Aquaculture International (2020) 28:73–84
https://doi.org/10.1007/s10499-019-00444-9

Effect of common mallow (*Malva sylvestris*) dietary supplementation on growth performance, digestive enzyme activities, haemotological and immune responses of common carp (*Cyprinus carpio*)

Soner Bilen[1] **· Abdelsalam M. O. Filogh**[2] **· Abobaker Barka Ali**[2] **· Osman Nezih Kenanoğlu**[1] **· Mehmet Arif Zoral**[2]

Received: 8 April 2019 / Accepted: 24 July 2019 / Published online: 12 August 2019

Abstract

The present study was designed to assess the effect of common mallow (*Malva sylvestris*) extract diet in common carp (*Cyprinus carpio*) health parameters such as growth performance, digestive enzyme activity, hematological profile, and immunological responses. In addition, we evaluated the possibility of controlling *A. hydrophila* infection in common carp during the common mallow extract diet (CM). Diets of common carp were supplemented with three different concentrations (0.1, 0.5, and 1 g kg^{-1}) of common mallow extract. They were fed with common mallow diet for 45 days. At the end of feeding, 0.1 g kg^{-1} CM diet group showed higher final weight and SGR ($p < 0.05$), whereas the 0.5 g kg^{-1} CM diet group showed significantly ($p < 0.05$) lower FCR rate. The digestive enzyme activity experiment was showed that amylase and lipase activity significantly increased ($p < 0.05$) in 0.5 g kg^{-1} CM diet. No hematological parameters changed in all experimental groups. Only 0.1 g kg^{-1} showed significantly decreased level of red blood cell. In immunological response experiment, serum lysozyme activity, nitroblue tetrazolium reduction, and myeloperoxidase activity significantly increased in different doses of common mallow diet during the 45 days. Result of challenge test with *A. hydrophila* showed that survival rate in 0.5 and 1 g kg^{-1} CM diet was significantly lower compared to control. The results elucidated that the CM diet could support nonspecific immune system and growth promoter in common carp. Hence, common mallow can be used in semi-intensive and intensive common carp culture.

Keywords *Malva sylvestris* · *Cyprinus carpio* · Growth promoter · Immune responses · Immunostimulant

✉ Soner Bilen
sbilen@kastamonu.edu.tr

Extended author information available on the last page of the article

Introduction

Common carp (*Cyprinus carpio*) is a major cultured fish species in intensive and semi-intensive aquaculture. It is third in total aquaculture production worldwide and cultured in over 100 countries (Bostock et al. 2010; FAO 2016). The increasing intensive aquaculture systems has accelerated serious problems such as diseases outbreak, malnourished fish, deterioration of water quality, and others (Naylor et al. 2000; FAO 2016; Munir et al. 2018).

The fish diseases such as bacterial and parasitic infections are usually managed using antibiotics and chemicals which lead drug resistance, immunosuppression, residue risk, and side effects for animal health and environment (Bilen et al. 2016; Zoral et al. 2017; Altunoglu et al. 2017; Rahman et al. 2019). These problems have stimulated research on new alternative material such as medicinal herbs. The herbs can be suitable candidate materials because they prevent diseases, stimulate growth, and are eco-friendly for environment (Reverter et al. 2014; Bilen et al. 2016; Zoral et al. 2017). Additionally, it is easy to prepare supplemented herb extract diets for oral treatment methods (Zoral et al. 2017). Many studies have been reported that medicinal herbs are alternative therapy on controlling major diseases in aquaculture industry (Reverter et al. 2014; Bilen et al. 2016; Zoral et al. 2017). They were reported to have antibacterial, antifungal, antiparasitic, and immunostimulant activities (Bilen et al. 2011; Reverter et al. 2014; Zoral et al. 2018). In addition, previous studies reported that medicinal herbs have a positive effect on growth rate, hematological (white and red blood cell), and digestive enzyme (amylase, lipase, trypsin, and others) activity in fish species (Wang et al. 2015; Bilen et al. 2016; Bilen et al. 2018).

Medicinal herbs are useful candidate material for nutritional manipulation to supporting fish health in aquaculture (Reverter et al. 2014; Bilen et al. 2018). Besides, medicinal herbs are easy to apply in oral treatment methods compared to other methods such as immersion and injection (Zoral et al. 2017). Thus, in the last decades, many studies have been focused to effects of medicinal herb treatment advantages on fish health (Reverter et al. 2014). This study was designed to determine advantages of common mallow on common carp health.

Malva sylvestris, commonly known as “common mallow” in Turkey, belongs to Malvaceae family and is native to Europe, Asia, and North Africa (Gasparetto et al. 2012). Common mallow has been used in traditional medicine, pharmacy, and food industry (Ballero et al. 2001; Gasparetto et al. 2012; Prudente et al. 2017). It contains flavonoids, terpenoids, phenol derivatives, vitamins C and E, and fatty acids (Gasparetto et al. 2012; Saad et al. 2017; Yarijani et al. 2019). Moreover, several authors reported that herb has some beneficial features such as anti-microbial, anti-inflammatory, antioxidant, anti-diarrheal, antiseptic, emollient, demulcent, antitussive, anticancer, and antiulcerogenic properties for animal health (Schulz et al. 2003; Gasparetto et al. 2012; Prudente et al. 2013; Benso et al. 2015; Delfine et al. 2017; Saad et al. 2017).

Here, we examined the efficacy of common mallow diet on growth performance, digestive enzyme activity, hematological profile, and immunological responses in common carp. In addition, we investigated antibacterial effect of common mallow against *A. hydrophila* infection in common carp.

Material and methods

Source of fish

Common carp (2.95 ± 0.01 g) were obtained from a local fish farm and transferred to indoor laboratory, the Faculty of Fisheries, Kastamonu University, Turkey. A total of 40 fish acclimated in tanks with 6.8–7.2 mg/L dissolved oxygen, pH 7.7–8.5, 0.2–.05 mg/L ammonium, and 0.02–0.04 mg/L nitrite. During the acclimation, fish did not show any disease symptoms or abnormalities. Water temperature was kept at 25–28 °C for 2 weeks. The tanks were aerated and cleaned daily. The fish were maintained with a natural photoperiod. Water parameters were monitored daily. Until experiment started, fish were fed commercial feed twice a day at a rate of 2% body weight.

Diet preparation and experimental design

Leaves of *M. sylvestris* were purchased from local herb market in Kastamonu, Turkey (41°25′ 50 N, 33°45′19 E). They were kept at 4 °C and used within 2 weeks. Then, the leaves were dried at room temperature for 2 days. After drying, 50-g leaf material was extracted with 1 L of 40% methanol (Sigma-Aldrich, St. Louis, MO, USA) in laboratory blender at room temperature, kept for 3 days. The mixer was filtered through 150-μm filter paper and then evaporated in rotary evaporator at 55–65 °C to remove the methanol. The final product was dissolved in distilled water and stored in plastic tubes at 4 °C (Bilen et al. 2016). The methanolic extract of *M. sylvestris* (CM) was diluted with 50 mL of distilled water. In the study, the fish feed was prepared previously formulated by Bilen and Müge Bilen (2013). After then, the CM was sprayed on the fish diet at concentrations of 0.1 (CM01), 0.5 (CM05), and 1 g kg^{-1} (CM1) and stored in plastic zip pack at − 20 °C until use.

Randomly selected fish were divided into 4 experimental diet groups (0.1, 0.5, and 1 g kg^{-1} CM diets and a control diet without any extract). A number of 40 fish were placed in each aquarium (100 L) with biological and mechanical filtration. Water parameters (temperature, oxygen, ammonium, nitrite, and pH) were monitored daily. The fish were fed twice a day ad libitum for 45 days. Any abnormal behavior or mortality was recorded in all the experimental groups.

Growth performance

The fish were weighed at the beginning (initial body weight) and at the end of the experiment (final body weight). After the 45-day feeding experiment, weight gain (WG, %), specific growth rate (SGR, %g/day), feed conversion ratio (FCR), and fish survival rate (%) were calculated according to the method described by Ricker (1979) as follows:

$$\text{Weight gain (WG\%)} = 100 \times (\text{final body weight} - \text{initial body weight})/\text{initial body weight}$$

$$\text{Specific growth rate (SGR, \%g/day)} = 100 \times [(\text{Ln final weight}) - \text{Ln (initial weight)}]/\text{days of the experiment}$$

$$\text{Feed conversion ratio (FCR)} = \text{feed intake (g)}/\text{weight gain (g)}$$

$$\text{Fish survival rate (\%)} = 100 \times (\text{fish number in each group remaining at the end of the experiment}/\text{initial number of fish})$$

Digestive enzymes activity

Digestive enzymes such as amylase, lipase, and trypsin activities were examined at the end of the feeding trials. Stomach and anterior part of intestine were homogenized in 1 mL cold double distilled water using a Potter Elvehjem homogenizer. After then, samples were centrifuged at 15,000 rpm for 20 min at 4 °C. Supernatants were evaluated by the Bradford method (1976). The supernatant was gently collected with a plastic pipet and stored at − 80 °C until analysis. Amylase activity was determined by using 2% starch (Sigma-Aldrich, St. Louis, MO, USA) as a substrate (Worthington 1991). Lipase activity was determined by hydrolysis of 4-nitrophenyl myristate (Sigma-Aldrich, St. Louis, MO, USA) (Gawlicka et al. 2000). Trypsin activity was measured by using benzoyl-dl-arginine-p-nitroanilide (Sigma-Aldrich, St. Louis, MO, USA) (Erlanger et al. 1961). All enzyme activity units were calculated as follows:

$$\begin{aligned}
&\text{Amylase} = \left[(\text{Sample}-\text{Blank})^2 \times 7.712\right] - [1.082 \times (\text{Sample}-\text{Blank})] \\
&\qquad + 0.082 = \text{Result}/\text{mg protein} \\
&\text{Lipase} = \left[(\text{Sample}-\text{Blank}) \times (0.2359 + 0.0153)^2\right]/\text{mg protein} \\
&\text{Trypsin} = [(\text{Last Result}-\text{First Result})/10\ \text{min}] \\
&\quad = \text{Absorption Result } [(\text{Absorption Result} \times 1\ \text{million})/8.800]/2 = \text{Result}/\text{mg protein}
\end{aligned}$$

Hematological analysis

At the end of feeding trials, fish were anesthetized by MS-222 (100 mg/L) for blood sampling. The blood samples were collected from caudal vein puncture with heparinized syringes and transferred EDTA tubes to test hematological parameters analysis. White blood cell (WBC × 10^7/mm^{-3}), red blood cell (RBC × 10^6/mm^{-3}) counts, hemoglobin (Hb, g/dL^{-1}), and hematocrit (Hct, %) were measured as described by Blaxhall and Daisley (1973). Blood indices included mean cell (MCV, fL), mean cell Hb (MCH, pg), and the mean cell Hb concentration (MCHC, %) that were measured according to Lewis et al. (2006).

Non-specific immune parameter analysis

At days 15, 30, and 45, three randomly selected fish from each group's aquariums were examined for serum lysozyme activity (LYS), nitroblue tetrazolium reduction assay (NBT), and myeloperoxidase activity (MPO). LYS and NBT were measured according to method used by Bilen et al. (2016). Myeloperoxidase activity (MPO) content in serum was determined as described by Sahoo et al. (2005) with some modifications according to method used by Bilen et al. (2016).

Challenge test

In the study, *A. hydrophila* strain SB-Ah1 previously isolated from diseased fish, with 1 × 10^8 CFUs mixed in 100 μL PBS was injected intraperitoneally to all experimental fish at the end of dietary administration. Survival rate of each experimental group was examined and recorded for 10 days.

Statistical analyses

Statistics were analyzed with SPSS software version 24.0 (IBM Corp., Armonk, NY, USA). All results of experiments were compared using one-way ANOVA. Before subject to statistical analyses, all data were checked for normal distribution and homogeneity of variance with the Kolmogorov-Smirnov test and Levene's test, respectively. Multiple comparisons were made using Tukey's multiple range test. Significance was set at $p < 0.05$.

Results

Growth performance

Only CM01 diet group showed higher final weight and SGR ($p < 0.05$) (Table 1). CM01 diet was increased WG rate, whereas other treatment groups (CM05 and CM1 diet) showed lower WG rate. CM05 diet group showed significantly ($p < 0.05$) lower FCR rate (Table 1).

Digestive enzymes activity

Lipase activity significantly increased ($p < 0.05$) in CM05 diet (Fig. 1). We observed that trypsin level was not changed in all experimental groups (Fig. 2). Amylase activity was significantly increased on CM05 groups compared to control (Fig. 3).

Hematological analysis

HGB, HCT, MCV, MCH, and MCHC were not significantly changed in all experimental groups (Table 2). Level of RBC was observed to significantly decrease in CM01 diet group.

Non-specific immune parameters analysis

At the end of 30 days, LYS significantly increased ($p < 0.05$) in fish fed with CM01 and CM05 diet groups when compared with the control (Table 3). Furthermore, we observed that LA level continued to be higher in CM01 diet group compared to other diets and control after 45 days.

MPO level significantly increased ($p < 0.05$) in fish fed with CM1 supplementation diets after 15, 30, and 45 days (Table 3). In addition, higher MPO level was observed in CM01 and CM05 diet groups compared to control in different sampling days.

NBT increased ($p < 0.05$) in all treatment groups at the end of 45 days (Table 3).

Challenge test

At the end of the study, after the fish challenged with *Aeromonas hydrophila*, all results are summarized in the Fig. 4. The results showed that the higher survival rate was found in control and CM01 group. While no differences were observed between control and CM01 group ($p > 0.05$), CM05 and CM01 groups' survival rate was significantly decreased ($p < 0.05$).

Table 1 Growth performances of common carp fed with common mallow (CM) aqueous methanolic extract, control diet (0 g kg^{-1}), CM01 (0.1 g kg^{-1}), CM0.5 (0.5 g kg^{-1}), and CM1 (1 g kg^{-1}) at the end of the 45-day experiment

	Control	CM01	CM05	CM1
Initial weight	3.09 ± 0.01	3.03 ± 0.02	3.05 ± 0.01	3.08 ± 0.01
Final weight	5.12 ± 0.16 ^{b}	6.16 ± 0.48 ^{a}	5.30 ± 0.07 ^{b}	4.56 ± 0.24 ^{c}
Feed intake	201.06 ± 13.48 ^{a}	222 ± 19.80 ^{a}	93.09 ± 6.51 ^{b}	103.59 ± 3.20 ^{b}
SGR	1.12 ± 0.08 ^{b}	1.57 ± 0.19 ^{a}	1.23 ± 0.03 ^{b}	0.87 ± 0.11 ^{c}
FCR	1.51 ± 0.03 ^{b}	1.44 ± 0.04 ^{b}	1.21 ± 0.01 ^{c}	2.85 ± 0.06 ^{a}
WG %	65.86 ± 5.60 ^{b}	103.71 ± 17.05 ^{a}	73.95 ± 1.97 ^{b}	47.98 ± 7.38 ^{c}
RPS	–	–	44.23 ^{a}	69.23 ^{a}

Data represent means from three replicates per treatment. Values are presented as the mean ± SE

Values in a row with the different superscript letters denote significant difference ($p < 0.05$)

Discussion

Results of the study indicate that administration of dietary methanolic extracts of CM can stimulate nitroblue tetrazolium reduction, lysozyme, and myeloperoxidase activity in the common carp (*Cyprinus carpio*). It was also demonstrated that the survival rate of *A. hydrophila* challenged common carp of CM05 and CM1 groups was lower than that of control groups.CM01 group's growth promotion was determined to be higher than control. The increased growth promotion and stimulated non-specific immune response could provide a better yield in the common carp.

Aquaculturists have focused on alternative agents including the use of herbs as growth promoters in fish for success production (Zoral et al. 2018). The common mallow at 0.1 g kg^{-1} significantly increased growth performance of the fish. FCR decreased slightly but not significantly after 45 days of CM01 diet. Additionally, FCR rate significantly decreased in CM05 diet. On the other hand, FCR rate was higher, whereas WG rate was lower in CM1 diet. When we examined digestive enzyme activity (lipase, amylase, and trypsin) between all experimental groups, we did not find decreasing enzyme activity in CM1 diet. According to this result, it is possible that CM1 diet was not consumed by fish. In our unpublished study, the common mallow diet in all doses has positive effect on growth rate in rainbow trout (*Oncorhynchus mykiss*). This difference could be explained by differences of fish species. Some studies reported that garden cress (*Lepidium sativum*) and rhubarb (*Rheum officinale*) diet increase growth rate in common carp (Xie et al. 2008; Bilen et al. 2018). According to our treatment groups, CM01 and CM05 diet except CM1 diet showed similar growth-promoting effect in common carp.

The digestive enzyme activities (lipase, amylase, and trypsin) are major players on growth parameters (Furne et al. 2005). The higher digestive enzyme activities can easily hydrolyze essential nutrient such as carbohydrates, lipids, and proteins (Bilen et al. 2018). Hence, the present study was undertaken to investigate advantages of common mallow on the digestive enzyme activity. In the study, lipase activity was significantly increased in CM05 diet. In addition, FCR rate decreased in the same dose. Some organic and inorganic components such as flavonoids and sugar can trigger enzyme activity (Krogdahl et al. 2005). For this reason, common mallow may have affected those enzyme activities. Similar result was also found by Amhamed et al. (2018) that amylase and lipase activity increased during the nettle-leaf goosefoot (*Chenopodium album*) diet in common carp.

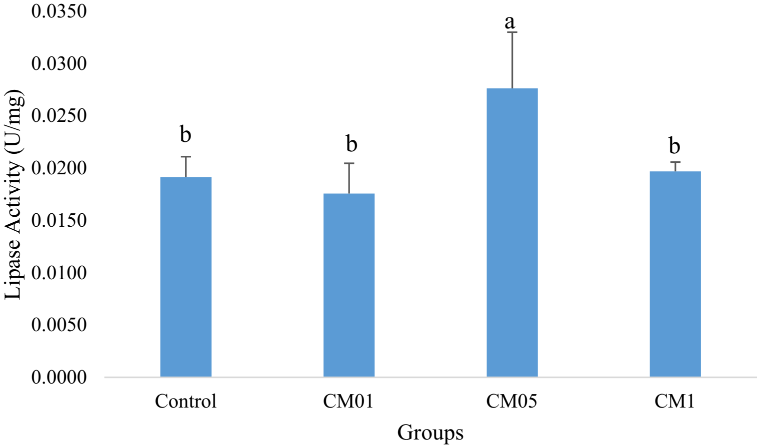

Fig. 1 Lipase enzyme activities of common carp fed a basal diet (control) and two diets supplemented with 0.1, 0.5, and 1 g kg^{-1} for 45 days. Data represent the mean ± SEM. Bars assigned with different superscript letters are significantly different ($p < 0.05$). Control: control diet (common mallow 0 g kg^{-1} feed), CM01 (common mallow 0.1 g kg^{-1} feed), CM0.5 (common mallow 0.5 g kg^{-1} feed), and CM1 (common mallow 1 g kg^{-1} feed)

There were no significant differences between the treatment groups fed with different levels of CM in blood parameters. Hematological results demonstrated that doses of CM01 to CM1 diet did not affect blood parameters in common carp. The efficacy of CM diet on hematological parameters may depend upon fish species, age, and conditions. It is possible that CM diet can make different results in different fish species.

The LYS level is an important indicator in nonspecific immune defense against bacteria and other pathogen species (Shoemaker et al. 2015; Altunoglu et al. 2017). In this study, LYS increased in treatment groups. Several studies have reported that various compounds in herb extracts can directly trigger the increase of LYS and support to immune system in fish (de Souza et al. 2019). For example, black cumin seed (*Nigella sativa*), tetra (*Cotinus coggygria*),

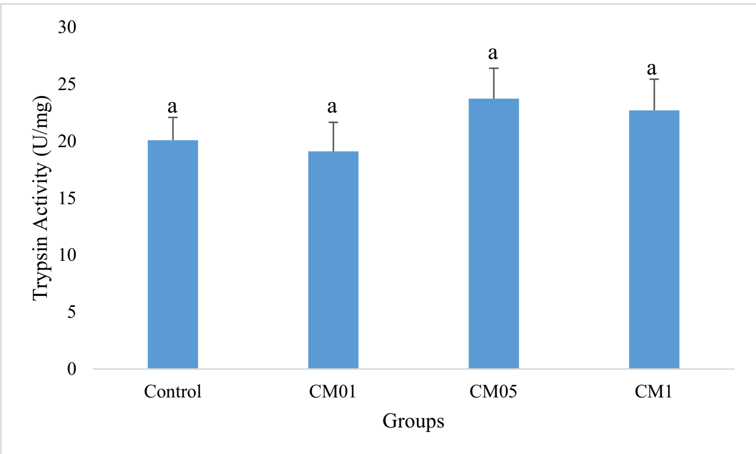

Fig. 2 Trypsin enzyme activities of common carp fed a basal diet (control) and two diets supplemented with 0.1, 0.5, and 1 g kg^{-1} for 45 days. Data represent the mean ± SEM. Bars assigned with different superscript letters are significantly different ($p < 0.05$). Control: control diet (common mallow 0 g kg^{-1} feed), CM01 (common mallow 0.1 g kg^{-1} feed), CM0.5 (common mallow 0.5 g kg^{-1} feed), and CM1 (common mallow 1 g kg^{-1} feed)

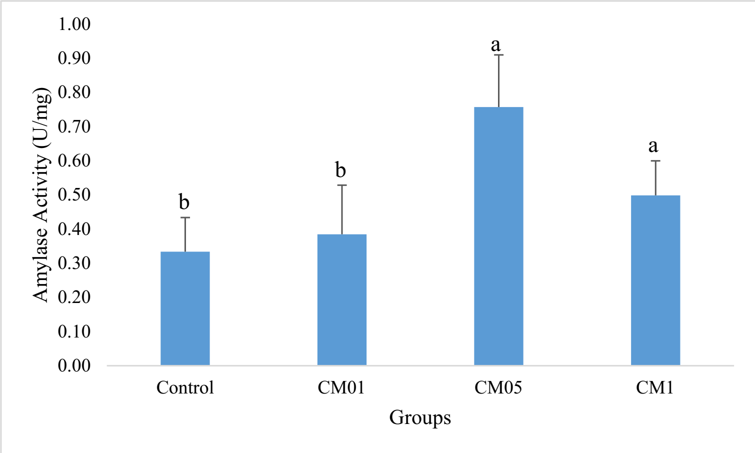

Fig. 3 Amylase enzyme activities of common carp fed a basal diet (control) and two diets supplemented with 0.1, 0.5, and 1 g kg^{-1} for 45 days. Data represent the mean ± SEM. Bars assigned with different superscript letters are significantly different ($p < 0.05$). Control: control diet (common mallow 0 g kg^{-1} feed), CM01 (common mallow 0.1 g kg^{-1} feed), CM0.5 (common mallow 0.5 g kg^{-1} feed), and CM1 (common mallow 1 g kg^{-1} feed)

and dill (*Anethum graveolens*) increased LYS activity (Awad et al. 2013; Bilen et al. 2014; Bilen et al. 2018). These herbs and our candidate herb contain various flavonoids components (Tubesha et al. 2011; Wang et al. 2015; Saad et al. 2017; Genovese et al. 2018). The flavonoids have direct effect to LYS level (Yang et al. 2012). Hence, it is necessary to identify which active flavonoid component affect LYS level.

The flavonoids can trigger and affect superoxide anion activity (Robak and Gryglewski 1988). Superoxide anion is important to prevent and control against microbial infections in living organisms (Bilen et al. 2018). The common carp fed with all doses of CM diets showed a significant increase in NBT level when compared to the control. Previous studies reported that NBT level increased during the oyster mushroom (*Pleurotus ostreatus*), tetra, and black cumin diets which contain flavonoids (Bilen et al. 2014; Bilen et al. 2016; Llaurado et al. 2016; Saad et al. 2017; Genovese et al. 2018). Thus, our results are consistent with the results of different herb species obtained from the previous studies.

White blood cells such as macrophages and neutrophils are important in host defense mechanism against pathogens such as bacteria, virus, fungus, and parasites (Johnston 1978).

Table 2 Hematology of common carp fed with different doses of the common mallow aqueous methanolic extract at the end of the 45-day experiment

	Control	CM01	CM05	CM1
RBC	1.91 ± 0.04 [a]	1.27 ± 0.32 [b]	1.86 ± 0.07 [a]	1.86 ± 0.01 [a]
HGB	8.25 ± 0.11	5.10 ± 1.27	7.50 ± 0.00	6.45 ± 0.53
HCT	174.90 ± 4.45	153.55 ± 0.67	155.40 ± 5.09	155.15 ± 2.72
MCV	43.15 ± 0.25	40.40 ± 0.21	40.40 ± 1.56	34.65 ± 2.72
MCH	43.15 ± 0.25	40.40 ± 0.21	40.40 ± 1.56	34.65 ± 2.72
MCHC	263.50 ± 4.60	264.00 ± 2.83	260.00 ± 1.41	224 ± 21.92

Values in a row with the different superscript letters denote significant difference ($p < 0.05$). Control: control diet (common mallow 0 g kg^{-1} feed), CM01 (common mallow 0.1 g kg^{-1} feed), CM0.5 (common mallow 0.5 g kg^{-1} feed), and CM1 (common mallow 1 g kg^{-1} feed)

Table 3 Immune responses of common carp fed with different doses of the common mallow aqueous methanolic extract at the end of the 45-day experiment

		Control	CM01	CM05	CM1
NBT	15 days	0.91 ± 0.05	0.94 ± 0.11	0.78 ± 0.07	0.94 ± 0.13
	30 days	0.62 ± 0.10 [b]	0.74 ± 0.06 [a]	0.65 ± 0.05 [b]	0.95 ± 0.14 [a]
	45 days	1.16 ± 0.07 [b]	1.44 ± 0.04 [a]	1.47 ± 0.24 [a]	1.51 ± 0.03 [a]
MPO	15 days	31.22 ± 5.66 [b]	67.73 ± 17.16 [b]	151.67 ± 2.37 [a]	137.27 ± 25.05 [a]
	30 days	16.49 ± 0.06 [c]	275.16 ± 61.44 [a]	183.36 ± 23.00 [b]	284.17 ± 22.78 [a]
	45 days	1111.27 ± 156.67 [b]	1400.26 ± 2.70 [a]	701.08 ± 58.59 [c]	1935.76 ± 55.25 [a]
LYS	15 days	80.22 ± 3.00	76.85 ± 3.09	73.44 ± 8.22	78.73 ± 2.60
	30 days	9.03 ± 1.09 [b]	16.39 ± 2.33 [a]	15.10 ± 5.54 [a]	11.74 ± 1.65 [b]
	45 days	12.61 ± 1.26 [b]	23.39 ± 4.51 [a]	14.73 ± 2.32 [b]	14.46 ± 2.8 [b]

Values in a row with the different superscript letters denote significant difference ($p < 0.05$). Control: control diet (common mallow 0 g kg^{-1} feed), CM01 (common mallow 0.1 g kg^{-1} feed), CM0.5 (common mallow 0.5 g kg^{-1} feed), and CM1 (common mallow 1 g kg^{-1} feed)

Neutrophil activity depends on some factors such as chemotactic, phagocytic, bactericidal functions, respiratory burst, and MPO level in fish (Dalmo et al. 1997; Palic et al. 2005). When the inflammation was caused by the pathogens in the body, MPO enzyme directly stimulates the macrophages and neutrophils (Grattendick et al. 2002; Lau et al. 2005). In our study, MPO level was significantly increased in all treatment groups. Many studies have reported that MPO activity increased in fish fed with various herb extracts. For example, guduchi (*Tinospora cordifolia*), dill, garden cress, nettle, and oyster mushroom increased MPO activity in fish (Alexander et al. 2010; Soner et al. 2016; Soner et al. 2018). MPO level may depend on flavonoids or other active components of herb extract such as LA and NBT.

We have shown that CM05 and CM1 diets resulted decreased survival rate of fish compared to control and CM01 diets. This result suggests that common mallow in oral treatment did not treat *A. hydrophila* infection during the CM01 to CM1 diet. Different treatment methods such as immersion or higher doses of common mellow extract in feed may treat and prevent the

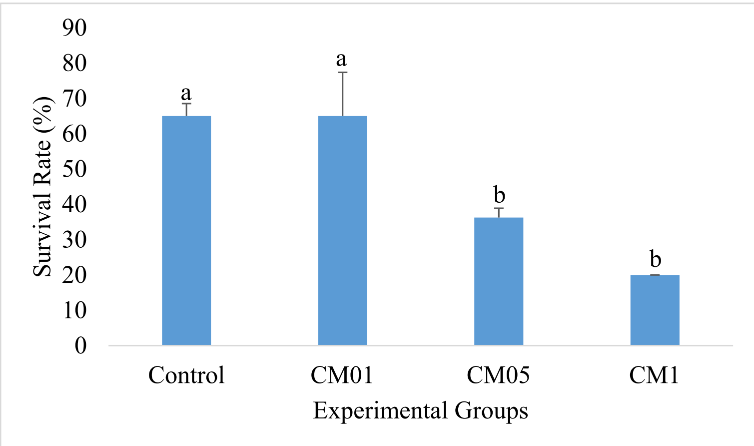

Fig. 4 Survival rate of the experimental groups after challenge with *A. hydrophila* at the end of the study. Data represent the mean ± SEM. Bars assigned with different superscript letters are significantly different ($p < 0.05$). Control: control diet (common mallow 0 g kg^{-1} feed), CM01 (common mallow 0.1 g kg^{-1} feed), CM0.5 (common mallow 0.5 g kg^{-1} feed), and CM1 (common mallow 1 g kg^{-1} feed)

A. hydrophila. Several studies reported that common mallow extract inhibited the growth of colonies of fungus such as *Aspergillus candidus*, *Aspergillus niger*, and *Fusarium culmorum* (Magro et al. 2006; Sleiman and Daher 2009; Gasparetto et al. 2011). Probably, in the future, investigation may be reported that common mallow extract will show antipathogenic activity against fungus species in different fish species.

In conclusion, our results show that common mallow is suitable candidate herb as a support nonspecific immune system, growth promoter in specific dose (CM05), and triggered digestive enzymes activity. However, increased non-specific immune parameter was not finalized with high survival rates against *A. hydrophila*. Further studies are needed to be determined if there are any protection effects of common mallow against different pathogenic bacteria. It is also clear from the study that common mallow dosage is very important for common carp and increased dosage caused high mortality rate against *A. hydrophila*. It is necessary to determine safety dose for fish health. Hence, toxicological and pharmacological studies are needed for drug safety. This and similar studies will help to find the advantages of a new candidate herb species for fish health.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study protocol was approved in advance by the local Ethics Committee for Animal Research Studies at the Kastamonu University (KUHADYEK-17.12.2017-2017.323).

References

Altunoglu YC, Bilen S, Ulu F, Biswas G (2017) Immune responses to methanolic extract of black cumin (*Nigella sativa*) in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 67:103–109

Alexander CP, Kirubakaran JW, Michael RD (2010) Water soluble fraction of *Tinospora cordifolia* leaves enhanced the non-specific immune mechanisms and disease resistance in *Oreochromis mossambicus*. Fish Shellfish Immunol 29:765–772

Amhamed ID, Mohamed GA, Almabrok AA, Altıef TAS, Bilen S (2018) Efficacy of dietary *Chenopodium album* extract on some health parameters, digestive enzymes and growth performance in juvenile *Cyprinus carpio*. Alinteri J Agric Sci 33(2):165–176

Awad E, Austin D, Lyndon AR (2013) Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture 388–391:193–197

Ballero M, Poli F, Sacchetti G, Loi MC (2001) Ethnobotanical research in the territory of Fluminimaggiore (South-Western Sardinia). Fitoterapia 72:788–801

Benso B, Rosalen PL, Alencar SM, Murata RM (2015) *Malva sylvestris* inhibits inflammatory response in oral human cells. An in vitro infection model. PLoS One 19(10):e0140331

Bilen S, Müge Bilen A (2013) Effects of different protein sources on growth performance and food consumption of goldfish, *Carassius auratus*. Iran J Fish Sci 12(3):717–722

Bilen S, Bulut M, Bilen AM (2011) Immunostimulant effects of *Cotinus coggyria* on rainbow trout (Oncorhynchus mykiss). Fish Shellfish Immunol. 30(2):451–455

Bilen S, Ünal S, Güvensoy H (2016) Effects of oyster mushroom (*Pleurotus ostreatus*) and nettle (*Urtica dioica*) methanolic extracts on immune responses and resistance to *Aeromonas hydrophila* in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 454:90–94

Bilen S, Özkan O, Alagöz K, Özdemir KY (2018) Effect of dill (*Anethum graveolens*) and garden cress (*Lepidium sativum*) dietary supplementation on growth performance, digestive enzyme activities and immune responses of juvenile common carp (*Cyprinus carpio*). Aquaculture 495:611–616

Bostock J, McAndrew B, Richards R, Jauncey K, Telfer T, Lorenzen K, Little D, Ross L, Handisyde N, Gatward I, Corner R (2010) Aquaculture: global status and tends. Phil Trans R Soc B 365:2897–2912

Dalmo RA, Ingebrightsen K, Bogwald J (1997) Nonspecific defense mechanisms in fish, with particular reference to the reticuloendothelial system (RES). J Fish Dis 20:241–273

Delfine S, Marrelli M, Conforti F, Formisano C, Rigano D, Menichini F, Senatore F (2017) Variation of *Malva sylvestris* essential oil yield, chemical composition and biological activity in response to different environments across southern Italy. Ind Crop Prod 98:29–37

de Souza EM, de Souza RC, Melo JFB, da Costa MM, de Souza AM, Copatti CE (2019) Evaluation of the effects of *Ocimum basilicum* essential oil in Nile tilapia diet: growth, biochemical, intestinal enzymes, haematology, lysozyme and antimicrobial challenges. Aquaculture 504:7–12

FAO (2016) Fisheries and Aquaculture Department, The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome

Furne M, Hidalgo MC, Lopez A, Garcia-Gallego M, Morales AE, Domezain A, Domezaine J, Sanz A (2005) Digestive enzyme activities in Adriatic sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. Aquaculture 250(1):391–398

Gasparetto JC, Martins CA, Hayashi SS, Otuky MF, Pontarolo R (2012) Ethnobotanical and scientific aspects of *Malva sylvestris* L.: a millennial herbal medicine. J. Pharm. Pharmacol. 64(2):172–189

Genovese S, Epifano F, Fiorito S, Taddeo VA, Preziuso F, Fraternale D (2018) Modulation of the phenylpropanoid geranylation step in *Anethum graveolens* cultured calli by ferulic acid and umbelliferone. Ind Crop Prod 117:128–130

Grattendick K, Stuart R, Roberts E, Lincoln J, Lefkowitz SS, Bollen A (2002) Alveolar macrophage activation by myeloperoxidase: a model for exacerbation of lung inflammation. Am J Respir Cell Mol Biol 26:716–722

Johnston JR (1978) Oxygen metabolism and the microbicidal activity of macrophages. Federation Proceeding: 2759-2764

Krogdahl A, Hemre GI, Mommsen TP (2005) Carbohydrates in fish nutrition: digestion and absorption in postlarval stage. Aquac Nutr 11(2):103–122

Lau D, Mollnau H, Eiserich JP, Freeman BA, Daiber A, Gehling UM (2005) Myeloperoxidase mediates neutrophil activation by association with CD11b/CD18 integrins. Proc Natl Acad Sci 102:431–436

Magro A, Carolino M, Bastos M, Mexia A (2006) Efficacy of plant extracts against stored products fungi. Rev Iberoam Micol 23:176–178

Munir MB, Hashim R, Nor SAM, Marsh TL (2018) Effect of dietary prebiotics and probiotics on snakehead (*Channa striata*) health: haematology and disease resistance parameters against *Aeromonas hydrophila*. Fish Shellfish Immunol. 75:99–108

Naylor LR, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000) Effect of aquaculture on world fish supplies. Nature 405:1017–1024

Palic D, Andreasen CB, Menzel BW, Roth JA (2005) A rapid direct assay to measure degranulation of primary granules in neutrophils from kidney of fathead minnow (Pimephales promelas Rafinesque, 1820). Fish Shellfish Immunol. 19:217–227

Prudente AS, Loddi AMV, Duarte MR, Santos ARS, Pochapski MT, Pizzolatti MG, Hayashi SS, Campos FR, Pontarolo R, Santos FA (2013) Pre-clinical anti-inflammatory aspects of a cuisine and medicinal millennial herb: *Malva sylvestris* L. Food Chem Toxicol 58:324–331

Prudente AS, Sponchiado G, Mendes DAGB, Soley BS, Cabrini DA, Otuki MF (2017) Pre-clinical efficacy assessment of *Malva sylvestris* on chronic skin inflammation. Biomed Pharmacother 93:852–860

Reverter M, Bontemps N, Lecchini D, Banaigs B, Sasal P (2014) Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture 433:50–61

Robak J, Gryglewski RJ (1988) Flavonoids are scavengers of superoxide anions. Biochem Pharmacol 37(5):837–841

Ricker WE (1979) Growth rates and models. In: Hoar WS, Randall DJ, Brett JR (eds) Fish physiology. Academic Press, New York, pp 677–743

Saad AB, Rjeibi I, Alimi H, Ncib S, Smida A, Zouari N, Zourgui L (2017) Lithium induced, oxidative stress and related damages in testes and heart in male rats: the protective effects of *Malva sylvestris* extract. Biomed Pharmacother 86:127–135

Schulz H, Schrader B, Quilitzsch R, Pfeffer S, Kruger H (2003) Rapid classification of basil chemotypes by various vibrational spectroscopy methods. J Agric Food Chem 51:2475–2481

Shoemaker C, Xu D-H, LaFrentz B, LaPatra S (2015) Overview of fish immune system and infectious diseases. In: Lee C-S, Lim C, Webster CD (eds) Dietary nutrients, additives, and fish health. Wiley Blackwell, NJ, pp 1–24

Sleiman NH, Daher CF (2009) *Malva sylvestris* water extract: a potential anti-inflammatory and anti-ulcerogenic remedy. Planta Med 75:1010–1010

Tubesha Z, Iqbal S, Ismail M (2011) Effects of hydrolysis conditions on recovery of antioxidants from methanolic extracts of *Nigella sativa* seeds. J Med Plant Res 5(22):5393–5399

Wang G, Wang J, Du L, Li F (2015) Effect and mechanism of total flavonoids extracted from *Cotinus coggygria* against glioblastoma cancer *in vitro* and *in vivo*. Biomed Res Int: 856349

Xie J, Liu B, Zhou QL, Su YT, He YJ, Pan LK, Ge XP, Xu P (2008) Effects of anthraquinone extract from rhubarb *Rheum officinale* bail on the crowding stress response and growth of common carp *Cyprinus carpio* var. *Jian* Aquaculture 281:5–11

Yang R, Yu L, Zeng H, Liang R, Chen X, Qu L (2012) The interaction of flavonoid-lysozyme and the relationship between molecular structure of flavonoids and their binding activity to lysozyme. J Fluoresc 22:1449–1459

Yarijani ZM, Najafi H, Shackebaei D, Madami SH, Modarresi M, Jassemi SV (2019) Amelioration of renal and hepatic function, oxidative stress, inflammation and histopathologic damages by *Malva sylvestris* extract in gentamicin induced renal toxicity. Biomed Pharmacother 112:108635

Zoral MA, Futami K, Endo M, Maita M, Katagiri T (2017) Anthelmintic activity of Rosmarinus officinalis against *Dactylogyrus minutus* (Monogenea) infections in *Cyprinus carpio*. Vet Parasitol 247:1–6

Zoral MA, Ishikawa Y, Ohshima T, Futami K, Endo M, Maita M, Katagiri T (2018) Toxicological effects and pharmacokinetics of rosemary (*Rosmarinus officinalis*) extract in common carp (*Cyprinus carpio*). Aquaculture 495:955–960

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Affiliations

Soner Bilen[1] • Abdelsalam M. O. Filogh[2] • Abobaker Barka Ali[2] • Osman Nezih Kenanoğlu[1] • Mehmet Arif Zoral[2]

[1] Faculty of Fisheries, Department of Aquaculture, Kastamonu University, Kastamonu, Turkey

[2] Institute of Science, Department of Aquaculture, Kastamonu University, Kastamonu, Turkey