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EFFECTS OF DIETARY SUPPLEMENTATION OF PEPPERMINT EXTRACT ON GROWTH PERFORMANCE, INTESTINAL MICROBIOTA, LIVER AND INTESTINE HISTOPATHOLOGY OF *CYPRINUS CARPIO*

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Abstract

This study aimed to evaluate the effects of *Mentha piperita* methanolic extract (MPE) on *Cyprinus carpio* intestinal microbiota, including total microorganisms gram-negative bacteria, lactic acid bacteria, and fungi count. Liver and intestinal histopathology, and the activity of liver enzymes, were also used to evaluate the possible side effects of MPE. A total of 96 healthy *C. carpio* fries (76.76±20.26 g) were allocated to four treatment groups with three replications in a completely randomized design. The fries were fed with diets containing 0, 0.5, 1, and 2% extract for 56 days at the rate of 2% of body weight during the experiment. Results showed a significant decrease in total microorganisms, enteric gram-negative bacteria, and total fungi counts ($P<0.05$). The total lactic acid bacteria count in 0.5% treatment was significantly lower than in control and 2% treated fish ($P<0.05$). MPE did not affect AST, leading to a significant increase in ALT levels. Simultaneously, ALP represented significantly higher activity in the control group ($P>0.05$). Microscopic findings revealed marked lesions, including congestion and cell degeneration in the livers of the three groups of fish fed with the extract. The intestinal folds were shortened and blunted in the treatment groups. Furthermore, the intestinal mucosa was necrotic, and the lamina propria was significantly thickened with mononuclear inflammatory cells ($P<0.05$). Although MPE significantly affects intestinal microbiota, its consumption at 2% is not recommended for *C. carpio* due to the lesions made in the liver and intestine.

Key words: carp, mentha, intestinal microbiota, liver, *Cyprinus carpio*

Industrial aquaculture is in pressing need of developing modern and environmentally friendly techniques to manage high-density culture systems and antibiotics acquisitions (Vijayaram et al., 2022). Thyme, savory, Persian shallot, oak acorn, common mallow, garlic, and pomegranate are all examples of safe herbal additives that have been studied for their potential benefits on fish health and welfare (Yousefi et al., 2022), resistance to diseases and environmental stressors (Ghafarifarsani et al., 2021 b, 2022 a; Raissy et al., 2022), environmental and intestinal microorganisms (Ahmadniaye Motlagh et al., 2019), immune system and antioxidant defense system (Ghafarifarsani et al., 2021 a). Intestinal microbial manipulation can improve aquaculture's growth, immunity, and reproduction performance. Besides probiotics, prebiotics, synbiotics, and antibiotics, organic salts and herbal additives are promising candidates for aquatics gut microbial manipulation. In recent years, the application of herbal additives in aquaculture has risen due to their affordability and lack of serious side effects on humans and the environment (Ahmadniaye Motlagh et al., 2020).

Peppermint, scientifically known as *Mentha piperita*, is a perennial herbaceous plant belonging to the genus *Mentha* (Lamiaceae), native to the Mediterranean region. The plant *M. piperita* is currently cultivated worldwide for food, medicine, and perfumery purposes (Mahendran and Rahman, 2020). Peppermint shoots contain essential oils, phenolic compounds, flavonoids, fatty acids, vitamins, minerals, and salicylic acid (Malekmohammad et al., 2021; Rita and Animesh, 2011). Monoterpenes are the main constituents of peppermint essential oil, the most important of which are menthol (30–55%), menthyl acetate (2 to 8–10%), and menton (32–34%). Other components are limonene (1–5%), isomentone (1.5–10%) and caron (maximum 1%). Menthol is known to be responsible for many of the peppermint's biological functions and its aroma. Several documented properties are attributed to peppermint, such as being antibacterial (Singh et al., 2015), antifungal (Hu et al., 2019), antiparasitic (Castro et al., 2013), antiviral (Loolaie et al., 2017), anti-inflammatory and wound healing (Loolaie et al., 2017), antioxidant, immune stimulant (de Souza Silva et al., 2019), triglyceride- and cholesterol-lowering (Li et

al., 2017), appetite activating (Talpur, 2014) and anesthetic (de Oliveira Hashimoto et al., 2016). There have been many reports of the herb's beneficial effects, and the methanolic isomers have not been reported to have any significant innate mutagenic, genotoxic, or embryotoxic properties. Nonetheless, some researchers have brought up the herb's relative toxicity, which should be taken into account (Malekmohammad et al., 2021).

Compared to other medicinal plants such as garlic (extensively studied in aquaculture), the utilization of peppermint in aquaculture has received less attention from researchers. Talpur (2014) was the first to study the dietary effects of peppermint on growth, survival, and immune response of Asian seabass, *Lates calcarifer*. The author also included fish resistance against *Vibrio harveyi* infection in their study. The experiment results showed that diets containing peppermint could significantly reduce mortality from *V. harveyi* bacteria and improve immune and growth parameters. Peppermint was mentioned to effectively reduce metabolic factors such as total lipids, triglycerides, and cholesterol content. In similar works, the positive effect of peppermint ethanolic extract on fish growth and immunity was demonstrated for Caspian white fish (*Rutilus frisii kutum*) (Adel et al., 2015 a), rainbow trout (*Oncorhynchus mykiss*) (Adel et al., 2016) and Caspian brown trout (*Salmo trutta caspius*) (Adel et al., 2015 b).

The studies conducted regarding the antimicrobial activity of peppermint in aquatic animals can be divided into two categories. The first category deals with the *in vitro* peppermint antimicrobial effects (Saharkhiz et al., 2012; Singh et al., 2015). In contrast, the second group concerns the *in vivo* condition to control specific infectious agents such as *Streptococcus agalactiae* (de Souza Silva et al., 2019), *Piscinoodinium pillulare* (de Oliveira Hashimoto et al., 2016) and *Vibrio harveyi* (Adel et al., 2016; Talpur, 2014). To the best of our knowledge, the experimental work presented here provides one of the first investigations into how peppermint extract might influence the intestinal microbiota of aquatic animals. There is only one published study regarding how intestinal microbials of *Carassius auratus* respond to pomegranate peel extract application (Ahmadniaye Motlagh et al., 2020). According to their results, treatments with pomegranate peel extract effectively reduced the gram-negative bacteria in favor of lactic acid bacteria (as beneficial bacteria). Another relevant case reported that subjecting broilers to a diet containing *Aloe vera* and peppermint could reduce *Escherichia coli* and increase lactic acid bacteria load (Darabighane et al., 2017).

The aim of this research was to evaluate the effects of *M. piperita* extract (MPE) on intestinal microorganisms (total count of microorganisms, enteric gram-negative bacteria count, total lactic acid bacteria count, as well as total fungal count) of common carp (*Cyprinus carpio*) as one of the most cultivated fishes in the world. We also explored the probable side effects on cholesterol level, liver and intestinal histopathology, and liver enzymes including alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST).

Material and methods

Preparation of methanolic extract of peppermint

Maceration and methanol solvent (80%) were used to obtain the extract from the dried leaves of *M. piperita*. After washing, drying, and grinding the peppermint leaves, 50 g of dried material was mixed with 300 ml of methanol in an Erlenmeyer flask, and shaken at 25°C for 24 hours. After extraction, the solution was passed through Whatman No. 41 filter paper, and the solvent was removed by using a rotary evaporator. The extract was concentrated and dried at 70°C in Ben Mari (Hossain et al., 2014).

Experimental design and experimental diets

A total of 96 healthy *Cyprinus carpio* (76.76±10.26 g) were purchased from a local carp hatchery and transported to the laboratory. After disinfection with formalin (5 ppm), the fish were subjected to an acclimatization period of 14 days, and then randomly distributed among 12 glass aquariums (120 L) at a density of 8 fish per tank. The experiment was carried out in the form of a completely randomized design (triplicated). Commercial fish feed (Beyza feed® Iran) was used during the 56 days of the experimental period. The nutrient compositions of feed were as follows (in percentages): crude protein 30.1±2.0, crude lipid 12.2±1.7, ash 2.72±0.59, crude fiber 5.5±0.95, ash 9±0.5. Total volatile nitrogen (TVN) and gross energy were 40 mg 100 g^{-1} and 3500±100 kcal/kg, respectively. To prepare the experimental diets, peppermint extract was sprayed on the basal diet at 0% (control), 0.5%, 1%, and 2% levels according to the literature (Adel et al., 2015 b). The sunflower oil (5 ml L^{-1}) was sprayed on the prepared feed to prevent the extract from oxidizing and leaching into the water. The prepared diets were air-dried and stored at 4°C until use. Feeding was performed at the rate of 2% of body weight, and all experiments were done according to Ferdowsi University of Mashhad animal ethics. Physicochemical parameters of rearing water such as temperature (25±2.7°C), pH (7.5±0.29), dissolved oxygen (7.12±0.33 mg/L) and total hardness (220.68±42.9 mg/L) were measured during the experiment period.

Growth performance

At the end of the trial, feeding fries was halted 24 h before sampling. To evaluate the effect of MPE on growth indices, all the fish were anesthetized using clove powder (0.50 g L^{-1}) and weighed with a digital scale (0.01 g). The growth rate was calculated as weight gain (g) = final weight − initial weight.

Intestinal microbial analyses

As for the microbial analyses, the last one centimeter of the intestine was removed and immediately immersed

in one ml of sterile ringer solution. Afterward, the microtubules containing the sample and the Ringer solution were stirred until a homogeneous mixture was obtained. One ml of the resulting mixture was added to nine ml of sterile Ringer solution, and other dilutions required for bacterial and fungal cultures were prepared from this solution. Microbial tests in this study included the total microorganism count, enteric gram-negative bacteria count, total bacterial lactic acid count, as well as total fungal count.

Total microorganisms count was identified by pour plate culturing of one ml of each dilution with 10–12 ml plate count agar medium and incubation at 30°C for 48 h (ISO 4833-1: 2013, 2013), enteric gram-negative bacteria count by pour plate culturing of 1 ml of each dilution with 10–12 ml MacConkey agar medium and incubation at 37°C for 24 h (ISO 21528-2: 2017, 2017), bacterial lactic acid count by pour plate culturing of 1 ml of each dilution with 10–12 ml MRSA agar medium and incubation at 30°C for 72 h (ISO, 1998), and total fungal count by surface plate culturing of 1 ml of each dilution on YGC agar medium and incubation at 25°C for five days (ISO 21527-2, 2008). Once incubation terminated plates containing 30 to 300 colonies were selected for counting.

Histopathological examination of the liver and intestine

The livers and intestines (posterior part) of *C. carpio* were removed and fixed in 10% neutral buffered formalin. The tissue specimens were routinely processed, dehydrated with ethanol at different concentrations, cleared in xylene, and embedded in paraffin. The paraffin-embedded specimens were sectioned at a thickness of 5 µm. Finally, the sections were stained with hematoxylin and eosin (H & E) and studied for any histopathological lesion under a light microscope (Olympus, Japan). Furthermore, a semi-quantitative scoring system proposed by Urán et al. (2008) was used for the evaluation of intestinal lesions of the fish. Based on lesion severity, scores ranging from one (normal) to five (distinct pathological condition) were assigned to each criterion including characteristics of the lamina propria, infiltration of inflammatory cells into the lamina propria and sub-epithelial mucosa as well as abundance of goblet cells (Table 1).

Cholesterol level and liver enzymes activity

In order to measure the liver enzymes activity, three fish per aquarium were randomly selected and blood samples were taken using a sterile heparinized syringe from the caudal venous. Blood was discharged into 2-ml plastic tubes and centrifuged at 3000 rpm for five min. The plasma was removed and kept at –70°C until use. Plasma cholesterol, ALT, AST and ALP were measured using Pars Azmun commercial kits (Tehran, Iran) according to the protocol provided by the producer. All the analyses were done by auto-analyzer Selectra E (Vital Scientific, Dieren, The Netherlands).

Statistical analysis

After determination of normality of data using Kolmogorov-Smirnov test and homogeneity of variance using Levene's test, the data were analyzed using one-way ANOVA. Tukey's test was used to compare means ($P<0.05$).

Results

Figure 1 illustrates the effect of MPE on *C. carpio* gut microbiota. The groups receiving the extract, showed a significant decrease in total microorganisms, enteric gram-negative bacteria and total fungi counts ($P<0.05$). The results also indicated that the total lactic acid bacteria count in 0.5% treatments was significantly lower than that of the control and 2% treated fish ($P<0.05$). Total fungi count exhibited a sharp decline in treatments with MPE ($P<0.05$).

The administration of MPE in the diet showed no significant effect on the final weight and weight gain (Table 2). Likewise, treatment of *C. carpio* fries with peppermint methanolic extract had no effect on AST, while it led to a significant increase in ALT. ALP represented significantly higher activity in the control group ($P>0.05$). No significant differences were observed in terms of plasma cholesterol content.

Table 1. Semi-quantitative scoring system used for the evaluation of intestinal lesions of *C. carpio* fed with *Mentha piperita* extract supplemented diets for 56 days

Criterion	Description
Lamina propria	Normal size LP (score 1), increased size of LP (score 2), medium size LP (score 3), large LP (score 4), largest LP (score 5).
Goblet cells	Scattered cells (score 1), increased number and sparsely distributed (score 2), diffused number widely spread (score 3), densely grouped cells (score 4), and highly abundant and tightly-packed cells (score 5).
Inflammatory cells	Few in SM basal with small quantity (score 1), increased number in SM and some migration into LP (score 2), increased migration into LP (score 3), diffused number in LP and SM (score 4), dense IC in LP and SM (score 5).

LP: lamina propria; SM: sub-epithelial mucosa; IC: inflammatory cells.

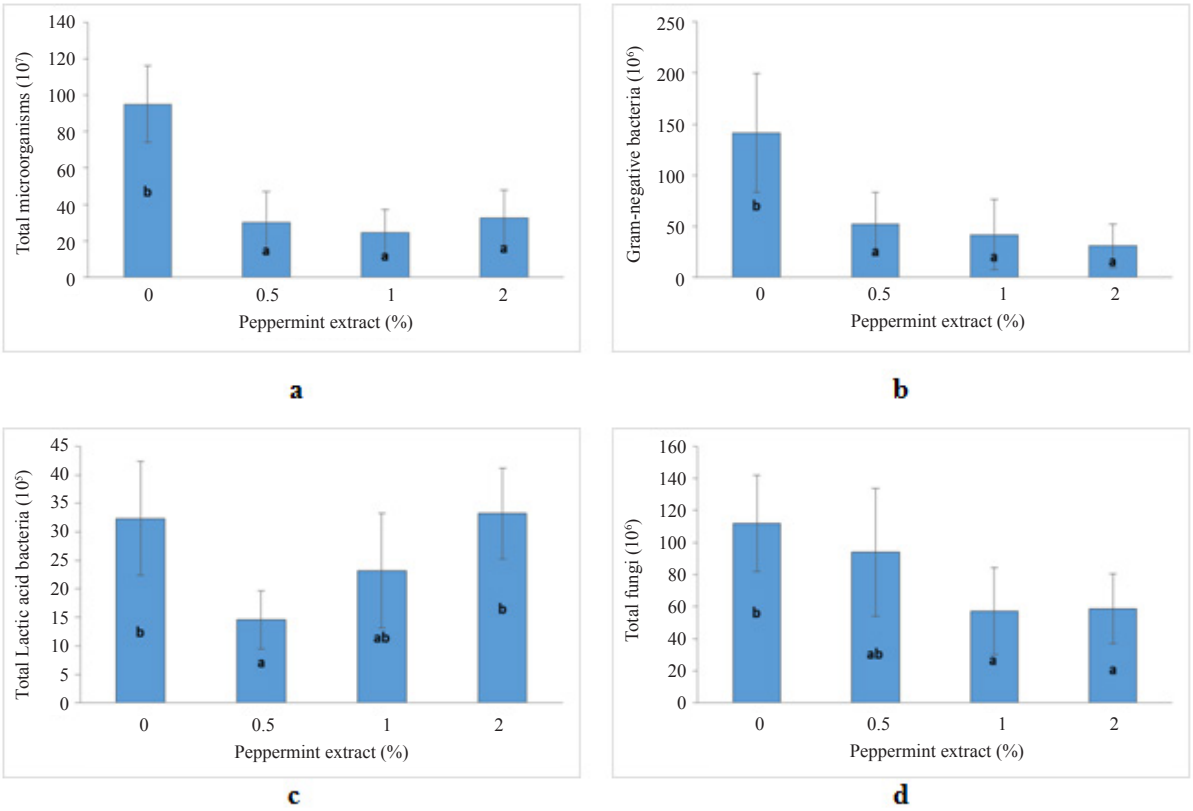

Figure 1. Total microorganisms (10^7) (a), enteric gram-negative (10^6) (b), total lactic acid bacteria (10^5) (c), and total fungi (10^5) (d), in the gut of *C. carpio* fed *M. piperita* extract-supplemented diets for 56 days (mean ± SD, n = 3)

Table 2. Growth and blood biochemical parameters of *C. carpio* fed with *Mentha piperita* extract supplemented diets for 56 days (Mean ±SD, n=3)

	Dietary *M. piperita* extract levels (%)			
	0.00	0.50	1.00	2.00
Initial weight	76.38±10.87	77.13±11.08	76.83±9.38	76.71±10.17
Final weight	88.78±15.15	93.12±16.37	90.14±14.47	93.50±17.47
Weight gain	12.4±2.28	16.00±4.57	13.31±3.10	16.80±7.30
AST (U/L)	194.16±89.22	244.79±80.94	271.76±96.45	220.57±82.04
ALP (U/L)	334.92±158.17 b	207.31±82.61a	155.12±87.00 a	159.15±82.89 a
ALT (U/L)	9.04±3.82 a	14.00±6.63 b	14.39±6.11 b	15.15±5.01 b
CHL (mg/dL)	186.03±35.22	163.51±40.78	174.20±53051	181.84±34.73

Means with different letters in the same row are significantly different (ANOVA, $P<0.05$). AST: aspartate aminotransferase; ALP: alkaline phosphatase; ALT; alanine aminotransferase; CHL: cholesterol.

Microscopical findings

In addition to vacuolar degeneration and fatty change, the livers of the fish fed with the extract in all the three groups showed marked lesions, including congestion and cell degeneration compared with the control group. Interestingly, there was an apparent increase in the number of the bile and pancreatic ducts (Figure 2 a). Additionally, prominent melanomacrophage centers located around the blood vessels and hepatopancreas hemopoietic tissue were observed particularly in the livers of the fish receiving 1 and 2% MPE (Figure 2 b). The majority of the pigments within macrophages were light in color with a minimal amount of pigments in macrophages.

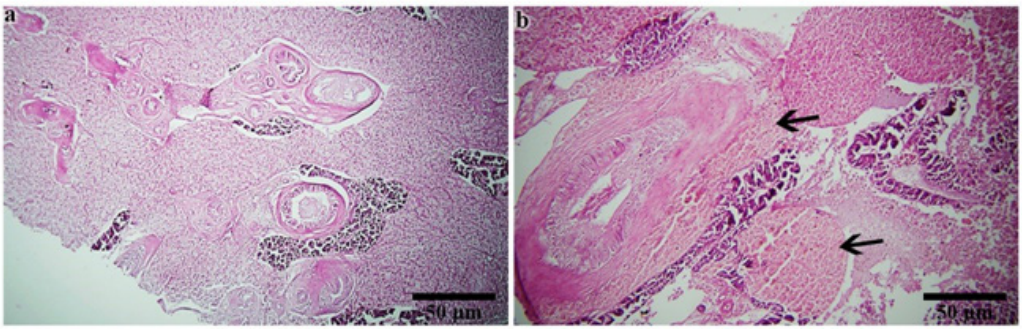

Figure 2. Prominent bile and pancreatic ducts (a), and extended melanomacrophage centers around the hepatopancreas hemopoietic tissue containing a bright pigment (arrows, b) are observed in the periportal area of the livers in the fish treated with the peppermint extract (H & E staining; scale bars = 50 µm)

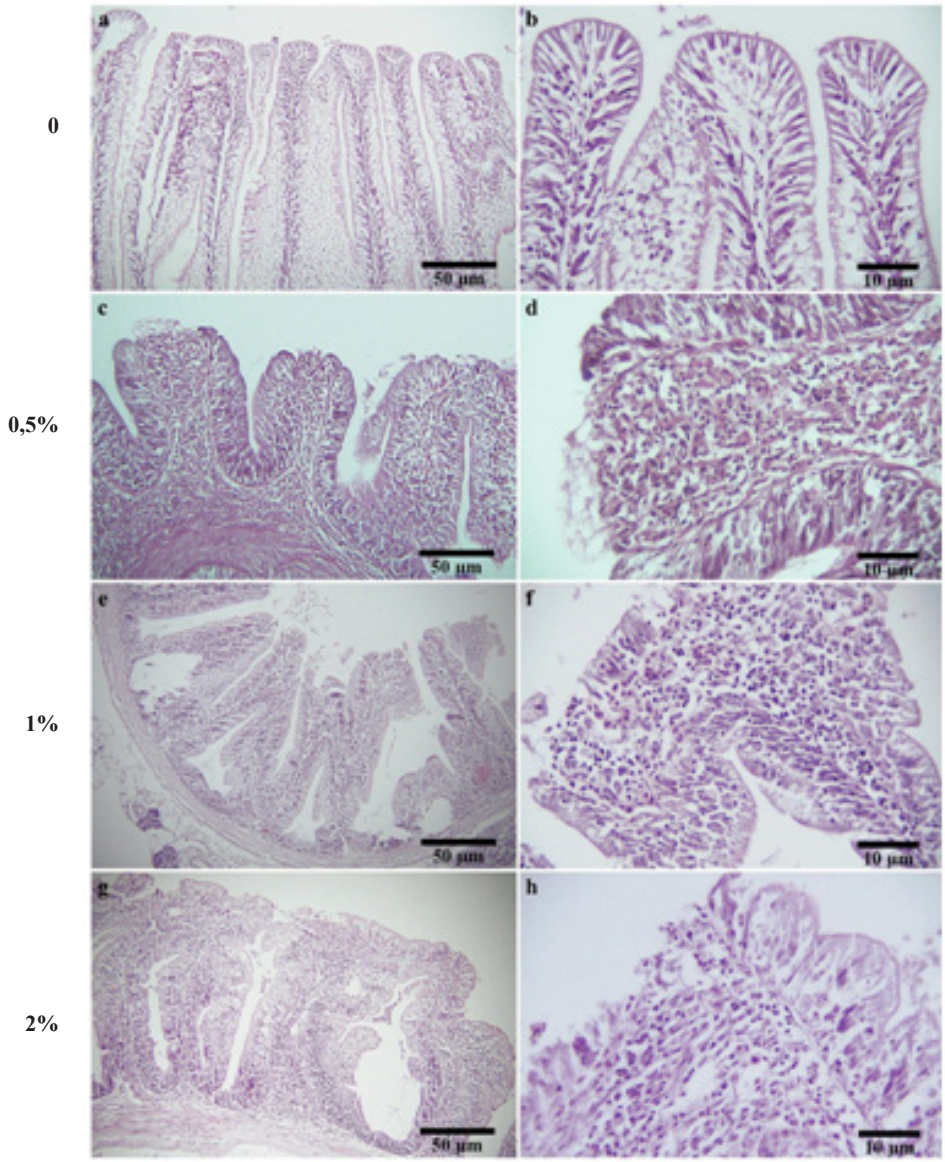

Figure 3. Microscopic morphology of the intestine in common carp (*C. carpio*) after feeding control diet (a and b) and a diet containing 0.5% (c and d), 1% (e and f), and 2% (g and h) peppermint extract (H & E staining, scale bars = 50 µm and 10 µm for the first and second column, respectively). Note the narrow and high intestinal folds with a normal simple columnar epithelium containing goblet cells. Infiltration of inflammatory cells is seen neither in the sub-epithelial mucosa nor in the lamina propria. A thin lamina propria is also seen in the fish fed the control diet (0 extract) (a and b). Sloughing of intestinal mucosa and short and blunt intestinal folds of common carp (*C. carpio*) affected by ingestion of the peppermint extract are clearly evident (c–h). There is heavy infiltration of mononuclear inflammatory cells such as lymphocytes and macrophages in the sub-epithelial mucosa and lamina propria. Additionally, the blood vessels in the lamina propria are congested (c–h). H & E staining, scale bars = 50 and 10 µm

Table 3. Semi-quantitative findings obtained from the histopathological evaluation of the intestine in *C. carpio* fed with *Mentha piperita* extract supplemented diets for 56 days

Criterion	Median (min–max)				P[a]
	0	0.5 %	1 %	2 %	
LP	1 (1–2)[b]	3 (2–4)	3 (2–5)	2 (2–3)	0.003
GC	2 (2–3)	3 (2–4)	2 (2–4)	2 (2–4)	0.056
IC	1 (1–2)[c]	3 (2–4)	3 (2–3)	2 (1–3)	0.003

Lamina propria (LP); goblet cells (GC); inflammatory cells (IC).
[a]Kruskal–Wallis non-parametric ANOVA.
[b]P<0.05 (compared with the 0.5%, 1%, and 2% groups by Mann–Whitney U test).
[c]P<0.05 (compared with the 0.5%, 1%, and 2% groups by Mann–Whitney U test).

Normal histological structure of the intestine characterized by simple and compound folds lined by high simple columnar epithelial cells and vacuolated goblet cells was observed in the control group. The folds were normal in length and shape, and a thin lamina propria with no inflammatory cells was observed (Figure 3 a and b). Conversely, in the treatment groups, the folds were shortened and blunted with necrotic and sloughed intestinal mucosa in some mucosal folds (MFs) (Figure 3 c–h). In addition to the histological changes in the morphology of the MFs, the lamina propria was significantly thickened by infiltration of a large number of inflammatory cells (P<0.05). The microscopic scores related to enteritis degree of the fish are presented in Table 3. Unlike the control group, mononuclear inflammatory cells, including lymphocytes, plasma cells and macrophages infiltrated into the sub-epithelial mucosa and invaded the lamina propria (P<0.05) and resulted in the expansion of the MFs in the treated fish. Nonetheless, the differences in the examined features were insignificant between the treatment groups (P>0.05). Congestion and hemorrhage were also evident. In a few sections of the intestine of the treatment groups, a catarrhal exudate was observed probably as a result of goblet cell hyperplasia.

Discussion

There is ample evidence that careful manipulation of the intestinal microbial composition of aquatic animals could lead to positive effects on fish growth, health and immunity. The results of this study showed that diets enriched with peppermint extract could reduce total microorganisms, gram-negative bacteria (as pathogenic bacteria) as well as total fungi count. Interestingly, the lowest peppermint extract level (0.5%), not the 2% treatment, caused the significantly largest reduction in lactic acid bacteria count (a representative of beneficial bacteria). Evidently, for the gram-negative bacteria and fungi, the 2% treatment had the most significant effect, while this treatment failed to reduce the lactic acid bacteria count. These findings have significant implications for achieving a beneficial and stable microbial composition in the intestine of aquatic animals. The study on mammalian models showed that peppermint application can often result in improving the normal function of the gastrointestinal tract (muscular action, secretory and transport processes of the enterocytes) and eliminating digestive problems such as indigestion, cramps, flatulence, etc. These benefits are partly attributed to improved reproduction of beneficial bacteria, secretion of digestive enzymes and hence better food digestion (McKay and Blumberg, 2006). In addition to antibacterial and fungal activities, it has been reported that peppermint essential oil has antiparasitic properties (Desam et al., 2019; Mahendran and Rahman, 2020; Malekmohammad et al., 2021).

The antibacterial effect of peppermint extract and its essential oils on gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) has been investigated by Singh et al. (2015). The antimicrobial activity of the essential oils was comparable to that of gentamicin, a well-known broad-spectrum antibiotic. Their results also indicated stronger antibacterial activities in petroleum ether, chloroform and ethyl acetate extracts compared with aqueous and ethanolic extracts. They finally concluded that the antibacterial activity of the peppermint extract was directly proportional to the concentration of the bioactive ingredients. For example, higher concentration of menthol is obtained via petroleum ether and chloroform extraction methods and the same applies to phenol or flavonoids in chloroform and ethyl acetate extraction methods (Singh et al., 2015). In a similar study examining the effect of pomegranate peel extract on the intestinal bacteria of *Carassius auratus*, it was found that the extract holds an outstanding potential to reduce gram-negative bacteria which corroborates our findings (Ahmadniaye Motlagh et al., 2020).

It is still unclear how the compounds obtained from peppermint perform their antimicrobial function. However, researchers believe that the antibacterial and antifungal properties of chemical compounds could be attributed to the production of free radicals such as reactive oxygen species (ROS) (Ghafarifarsani et al., 2021 a, 2022 b; Raissy et al., 2022). It has also been suggested that menthol in the extract can disturb the cell balance and facilitate cellular cytoplasm discharge by disturbing the lipid particles in the cell wall of fungi (Desam et al., 2019; Sharifzadeh et al., 2017).

There is a vast variety of reported adverse reactions to medicinal herbs. These toxic properties may occur by various mechanisms, including direct toxicity, contamination, and drug complications. Menthol, polgon, menthone, and menthofuran have been identified as the four most prominent potentially hazardous chemicals in MPE (Malekmohammad et al., 2021). The effects of peppermint extract on vital organs such as the liver, intestine and nervous system are well established (Ibrahim et al., 2000). For example, rats receiving 40 to 100 mg/kg peppermint oil between 28 and 90 days, showed nephropathy and cyst-like lesions in the cerebellum. Ghaly et al.

(2017) found that supplementation of broiler diets with *M. piperita* alone and in combination with *Nigella sativa* for 6 weeks caused renal and hepatic lesions in the microscopic examination including nephritis and vacuolar degeneration, respectively. Nonetheless, they did not observe significant alteration in the ALP, AST, and ALT activities.

Menthol glucuronide is the major biliary metabolite of menthol in the liver, and it is discharged in the urine and feces (Malekmohammad et al., 2021). It follows that the liver is one of the first organs to be at risk because it is involved in the detoxification of numerous plant metabolites. In line with these research works, our results illustrated that the treatment of carps with MPE caused some histopathological lesions such as congestion and cell degeneration in the liver tissues. Microscopic examination of the intestine showed the damage to the epithelium and villi, shortened mucosal folds as well as an increase in inflammatory cells infiltrated into the lamina propria like lymphocytes and macrophages. Akdogan et al. (2003) showed that *M. piperita* induced some histopathological lesions in the kidney tissues of rats including hydropic degeneration, necrosis of tubular epithelial cells, tubular dilatation, and enlarged Bowman capsules. Additionally, they demonstrated nephrotoxicity of *M. spicata*, another species of peppermint, so that it caused focal mononuclear nephritis in addition to hydropic degeneration, atrophy of tubules and glomerules as well as cellular degeneration and necrosis. They suggested that increased amounts of superoxide radicals, ROS, produced during the peppermint metabolism could inflict lipid peroxidation and widespread tissue destruction (Akdogan et al., 2003). Likewise, one probable explanation for diminished bacterial counts and the intestinal damage triggering immune/inflammatory response in the fish fed with different concentrations of MPE in this study could be the production of superoxide free radicals. The ROS could injure the bacterial and fungal cell walls and also the normal cells and cause cell death or necrosis. Moreover, the livers of these fish showed fatty change, and melanomacrophage centers were more prominent compared to the fish that did not receive MPE, probably due to the same mechanism.

ALP, AST, and ALT are non-functional enzymes, mainly found in the liver and kidney (Yousefi et al., 2020). Increased circulating levels of these enzymes is a sign of tissue damage. Based on the biochemical analysis of plasma, the plasma level of AST did not change, whereas ALT, and ALP activities increased and decreased, respectively, in response to peppermint extract application. Rainbow trout (Adel et al., 2015 b), Caspian salmon (Adel et al., 2016) and broilers (Khodadust et al., 2015) also showed no changes in liver enzymes in response to peppermint (1, 2, and 3%). The increase in ALT activity may be related to the liver tissue damage that was confirmed by histopathological examination. However, the decrease in ALP activity in this study is not yet well understood and additional studies are required. Circulating fat levels can be considered a health indicator (Hoseini et al., 2018). Although the administration of peppermint in the diet of rohu (*Labeo rohita*) (Padala et al., 2021) and Asian bass (*Lates calcarifer*) (Talpur, 2014) reduced cholesterol, the results of the current experiment did not show cholesterol-lowering effects for peppermint. The same was true for *Salmo trutta caspius* and rainbow trout (Adel et al., 2015 b). Perhaps the different physiological responses of the different species to herbal medicines can be attributed to these contradictory results (Adel et al., 2016).

While in previous similar studies, peppermint in *L. calcarifer*, *Rutilus frisii kutum,* and *L. rohita* increased growth rate (Adel et al., 2015 a; Padala et al., 2021; Talpur, 2014), we did not notice any significant increase in the final weight after 56 days of feeding with MPE administered diets, despite improvement in the microbial composition of the intestine. It is probably because of the damage to the intestine epithelium and villi interfering with the absorption. In addition, accumulation of fat vacuoles within hepatocytes might affect adversely growth. Similarly, Roslan et al. (2016) showed that dietary peppermint oil caused some histopathological lesions in the hepatopancreas of crabs and it had no positive effect on the crab growth. Likewise, the addition of menthol, the most important constituent of *M. piperita*, to the carp diet did not increase growth in another experiment (Hoseini et al., 2019).

Conclusion

In this study we have attempted to establish a new concept of herbiotics in aquaculture. Herbiotics can be defined as herbal additives which can favorably influence host intestinal microbiota balance without adversely impacting other organs. The results revealed that peppermint extract improves the microbial composition of *C. carpio* intestine, which provides a reasonable foundation for further researches. Caution must be exercised as to how the introduced treatments could damage the liver and intestinal tissues.

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Ethical approval

All experiments were done according to Ferdowsi University of Mashhad animal ethics.

Consent for publication

All authors review and approve the manuscript for publication.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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