responses (Trichet, 2010; Omidzahir and Fallah, 2023). Studies have shown that medicinal plants with bioactive compounds as food supplements in the fish diet can strengthen the immune system against stress factors (Amar et al., 2004). Medicinal plant extracts have antifungal, antiparasitic, antibacterial, and antiviral properties, which have been investigated in many studies (Kordali et al., 2005). Using natural compounds such as plant extracts has been effective as an approach to increasing growth performance, immune system stimulation, and resistance to diseases in aquatic animals. For example, Shalaby et al. (2006) showed that adding garlic (*Allium sativum*) to the Nile tilapia (*Oreochromis niloticus*) diet increased food intake, specific growth

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rate, and final weight. Bello et al. (2012) reported the favorable effect of walnut (*Tetracarpidium conophorum*) leaves on growth performance and food digestion in *Clarias gariepinus* Juveniles. The impact of basil (*Ocimum basilicum*) extract as a food supplement in common carp (*Cyprinus carpio*) increased white blood cells and red blood cells, hematocrit, hemoglobin, total protein, globulin, and albumin (Amirkhani et al., 2013). Taleghani et al. (2019) reported the proper effect of damask rose (*Rosa damascene*) extract to decrease the toxicity of Zn on the liver of common carp.

The hyssop (*Hyssopus officinalis*), a member of the Lamiaceae family, is a medicinal plant (Fathiazad and Hamedeyazdan, 2011; Alinezhad et al., 2013). It has antibacterial and antifungal effects and is used in the treatment of viral infections (Kazazi et al., 2007). It contains anti-inflammatory, antispasmodic, and antioxidant properties (Fathiazad and Hamedeyazdan, 2011). *Hyssopus officinalis* consists of several polyphenol compounds, mainly flavonoids such as apigenin, quercetin, diosmin, luteolin, tannin, thymol, gammaterpinene, parasmin, carvacrol, and myrtonic acid (Hatipoğlu et al., 2013). Each of these compounds is the source of specific therapeutic effects such as antibacterial, antifungal, and anti-inflammatory effects (Miyazaki et al., 2003). Flavonoids can inhibit enzymes involved in producing free oxygen radicals, such as cyclooxygenase, lipoxygenase, microsomal monooxygenase, and glutathione S-transferase (Fathiazad et al., 2011; Vlase et al., 2014). Studies have shown that these compounds are free radical scavengers and have antioxidant properties (Alinezhad et al., 2013).

Despite a long history of medicinal use of *H. officinalis*, very limited research has been reported on the effect of *H. officinalis* in fish. Thus, the present study tried to investigate the effects of *H. officinalis* extract as a food supplement on serological parameters, growth performance, and survival of common carp against *Aeromonas hydrophila.*

Materials and Methods

Fish preparation: In this study, juvenile common carps with an average weight of 24.16±1.03 g and length of 12.49±1.25 cm were obtained from a local fish farm. In order to adapt to the new environmental conditions, the fish were kept in the laboratory aquariums for two weeks and fed daily 5% of their body weight during the adaptation period.

***Hyssopus officinalis* extract preparation:** To prepare *H. officinalis* extract, the leaves of *H. officinalis* were separated and made into powder using a grinder. Then, *H. officinalis* powder was added to 70% ethanol at a temperature of 40°C. After that, the content was filtered using Whatman paper. Then, the dry extract was obtained by rotary evaporator at 40°C. The dry extract was kept in the refrigerator until use (Tahir et al., 2022).

Experimental setup: After a 2-week acclimatization period, 120 fish were divided into 4 treatments with three replications. Treatment 1 was the control, and the fish were fed a basal diet without the extract. Treatments 2, 3, and 4 received *H. officinalis* extract at concentrations of 250, 500, and 750 mg/kg, respectively. Different concentrations of the extract were sprayed onto the basal diet separately. The diet was mixed with *H. officinalis* extract, dried at room temperature, and then pelletized (Li et al., 2019). Ingredients and composition of basal diets are shown in Table 1. The fish were fed 5% of their body weight twice daily for 60 days, and the amount of food consumed during the experiment was recorded. Biometry of fish was performed at the beginning of the experiment, after 30 days, and at the end of the experiment, after 60 days.

Growth indices: Growth indices, including weight gain, length gain, specific growth rate (SGR), and food conversion ratio (FCR), were calculated using the following formulas (Jagruthi et al., 2014):

WG (g) = final weight (W2, g) - initial weight (W1, g)

LG (cm) = final length (L2, cm) - initial length (L1, cm)

SGR (%) =100 (ln final weight– ln initial weight) / number of days

FCR = feed intake (g)/weight gain (g)

Serological parameters: To analyze serological parameters, six fish were randomly selected from each

Table 1. Ingredients and composition of basal diets (%) used in this study.

Ingredients	(%)
Wheat meal	38.2
Fish meal	16
Corn meal	15.5
Soybean meal	15
Wheat gluten	9.3
Fish oil	5
Vitamin mix	0.5
Mineral mix	0.5
Composition	**(%)**
Dry matter	90
Crude protein	35.7
Crude ash	10.5
Moisture	10
Crude lipid	6.4
Crude fiber	5.5

treatment, and blood was collected through the caudal vein. Blood samples were poured into tubes without anticoagulant, and transported to the laboratory on ice. Serological parameters, including total protein, albumin, immunoglobulin, glucose, urea, creatinine, cholesterol, triglyceride, lipase, amylase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) enzymes, were measured by an autoanalyzer using a biochemical kit (Pars azmoon, Iran). Cortisol was measured by a biochemical kit (Monobind, USA) using an ELISA reader. Total antioxidant capacity (TAC) was measured using a commercial kit (Navand Salamat, Iran) according to the manufacturer's protocol. The measurement of serum malondialdehyde (MDA) level was evaluated based on the thiobarbituric acid reaction by a commercial kit (Navand Salamat, Iran).

Challenge test with *Aeromonas hydrophila*: After 60 days, the challenge test was performed with *Aeromonas hydrophila* for two weeks. For this purpose, the active culture medium of *A. hydrophila* (IBRC-M number 10814) was prepared from the Microorganisms Bank (Tehran, Iran). To challenge the fish with *A. hydrophila*, fish from each experimental treatment were injected intraperitoneally with 0.1 ml of live *A. hydrophila* (1×10^8 CFU/ml). The number of fish mortality and the survival rate of each experimental treatment were recorded for two weeks. Relative Percent Survival (RPS) was calculated based on the following formula (Sharma et al., 2010):

RPS% = (Number of surviving fish after challenge / Number of fish injected with bacteria) × 100

Statistical analysis: To analysis the serological parameters, growth performance, and survival of fish under different experimental treatments, the normality of each dataset was evaluated separately using the Kolmogorov-Smirnov test. Then, one-way analysis of variance and Duncan's post hoc test were used at the significance level of 0.05 by SPSS software version 22.0.

Results

Growth indices analysis: The growth indices showed that weight gain, length gain, and specific growth rate (SGR) increased in the treatments containing *H. officinalis* extract as a food supplement compared to the control treatment. The highest value was observed in 750 mg/kg *H. officinalis* extract. However, there are no significant differences between the treatments ($P>0.05$; Table 2). The food conversion ratio (FCR) decreased in the treatments receiving *H. officinalis* extract in the diet. The lowest rate of FCR was observed in 750 mg/kg *H. officinalis* extract, although there was no significant difference in FCR of all treatments ($P>0.05$; Table 2).

Serological analysis: The results showed that the amount of total protein increased in the treatments supplemented with *H. officinalis* extract. There was a significant difference between 500 and 750 mg/kg *H. officinalis* extract and other treatments ($P<0.05$; Fig. 1a). Immunoglobulin also showed an increasing trend with increasing concentrations of *H. officinalis* extract. The highest amount of Immunoglobulin was observed in 750 mg/kg *H. officinalis* extract, which was a significant difference compared to other treatments ($P<0.05$; Fig. 1A). There was no significant difference in the amount of albumin in all treatments ($P>0.05$; Fig. 1A). The amount of glucose and cortisol in the treatments fed with *H. officinalis* extract showed a decreasing trend. However, there was no significant difference among the treatments ($P>0.05$; Fig. 1B). Cholesterol and triglyceride decreased in the

Table 2. Growth indices in the treatments supplemented with different concentrations of *Hyssopus officinalis* extract in the *Cyprinus carpio* diet.

Growth indices	*Hyssopus officinalis* extract (mg/kg)			
	0	250	500	750
W1 (g)	24.26±1.60	23.96±1.73	24.20±1.85	24.23±2.05
W2 (g)	54.33±2.60	56.16±3.40	55.96±4.39	57.46±2.28
L1 (cm)	12.49±0.15	12.67±0.31	12.48±0.65	12.32±0.54
L2 (cm)	14.85±0.31	15.22±0.34	15.15±0.35	15.38±0.46
WG (g)	30.07±1.00	32.20±1.67	31.76±2.54	33.23±0.23
LG (cm)	2.36± 0.16	2.55±0.03	2.67±0.30	3.06±0.08
SGR (%)	1.34±0.78	1.39±0.05	1.38±0.13	1.45±0.06
FCR	2.94±0.40	2.77±0.23	2.81±0.35	2.64±0.17

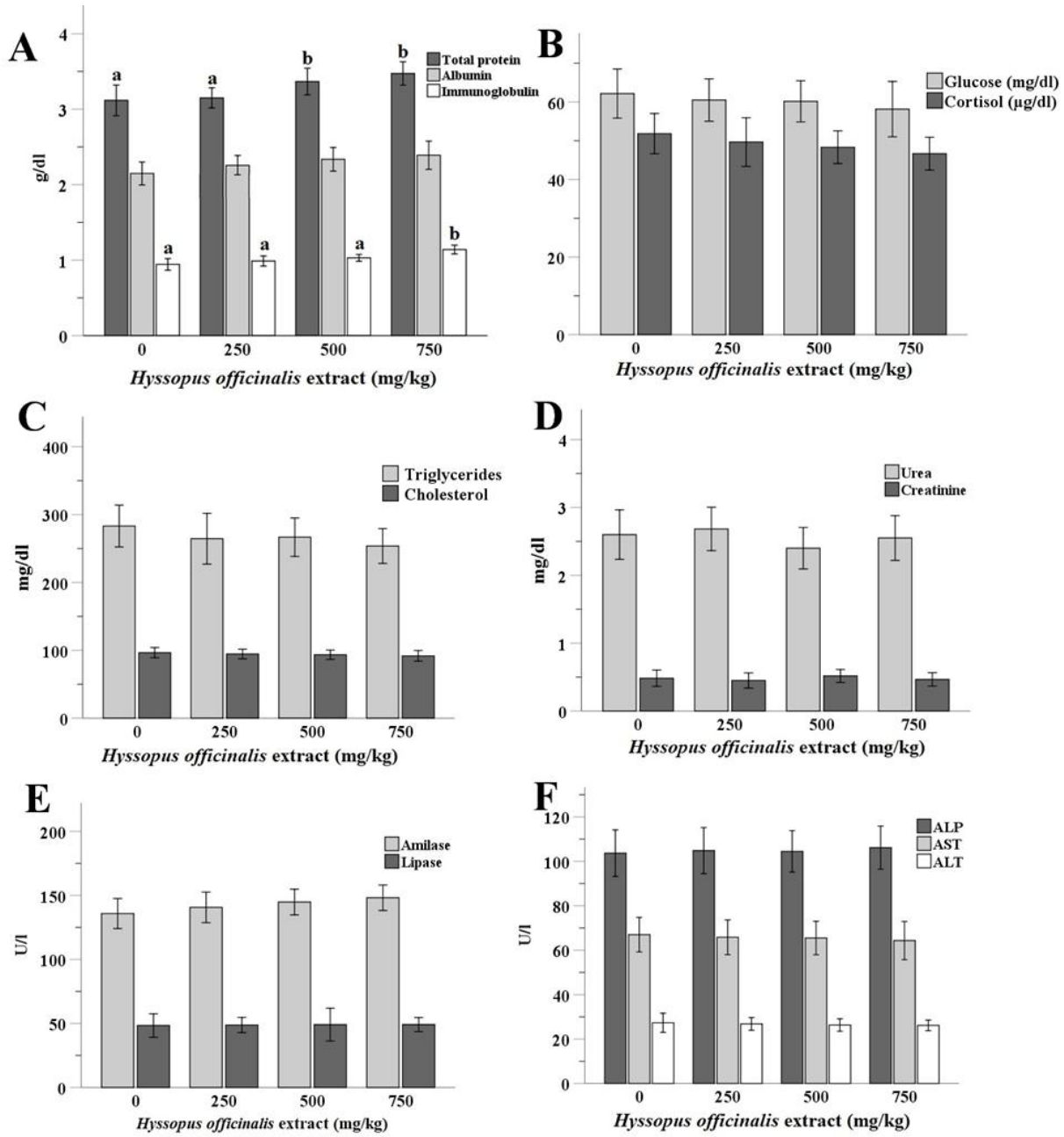

Figure 1. Serological parameters including A: total protein, immunoglobulin, albumin; B: glucose, cortisol, C: cholesterol, triglyceride; D: creatinine, urea; E: lipase, amylase; F: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) in the treatments supplemented with different concentrations of *Hyssopus officinalis* extract in the *Cyprinus carpio* diet. Different letters above the bars indicate significant differences among treatments (P<0.05). Data are presented as mean±SE.

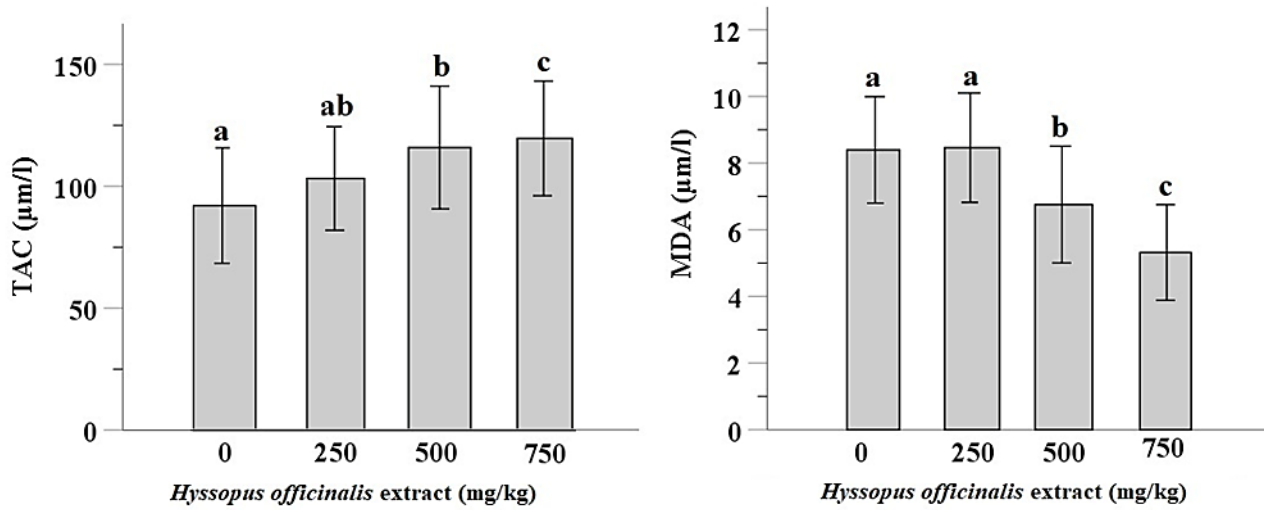

Figure 4. Total antioxidant capacity (TAC) and malondialdehyde (MDA) in the treatments supplemented with different concentrations of *Hyssopus officinalis* extract in the *Cyprinus carpio* diet. Different letters above the bars indicate significant differences among treatments (P<0.05). Data are presented as mean±SE.

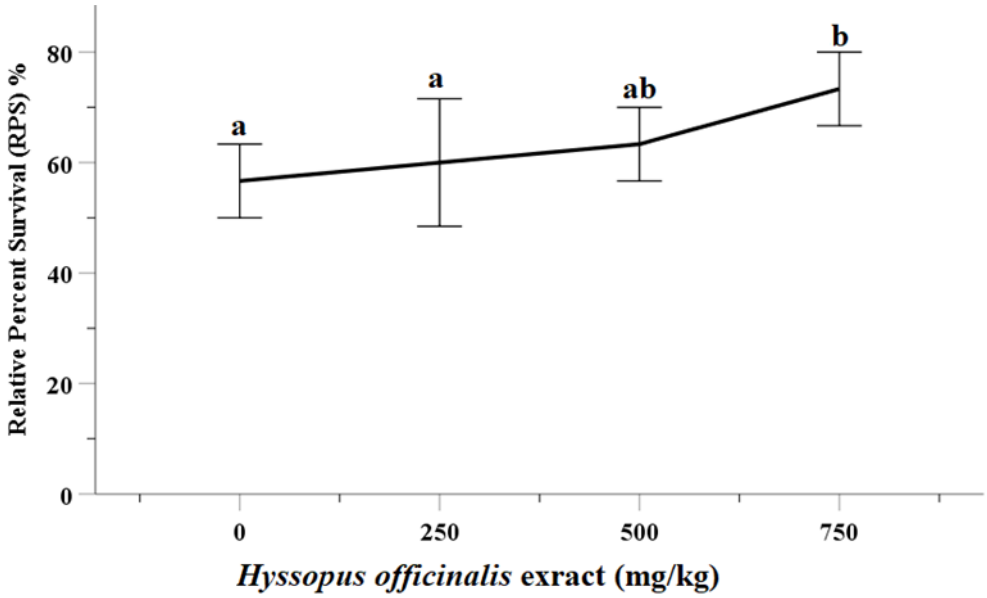

Figure 4. Relative survival percentage (RSP) in the treatments supplemented with different concentrations of *Hyssopus officinalis* extract in the *Cyprinus carpio* diet. Different letters above the bars indicate significant differences among treatments (P<0.05). Data are presented as mean±SE.

treatments supplemented with *H. officinalis*, although, it was not significantly different from other treatments (P>0.05; Fig. 1C). There was no significant difference in creatinine and urea levels in the treatments receiving *H. officinalis* extract in diet compared to the control treatment (P>0.05; Fig. 1D). Lipase and amylase digestive enzymes increased following increasing the concentration of *H. officinalis* extract in the diet, but no significant difference was observed among treatments (P>0.05; Fig 1E). There was no significant difference in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels in all treatments (P>0.05; Fig. 1F).

Total antioxidant capacity and malondialdehyde analysis: Serum total antioxidant capacity (TAC) increased in the treatments that received *H. officinalis* extract as a food supplement. The highest amount of TAC was observed in 750 mg/kg *H. officinalis* extract, which was significantly different compared to other treatments (P<0.05; Fig. 2). The amount of serum malondialdehyde (MDA) decreased in *H. officinalis*

extract-supplemented treatments. The lowest MDA was observed with the 750 mg/kg *H. officinalis* extract, which was significantly lower than that of other treatments ($P<0.05$; Fig. 2).

Challenge test with *Aeromonas hydrophila*: The results showed that the relative survival percentage (RSP) increased in the treatments fed *H. officinalis* extract. The highest RSP was observed with 750 mg/kg *H. officinalis*, which was significantly higher than the 250 mg/kg *H. officinalis* extract and control treatments ($P<0.05$; Fig. 3).

Discussions

The present study indicated that the growth indices, including weight gain, length gain, SGR, and FCR, in the treatments supplemented with *H. officinalis* extract have no significant difference compared to the control treatment. In other studies, plant supplements have been used in fish diets, with no significant effects on growth indices observed (Hernández et al., 2016; Baba et al., 2018). In the study by Yousefi et al. (2022), which investigated the effect of *H. officinalis* extract on the diet of rainbow trout (*Oncorhynchus mykiss*), no significant differences in growth indices were observed among treatments.

Evaluation of biochemical parameters in this study showed that the amount of total protein significantly increased in 500 and 750 mg/kg *H. officinalis* extract compared to the other treatments. Also, Immunoglobulin increased in the *H. officinalis* extract-supplemented treatments, with a significant difference at 750 mg/kg *H. officinalis* extract compared to the others. Analysis of the total protein, albumin, and globulin is used as an index to assess the immune and nutritional status (Abdel-Tawwab et al., 2007). Studies have shown that increasing total protein, albumin, and globulin levels in fish blood serum strengthens the immune system. Globulins are the main components of serum proteins, including immunoglobulins, and one of their important roles is in the immune response (Yu et al., 2008; Yıldız et al., 2009). In the current study, increases in total protein and immunoglobulin levels indicate a favorable effect of *H. officinalis* extract on the immune system of the studied fish. In the study by Yousefi et al. (2022), *H. officinalis* extracts significantly increased total protein and albumin in rainbow trout. In some other studies, increased serum protein levels have been reported following use of plant supplements (Banaee et al., 2011; Liu et al., 2012). Supplementing rainbow trout diets with Silymarin (*Silybum marianum* extract) increased total protein and globulin levels (Banaee et al., 2011). Studies by Xie et al. (2008) and Liu et al. (2012) indicated that *Rheum officinale* as dietary supplementation increased serum total protein and albumin in *Megalobrama amblycephala* and *C. carpio*, respectively. Also, an increase in total serum protein and immunoglobulin has been reported following the use of *Urtica dioica* in the diet of rainbow trout (Adel et al., 2017).

In the present study, the serum total antioxidant capacity (TAC) significantly increased in the treatment containing *H. officinalis* extract. On the other hand, serum malondialdehyde (MDA) levels decreased in the treatments supplemented with *H. officinalis* extract. TAC shows the ability to resist oxidants, which protects biomolecules against oxidation. Antioxidants reduce oxidative stress by scavenging reactive oxygen species (Martínez-Álvarez et al., 2005: Lee et al., 2009). Free radicals produce secondary oxidized products by attacking and oxidizing different molecules. Lipids are the most important molecules that are targeted by reactive oxygen species. Oxidative degradation of lipids is called lipid peroxidation, which takes place on unsaturated fatty acids, and its final product is malondialdehyde (Oropesa et al., 2009). Therefore, MDA measurement can assess lipid peroxidation and serve as an indicator of oxidative stress in organisms. In the present study, an increase in TAC and a decrease in MDA in treatments receiving *H. officinalis* extract may be due to the presence of polyphenolic and phenolic compounds, which can inhibit free radicals and exhibit antioxidant properties. *Hyssopus officinalis* consists of polyphenolic compositions, mainly flavonoids such as apigenin, quercetin, diosmin, luteolin, and also phenolic compositions such as chlorogenic, protocatechuic, ferulic, syringic,

p-hydroxybenzoic, and caffeic acids (Fathiazad and Hamedeyazdan, 2011).

In the present study, the challenge test against *A. hydrophila* showed increased fish survival in *H. officinalis* extract-supplemented treatments. Antibacterial effects of *H. officinalis* against *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* have been reported (Etminan et al., 2021). The antibacterial effects of *H. officinalis* are due to the compounds such as tannin, thymol, and carvacrol (Hatipoğlu et al., 2013). Thus, from the results of the present study, it can be concluded that a dietary supplement containing *H. officinalis* extract stimulated the immune responses and increased the percentage of common carp fish survival against *A. hydrophila.*

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