

Dietary Effect of Borage (*Borago officinalis*) Powder on Growth Performance, Immune Response, and Blood Biochemical Parameters in Common Carp (*Cyprinus carpio*) Juvenile

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Abstract

In this study, the effect of borage (*Borago officinalis*) powder on juveniles of common carp (*Cyprinus carpio*) were investigated. The diets contained control (0), 0.5%, 1.5%, and 2.5% of borage powder. The fish with the initial average weight of 8.40 ± 1.37 were allotted to 12 circular tanks of 300 L capacity at a density of ten fish per each tank and fed to apparent satiety three times a day. The growth performance of the fish showed no significant difference compared to the control group ($P > 0.05$). By adding 1.5% borage powder to the control diet the mucosal immunity of fish in terms of lysozyme activity (14.26%), total protein (69.64%), immunoglobulin (30.78%), and humoral immunity in term of lysozyme activity (9.7%), complement components C3 (8.27%), C4 (21.64%), and total protein (9.33%) were increased significantly ($P < 0.05$). A total number of red blood cell (12.39%), mean corpuscular volume (MCV, 8.99%), mean corpuscular hemoglobin (MCH, 10.22%), and hemoglobin (2.69%) showed a significant increase in the fish fed supplemented diet with 1.5% borage powder compared to the control ($P < 0.05$). The results showed supplemented diet with 1.5% dietary borage powder induced high immunity and survival rate of juvenile common carp.

Introduction

In the aquaculture industry, improve fish growth and disease free fish considered as the important factors. However, extra use of several antibiotics to control diseases and increase fish growth in aquaculture induced drug resistant bacteria and damaging to the environment and human health by making toxic substances (Shakya, 2017). Many studies showed that addition herbs in fish diet has positive influence on growth and health of fish (Chakraborty *et al.*, 2014). The addition of herbs in fish diet is inexpensive and ecofriendly (Shakya, 2017).

Researchers stated that bioactive compounds of various herbal plants, such as flavonoids, carotenoids, saponins, steroids, phenolic compounds, sulfide, lectin, the polypeptides, poly a non-starch polysaccharide, and some oligosaccharides are able to stimulate growth, reduce stress, motivate appetite, boost immunity, and avoid infections in fish (Citarasu 2010; Shakya 2017; Shekarabi *et al.*, 2021; Shekarabi *et al.*, 2022a). In addition, it was stated that herbal plants are suitable antimicrobial agents to stimulate both specific and non-specific immunity in fish by modulating the functions of the immune cells including T-cells, B-cells, increasing cytokine production, immune related gene expression,

and increasing antibody production (Citarasu, 2010; Chakraborty & Hancz, 2011; Pandey *et al.*, 2012).

Borage (*Borago officinalis*) also known as starflower is one of the most popular medicinal herbs, from the distant past and has many uses in traditional medicine. It belongs to Boraginaceae family with phytochemical contained phenolic acid, gamma-linolenic acid, pyrrolizidines alkaloid and sterols (Zemmouri *et al.*, 2019). The flowers of this plant have been used as anxiolytic, sedative (Rabbani *et al.*, 2004; Sayyah *et al.*, 2006), analgesic of inflammatory and antioxidant in medicine (Ranjbar *et al.*, 2006). Unfortunately, there is not any the knowledge about the effects of borage powder on fish performance except scanty information about the effects of dietary borage oil on the fish performance.

In addition, the common carp (*Cyprinus carpio*) is one of the most important species in Iran and the world. This is suitable for aquaculture because of its fast growth and easy reproduction. Therefore, further detection of commercially available dietary herbal additives to improve performance and disease resistance of the fish is needed.

Consequently, the aim of this study was to investigate the effects of borage powder on the growth performance, body composition, blood biochemical parameters, and immune indices of juveniles' common carp.

Material and Method

Diet Preparation

Ingredients and proximate composition of the basal diet are shown in Table 1. Experimental diets were formulated as a control (basal diet), and three other diets contained 0.5, 1.5, and 2.5% borage powders by replacing filler in basal diet (Ghadikolaei *et al.*, 2018; Ghafarifarsani *et al.*, 2021; Effendi *et al.*, 2022). The borage was supplied from local market after confirming of horticultural science, and then it was dried at room temperature. Dry ingredients were weighed, ground (100 µm particle sizes), and mixed thoroughly. Sunflower oil and water were added to the dry ingredients, mixed again until the dough was formed. Then, the prepared dough was pelleted using a meat chopper machine, which it was dried at room temperature for 24 h, and ground into desirable particle sizes. The diets were broken up, sieved into a proper pellet size, and stored at -20°C.

Experiment Fish and Feeding Conditions

The experiment was done in the lab of Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran. Juveniles of common carp were obtained from a private aquaculture center. The fish were acclimated to laboratory condition for 2 weeks before starting the feeding trial. Juvenile fish with

initial mean weight of 8.40±1.37 g were allocated randomly into 300 L circular tanks with 10 fish per each tank with three replicates. The replicates were hand-fed with one of the test diets to apparent satiety three times a day (9:00, 13:00 and 17:00) for 56 days. During the experimental period, 25% of the total water volume of each tank was replaced with filtered fresh water (28±0.5°C) every two days (Mohammadiazarm & Maniat, 2021). The tanks were siphoned once daily before the first feed. The uneaten feed was collected and weighed to determine the feed intake level (Staessen *et al.*, 2020). Mean water temperature was 28.35±0.2°C, dissolved oxygen was 6.35±0.19 mg/L, and the pH was 8.13±0.19. The photoperiod was left under natural conditions during the feeding trail.

At the end of the experiment, feeding was stopped 24 h prior to weighing or blood sampling to reduce stress on the fish. All fish were individually weighed at the termination of the experiment after anesthetizing with 2-phenoxyethanol 0.3 mL/L (Sigma Chemicals, St.90 Louis, MO, USA) then growth and feed utilization were calculated by the formula as follows: (Mousavi *et al.*, 2016):

$$\text{Weight gain (\%)} = \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{initial weight (g)}} \times 100$$

$$\text{Specific growth rate (SGR \% / day)} = \frac{[\ln(\text{final weight (g)}) - \ln(\text{initial weight (g)})]}{\text{time of trial}} \times 100$$

$$\text{Food conversion ratio (FCR)} = \frac{\text{feed consumption (g)}}{\text{weight obtained (g)}}$$

$$\text{Survival rate} = \frac{\text{number of fish at the end of trial}}{\text{number of fish at the beginning of trial}} \times 100$$

Furthermore, three fish from each replicate tank were randomly sampled for biochemical body composition and stored at -25°C for a short time until analysis.

Biochemical Body Composition

The biochemical composition of basal diet and the pooled samples of three fish from each replicate tank were assayed with the standard methods (AOAC, 2000). The moisture content was assayed by an oven at 110°C and protein content determined by the Kjeldahl technique (Kjeltec TM2300, Foss, Sweden). In addition, the fat content was assayed by ether removal with a Soxhlet (Soxtec TM8000, Foss, Sweden) and ash was defined by burning in a muffle furnace at 550°C for six h.

Sample Preparation

At the end of feeding trial, the pooled blood samples of three anesthetized fish with 2-phenoxyethanol at 0.3 mL/L (Sigma Chemicals, St. Louis, MO, USA) from each replicate tank were randomly

obtained from the caudal vein with heparinized syringes (Velisek *et al.*, 2007). Then the samples were centrifuged at 3000 ×g for 15 min at 4°C. After that, the supernatants were collected and stored at -80°C for future analysis. In addition, skin mucus samples were obtained from three anesthetized fish per replicate tank into separate zipper bags contained 50 mL of 50 mM NaCl for 2 min. Then, samples were centrifuged at 1500×g for 10 min at 4°C, and supernatant was kept at -80°C until analyses (Motlagh *et al.*, 2020).

Blood Hemato-biochemical and Immunological Assay

Blood hemoglobin (Hb), red blood cell counts (RBC), and hematocrit (Hct) were determined by cyanomethemoglobin method, microhaematocrit method, and blood cell counter method, respectively as described by Sandnes *et al.* (1988). The blood indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from individual hematological analyses according to Witeska *et al.* (2022) with following formula.

$$\text{MCV (fl)} = \text{Hct} \times 10 / \text{RBC}$$

$$\text{MCH (pg)} = \text{Hb} / \text{RBC}$$

$$\text{MCHC (g/dL)} = \text{Hb} \times 100 / \text{Hematocrit}$$

The biochemical parameters of plasma containing glucose, triglycerides, total cholesterol, and albumin were assayed using diagnostic kits (Pars Azmoon Co., Karaj, Iran) and auto-analyzer (Mindray BS-200, Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) following the producer's manner.

Total protein content of the plasma and mucus samples were determined according to the method of Lowry *et al.* (1951) with a commercial kit (ArsamFaraZist, Urmia, Iran). Globulin was calculated by subtracting albumin from the total protein.

Lysozyme activity in plasma and mucus samples were determined according to the method of Demers and Bayne (1997) by the lysis of the lysozyme sensitive Gram-positive bacterium, *Micrococcus lysodeikticus* (Sigma). The concentrations of complement components (C3 and C4) in plasma were measured according to immunoturbidimetry method using a commercial kit (Pars Azmoon Co., Karaj, Iran) at 340 nm (Reyes-Becerril *et al.*, 2008).

Furthermore, total immunoglobulin (Ig) in mucus samples was determined with the method of Siwicki *et al.* (1994). Briefly, 100 µl of the mucus were added to the same volume of 12% polyethylene glycol, and then the supernatant fraction samples were separated after incubating samples for 2 h at room temperature and centrifuging at 3000 ×g for 15 min. Finally, the residual protein was assayed, and it was reduced from the total mucus protein concentration.

Statistical Analysis

Data were subjected to one-way ANOVA to test the effect of borage on the fish performance. Kolmogorov-Smirnov and Levene's tests were performed to confirm the normality of data and homogeneity of variance. When significant differences were found in one-way ANOVA, Duncan's multiple range test was used to rank the groups. All statistical analyses were performed using SPSS version 16 (SPSS, Chicago, IL USA) with a significant level of (P<0.05). The values presented are mean ± standard error (SE).

Table 1. Ingredient and proximate composition of basal diet.

Ingredients	(% of Dry Matter)
Fish meal ^a	10.00
Soybean meal	35.72
Corn gluten	10.00
Wheat flour	32.50
Sunflower oil	3.28
Vitamin premix ^c	2.00
Mineral premix ^d	2.00
Binder ^f	2.00
Filler (cellulose)	2.50
Proximate composition (DM %)	
Protein	34.50
Lipid	4.85
Ash	5.77
Gross energy (Kcal g ⁻¹)	2.81

Cloponella meal^a, Iran

Kilka oil^b, Mazandaran Co, Iran

Vitamin premix^c (composition per 1kg): A=1600000 IU, D3=400000 IU, E=40000 mg, K3=2000 mg, B1=6000 mg, B2=8000 mg, B3=12000 mg, B5=40000 mg, B6=4000 mg, B9=2000 mg, B12=8 mg, H2=40 mg, C=60000 mg, Inositol=20000 mg

Mineral premix^d (composition per 1kg): Iron:6000 mg, Zinc:10000 mg, Selenium:20 mg, Cobalt:100 mg, Copper:6000 mg, Manganese:5000 mg, Iodine:600 mg, CoCl2:6000 mg

Binder^f: Amet Binder (Component: Crude Protein: 71.98%, Crude Fiber: 0.9%, Ash: 17.8%, Moisture: 9.55%)

Results

The effects of dietary graded levels of borage powder on growth performance of fish are illustrated in Table 2. The final weight, weight gain percent (WG%), specific growth rate (SGR), and food conversion ratio (FCR) of the fish fed supplemented diets with different levels of borage powder were not significantly different from the control group ($P>0.05$). The survival rate of fish fed supplemented diet with 1.5% borage powder was significantly different from the control group ($P<0.05$). The proximate compositions of the whole body of fish displayed no significant differences among all the treatments (Table 3, $P>0.05$).

The results of blood biochemical parameters, hematology, and some immune parameters of fish are shown in Table 4. The amount of glucose was

significantly reduced in the fish fed supplemented diet with 2.5% borage powder compared to the control group ($P<0.05$), while other treatments showed intermediate amount of glucose without significant difference compared to the control group ($P>0.05$). There was no considerable difference in the amount of total cholesterol and triglyceride between control and other treatments ($P>0.05$).

The content of serum protein was enhanced significantly in the fish fed supplemented diet with borage powder compared to the control group ($P<0.05$). There were no significant differences in the levels of albumin and globulin of fish fed supplemented diet with borage powder ($P>0.05$).

The hemoglobin was significantly greater in fish fed supplemented diet with 1.5% borage powder than the control group ($P<0.05$). Nevertheless, the hematocrit

Table 2. Growth performance and feed utilization of juvenile carp fed the experimental diets for 56 days

Parameters	Control	Borage 0.5	Borage 1.5	Borage 2.5
Final weight (g)	20.30±0.24 ^{ns}	20.44±0.25	20.67±0.32	20.67±0.28
Weight gain (%)	142.33±1.05 ^{ns}	143.70±4.33	145.06±2.63	145.90±3.42
SGR	1.58±0.01 ^{ns}	1.59±0.03	1.60±0.02	1.60±0.02
FCR	1.71±0.04 ^{ns}	1.72±0.09	1.73±0.01	1.76±0.05
Feed intake (g/ind)	20.35±0.10 ^{ns}	20.70±0.07	21.22±0.30	21.59±0.50
Survival (%)	90.00±0.00 ^b	93.33±3.33 ^{ab}	100±0.00 ^a	96.66±3.33 ^{ab}

A different lowercase in the same row denotes statistically significant differences (mean of three replicate tanks ± SE, $P<0.05$). ns, not significant differences ($P>0.05$).

Table 3. Proximate composition of whole body of juvenile carp fed the experimental diets for 56 days (wet weight %).

Parameters	Control	Borage 0.5	Borage 1.5	Borage 2.5
Protein	14.19±0.22 ^{ns}	14.18±0.24	14.37±0.20	14.35±0.19
Moisture	71.16±0.40 ^{ns}	71.27±0.40	71.19±0.33	71.21±0.40
Lipid	10.28±0.17 ^{ns}	10.26±0.16	10.18±0.13	10.11±0.19
Ash	3.11±0.11 ^{ns}	3.11±0.12	3.14±0.10	3.13±0.12

A different lowercase in the same row denotes statistically significant differences (mean of three replicate tanks ± SE, $P<0.05$). ns, not significant differences ($P>0.05$).

Table 4. Hemato-biochemical and immune indices of juvenile carp fed the experimental diets for 56 days.

Parameters	Control	Borage 0.5	Borage 1.5	Borage 2.5
Glucose (mg/dl)	72.86±3.01 ^a	65.90±6.07 ^{ab}	64.17±5.26 ^{ab}	60.77±2.96 ^b
Cholesterol (mg/dl)	169.29±7.15 ^{ns}	160.19±4.28	151.84±11.46	147.14±24.29
Triglyceride (mg/dl)	254.02±24.63 ^{ns}	234.07±4.62	230.69±31.00	226.44±31.89
Protein (g/dl)	4.50±0.35 ^b	4.57±0.28 ^a	4.92±0.38 ^a	4.70±0.36 ^a
Albumin (g/dl)	1.27±0.06 ^{ns}	1.30±0.05	1.35±0.05	1.32±0.05
Globulin (g/dl)	3.23±0.30 ^{ns}	3.26±0.23	3.57±0.32	3.37±0.31
Hb (g/dl)	9.66±0.10 ^b	9.81±0.14 ^{ab}	9.92±0.07 ^a	9.84±0.13 ^{ab}
Hct (%)	37.75±1.30 ^{ns}	37.83±0.61	38.27±0.09	37.83±0.58
RBC(10 ⁶ μL)	1.21±0.08 ^b	1.32±0.09 ^{ab}	1.36±0.05 ^a	1.35±0.05 ^a
MCV(fL)	278±7.06 ^b	283±10.60 ^b	303±0.10.1 ^a	285±13.70 ^{ab}
MCH(pg)	72.45±2.16 ^b	73.41±2.88 ^{ab}	79.86±4.50 ^a	74.32±3.80 ^{ab}
MCHC(g/dl)	26.26±0.61 ^{ns}	25.99±0.07	26.03±0.13	25.94±0.13
Lysozyme (units/ml)	19.39±0.44 ^b	20.56±1.36 ^{ab}	21.27±0.36 ^a	20.70±1.48 ^{ab}
C3 (mg/ml)	9.43±0.26 ^b	9.90±0.23 ^b	10.21±0.18 ^a	10.12±0.14 ^a
C4 (mg/ml)	1.94±0.19 ^b	2.23±0.14 ^{ab}	2.36±0.13 ^a	2.25±0.17 ^{ab}

A different lowercase in the same row denotes statistically significant differences (mean of three replicate tanks ± SE, $P<0.05$). ns, not significant differences ($P>0.05$).

(Hct %) values of fish did not show a significant difference among all the treatments ($P>0.05$). A total number of red blood cells (RBC) of fish fed supplemented diet with 1.5 and 2.5% dietary borage powder significantly enhanced compared to the control group ($P<0.05$). Furthermore, MCV and MCH of the fish fed supplemented diet with 1.5% borage powder increased significantly compared to control group ($P<0.05$). Nevertheless, MCHC of the fish were not significantly affected by different levels of dietary borage powder compared to the control group ($P>0.05$). Based on the results of plasma immune parameters, lysozyme activity in the plasma of the fish fed supplemented diet with 1.5% borage powder significantly enhanced compared to the control group ($P<0.05$). The concentration of C3 in plasma of the fish fed supplemented diet with 1.5 and 2.5% dietary borage powder showed significant increase compared to control group ($P<0.05$). In addition, the concentration of C4 in the fish fed supplemented diet with 1.5% borage powder enhanced significantly compared to the control group ($P<0.05$).

Mucosal immune indices (Figures 1) showed that the fish fed the supplemented diets with different levels of borage powder had significantly higher total protein, total Ig, and lysozyme activity compared to the control group ($P<0.05$). Therefore, the higher mucosal immune indices were observed in fish fed supplemented diet with 1.5% dietary borage powder.

Discussion

The growth performance, feed utilization, and final body composition of the fish were not affected by the diets supplemented with borage powder in this experiment. However, it was stated that the medicinal herbs have diverse groups of phytochemicals such as phenolics, flavonoids, alkaloids, polysaccharides, volatile oils, and proteoglycans that act as growth promoting with improved digestibility, gut microbial community, and secretion of endogenous digestive enzymes (Chakraborty *et al.*, 2014). Unfortunately, there is not any study about the effects of borage powder on the fish performance. Nevertheless, the improved growth of fish with other medicinal herbs were reported. For example, tilapia (*Oreochromis mossambicus*) fed diets supplemented with acetone extracts of *Cynodon dactylon*, *Aegle marmelos*, *Withania somnifera*, and *Zingiber officinale* showed a significant increase in growth performance (Immanuel *et al.*, 2009). The dietary ginseng herb (Ginsana G115) increased the growth performance and feed efficiency in the Nile tilapia (*Oreochromis niloticus*) juveniles (Goda, 2008). Lee *et al.* (2005) stated the improved growth in rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with maca meal (powdered tuber of *Lepidium meyenii*) or its methanol extract.

Unlike these reports, the dietary inclusion of some herbs had not much impact on growth response as

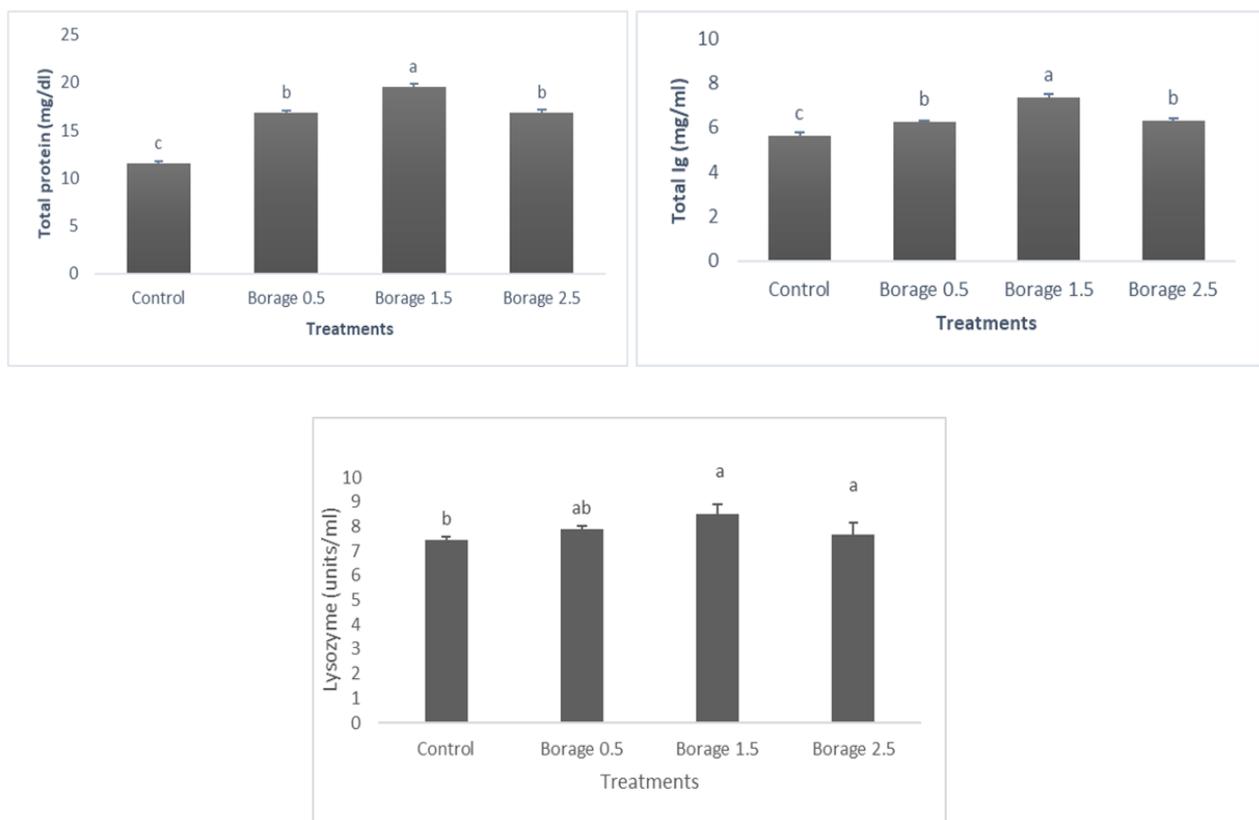


Figure 1. Mucosal immune parameters of juvenile carp fed the experimental diets for 56 days. A different lowercase in the column denotes statistically significant differences (mean of three replicate tanks \pm SE, $P<0.05$).

indicated in juvenile pikeperch (*Sander lucioperca*) fed on two medicinal herbs *Astragalus radix* and *Lonicera japonica* (Zakeş *et al.*, 2008), and common carp which received willow herb, *Epilobium hirsutum* (Pakravan *et al.*, 2012). Furthermore, in similarity with our result the survival rate, feed intake, and final carcass composition of the Japanese flounder fed with herbal mixture, *Massa medicata*, *Crataegi fructus*, *Artemisia capillaries*, and *Cnidium officinale* did not show significant difference among all the treatments (Ji *et al.*, 2007a). It was reported that the effectiveness of herbal additives in aquaculture depends greatly on factors such as the plant part, method of preparation, method of application, duration of experiment, dosage in relation to species, and age (Bahrami Babaheydari *et al.*, 2015; Oniovosa *et al.*, 2017).

Any change in the physiological status of the fish, from diet, can cause changes in blood parameters (Esmaili, 2021). In this regards, total plasma protein of the fish fed different levels of borage powder in diet significantly increased compared to the control. Ghafarifarsani *et al.* (2021) observed similar results when common carp fed supplemented diet with mixture of herbs contained *Malvae sylvestris*, *Origanum vulgare*, and *Allium hirtifolium*. It was reported that the herbal additives promoters the transcription rate that leads to increased RNA, total amino acid, and production of proteins in the cells (Citarasu, 2010).

In addition, the triglyceride and cholesterol levels had slightly decreased but they were not significant different from control. Similar results were observed by using four medicinal plants *Cynodon dactylon*, *Aegle marmelos*, *Withania somnifera* and *Zingiber officinale* for Mozambican tilapia (*Oreochromis mossambicus*) (Immanuel *et al.*, 2009). Feeding aquatics with diets containing phytochemicals can affect fat metabolism, which helped the fish to utilize lipid effectively as a source of energy (Ji *et al.*, 2007a, b). It means that other sources of energy like protein can be used more effectively for somatic growth (Zakes *et al.*, 2008).

Furthermore, the amounts of albumin, globulin were not different among treatments. The non-significant difference in the levels of these biochemical parameters suggests that borage powder used in this study may not interfere significantly with the metabolism of these biochemical parameters. This supports earlier report by Eenenebeaku *et al.* (2021) who studied aqueous and methanol leaves, stem and roots extracts of *Chasmanthera dependens* (Hochst) and *Dictyandra arborescens* (Welw.) in rat.

On the other hand, the significant enhanced hemoglobin, RBC, MCV, and MCH were seen in the fish fed supplemented diet with 1.5 % borage powder. In this regards, different studies reported enhanced hematocrit, hemoglobin, erythrocyte and lymphocytes count in the fish by herbal additives such as the effects of ginger at 1000 mg/35L on African catfish (*Clarias gariepinus*) juveniles (Soosean *et al.*, 2010), Mangosteen (*Garcinia mangostana*) at 0.5% diet on African catfish,

ginger and garlic at 1% diet on *Huso huso* (Gholipour Kanani *et al.*, 2014), powdered ginger rhizome (*Zingiber officinale*) at 1% diet on rainbow trout (Haghighi *et al.*, 2013), Methanol extract of tetra (*Cotinus coggygia*) at 1g/kg feed on Koi carp (Bilen *et al.*, 2013), Nettle or *Urticadioica* at 12% diet fon *H. huso* (Lim *et al.*, 2000), *Azadirachta indica*, *Ocimum sanctum* and *Curcuma longa* at 400 or 800mg/kg diet on Goldfish (*Carassius auratus*) (Harikrishnan *et al.*, 2010), *Echinacea purpurea* on Rainbow trout (Oskoi *et al.*, 2012), *Mentha piperita* at 0.5 % diet on Asian seabass (*Lates calcarifer*) (Talpur, 2014), and *Epilobium hirsutum* (willow herb) on common carp fingerlings (Pakravan *et al.*, 2012).

Mode of action of medicine plant on hematological indices could be due to the effect of phytoconstituents such as vitamin C on the increase of iron absorption from intestine of fish (Lim *et al.*, 2000). In addition, increased levels of MCH and MCHC indicates that phytoconstituents stimulated the secretion of erythropoietin which stimulates the stem cells in the bone marrow to produce RBC (Enebeaku *et al.*, 2021). On the other hand, an increase in hematological indices shows immune system stimulation and function of organs related to blood cell formation such as thymus, spleen, and bone marrow (Iheanacho *et al.*, 2017).

The significant enhanced immunity indices and survival rate were seen in fish fed with 1.5% dietary borage powder that could be related to phytochemical compound of borage. It was reported that medicinal herbs containing antimicrobial agents and stimulate both specific and non-specific immunity in fish (Citarasu 2010; Chakraborty & Hancz, 2011; Pandey *et al.*, 2012; Shekarabi *et al.*, 2022b).

In this regards, Shekarabi *et al.* (2021) reported that the used of 3 g/kg dandelion (*Taraxacum officinale*) flower extract in diet of rainbow trout induced enhanced total leukocyte, lymphocyte counts, complement components, immunoglobulin M, total protein, lysozyme activity, enzymatic activities, and skin mucus protein. In addition, the transcription levels of interleukin-1 β and interleukin-6 genes Interleukin-8, and lysozyme gene expression levels were increased in the fish fed with 3 and 4 g/kg dandelion with high resistance to *Streptococcus iniae*. It was reported the enhanced the activity of the complement system C3 and C4 through improvement of liver functions by herbal medicine such as *Polygonum minus* extracts, *Aloe vera*, and *Berberis vulgaris* in rainbow trout and Siberian sturgeon (*Acipenser baerii*), respectively (Adel *et al.*, 2020; Shekarabi *et al.*, 2022a; Bazari Moghaddam *et al.*, 2017). The mixture of three medicinal plant compounds, azadirachtin (a tetranortriterpenoid), camphor (a terpenoid), and curcumin (a polyphenol), in diet of major Indian carp (*Cirrhina mrigala*) significantly increased serum lysozyme activity (Harikrishnan *et al.*, 2009). Yuan *et al.* (2007) reported the increased expression of the immune response genes (interleukin-1b, tumour necrosis factor-a, Lysozyme-C) in the head kidney, gill and spleen of the common carp with

Astragalus polysaccharides.

It was reported that, common carp fed diets containing a mixture of *Astragalus membranaceus* (root and stem), *Polygonum multiflorum*, *Isatis tinctoria* and *Glycyrrhiza glabra* (0.5 and 1%) showed increased macrophage phagocytic activity, respiratory burst, levels of total protein, albumin, globulin and nitric oxide synthetize activity in the serum, but no significant difference was found in lysozyme activities (Yuan *et al.*, 2007). Jian & Wu (2004) studied the effect of a traditional Chinese medicine from *Astragalus* root and Chinese *Angelica* root on non-specific immunity of Jian carp (*C. carpio* var. Jian) and seen significant increases in blood phagocytic cell activity and plasma lysozyme activity in fish. In addition, supplemented diets with plant extracts from four Chinese herbs (*Rheum officinale*, *Andrographis paniculata*, *Isatis indigotica*, and *Lonicera japonica*) increased phagocytosis in the white blood cells of *C. auratus* (Chen *et al.*, 2003). Therefore, similar to these reports, borage powder is a useful dietary supplement which can improve both skin mucosal immunity, innate immune responses, and survival rate due to presence of bioactive molecules.

Conclusion

The results of the experiment recommended supplemented diet with 1.5% of borage powder for enhanced immunity indices of juvenile common carp fish. In addition, further studies on borage compounds, and their effects on fish immunity, nutrient absorption, and the mechanism of action may provide more knowledge, particularly in producing organic agricultural by-product additives to support sustainable aquafeed.

Ethical Statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Khorramshahr University of Marine Science and Technology (IR.KMSU.REC.1394.003).

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Author Contribution

Parisa Cheraghi, Investigation, and Methodology; Hamid Mohammadiazarm, Supervision, Methodology, Writing-review and editing; Milad Maniat, Fish lab assays and Formal Analysis.

Conflict of Interest

Authors declare that there is no conflict of interest.

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