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Improvement of non-specific immunity, growth, and activity of digestive enzymes in *Carassius auratus* as a result of apple cider vinegar administration to diet

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Abstract This study was conducted to evaluate the effects of apple cider vinegar (ACV) administration on non-specific immunity of serum and skin mucus, growth indices, and activity of digestive enzymes (amylase, lipase, and protease) in *Carassius auratus*. For this purpose, 180 fish (weighing 7.35 ± 0.19 g) were allocated to 4 treatment groups with 3 replications in a completely randomized design. Fish were fed for 105 days using a basal diet supplemented with 0% (control), 1% (T 1), 2% (T 2), and 4% (T 3) ACV (contained 5% acetic acid). Results showed a significant increase in lysozyme activity, ACH50, and total immunoglobulin of skin mucus in fish fed with T2 diet ($p < 0.05$). Total immunoglobulin and lysozyme activity were significantly lower in the serum of fish fed with control diet than those fed with the mentioned treatment ($p < 0.05$). The highest value was observed in fish fed with T2 diet. Minimum ($p < 0.05$) complement activity (1.52 ± 0. 25 U ml^{-1}) was observed in fish fed with control diet. The mean of the final weights (17.35 ± 1.39 g), daily growth (1.0 ± 0.01 g), and specific growth rate (2.19 ± 0.14) was significantly higher in T3 diet group than the controls ($p < 0.05$). While the highest amylase-specific activity was observed in the controls ($p < 0.05$), there was a significant increase in specific activity of protease, lipase, and alkaline phosphatase in T2 diet group ($p < 0.05$). According to the results of this study, the inclusion of a limited quantity of ACV (4%) into the diet can improve immunity and growth parameters in *C. auratus*.

Keywords Non-specific immunity · Apple cider vinegar · Organic acids · Digestive enzyme

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Introduction

The prevalence of various diseases in aquaculture industry has led to a widespread use of disinfectants and antibiotics. However, environmental considerations and concerns about the transfer of pharmaceutical ingredients to the humans and emergence of bacterial resistance have pushed researchers and manufacturers to use "safe" materials. These safe substances can be beneficial for aquatic organisms by improving their immune system or coping with pathogens. Various prebiotics, probiotics, and synbiotics have been introduced to improve immunity of the aquatics, but in addition to their high benefits, high cost of these materials has always been the main problem for farmers (Ahmadniaye Motlagh et al. 2019a). In this regard, the need for introducing economic and available alternatives seems reasonable, so far a great attention has been paid to the use of some additives such as medicinal plants extracts, essential oils, and organic acids.

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In nature, organic acids are usually derived from carbohydrate-fermenting bacteria present in the food. Organic acid is an organic compound with acidic properties comprising of one or more carboxyl groups, which is often used as antimicrobial compounds in the food industry (Romano et al. 2015). Various types of organic acid such as acetic acid, butyric acid, citric acid, lactic acid, malic acid, sorbic acid, formic acid, propionic acid, as well as their salts are used to improve the health of farm animals and poultry (Luckstadt 2008). Organic acid has been used as an anticorruption factor and food preservative since a long time ago (Ng et al. 2015). Currently, antibacterial or inhibitory effect of the organic acids has been proven; these properties can change the gut bacteria composition in favor of beneficial bacteria. Moreover, reducing pH of digestive tract and increasing intestinal discharge rate; enhancing activity of digestive enzymes; influencing on gut morphology; and increasing solubility of minerals (such as phosphorus, amino acids, and fats) in the stomach and intestine are among the beneficial effects regarding the administration of organic acids in fish or shellfish diets (Da Silva et al. 2013).

Acetic acid as one of organic acids is widely used in aquatic and farm animal studies. Acetic acid is considered as the main ingredient in apple cider vinegar (ACV) (3–9% v/v). ACV is a natural product obtained from apple fermentation. It contains flavonoids and polyphenolic compounds, other organic acids, vitamins, and minerals, which have pharmacological functions such as antioxidant activity, antidiabetic, cholesterol-lowering, and blood pressure-lowering effects without known adverse effects (Nazıroğlu et al. 2014).

Positive effects of organic acids on the activity of digestive enzymes (protease and amylase) in hybrid tilapia (Li et al. 2009) and *Sciaenops ocellatus* (pepsinogen, trypsin, lipase, and amylase) (Castillo et al. 2014) have been demonstrated. Organic acids and their salts can be used as growth promoters and disease inhibitor in aquaculture. The addition of sodium acetate, sodium butyrate, sodium citrate, sodium formate, sodium lactate, and sodium propionate to diets of *Litopenaeus vannamei* has been found to inhibit growth of *Vibrio harveyi*, *V. alginolyticus*, and *V. anguillarum* in the intestine (Da Silva et al. 2013). Results of a study on the effect of different levels of ACV on growth performance, immunity, and gastrointestinal microbiota of the *L. vannamei* showed that treatment with 1, 2, and 4% ACV did not improve growth indices, but increased expression of the prophenoloxidase (proPo) and lysozyme (Lys) genes, also total bacterial and vibrio count, significantly decreased in the vinegar-fed shrimp intestine compared with the controls (Pourmozaffar et al. 2019). Immune-boosting effect of ACV on common carp (*Cyprinus carpio*) has been demonstrated by administration of ACV separately and combined with *Lactobacillus casei* during an 8-week trial. In this study, researchers concluded that the application of ACV either solely or in combination with *Lactobacillus casei* might increase the serum and mucus total immunoglobulin and promote lysozyme and complement activity in the serum (Safari et al. 2017). *Carassius auratus* is an ornamental and popular species that is used as a model in biological experiments due to its easy growing conditions and high ability to withstand laboratory conditions. To the best of our knowledge, no study was conducted regarding the administration of ACV to the diet of *C. auratus*. Thus, the present experiment was conducted to investigate the effect of ACV on skin and serum immunity, growth performance, and activity of digestive enzymes in the *C. auratus*.

Materials and methods

Experimental diets and design

This experiment conducted as a completely randomized design in Fisheries Laboratory of Faculty of Natural Resources and Environmental Sciences of Ferdowsi University of Mashhad. In order to run the trial, 180 individuals of *C. auratus* fry (7.35 ± 0.11 g) were selected. After 10 days, acclimatization to laboratory conditions, the fish were randomly divided into 100-l aquariums. The ornamental fish feed (Energy® Iran) was used with the nutrient compositions as follows (%): dry matter 69.74 ± 2.25, crude protein 40.92 ± 1.37, crude lipid 3.72 ± 0.70, ash 2.72 ± 0.59, crude fiber 3.15 ± 0.95, and nitrogen-free extract 19.23 ± 1.12. To prepare the experimental diets, apple cider vinegar was added to the basal diet with the levels of 0% (control), 1% (T 1), 2% (T 2), and 4% (T 3). The experimental diets were also coated with gelatin (4 g kg diet^{-1}), a protective agent to avoid the garlic extract from leaching into the water. The control diet was prepared using only gelatin, without adding ACV (Ahmadniaye Motlagh et al. 2019b). The prepared diets were exposed to air for 3 h to dry and kept at 4 °C until consumption.

Feeding was performed at 3% body weight for 105 days. All experiments were done according to Ferdowsi University of Mashhad Laboratory Animal Ethics.

Apple cider vinegar

Apple cider vinegar was purchased from Golchekan Zamani Company (Mashhad, Iran). According to the manufacturer, the product contains water, condensed apple juice, 5% acetic acid, 0.25% salt, 70 mg kg^{-1} sodium metabisulfite, 0.052% sugar, 0.052% total carbohydrate, 0.3% protein, 0% fat, and 0% saturated and trans-fatty acids. pH value was 2.61 ± 0.08.

Sampling

Fish were not fed 48 h before sampling. Three fish were selected from each replicate (9 fish per a treatment) and anesthetized with clove powder (0.50 g L^{-1}). Blood sampling was carried out by a capillary tube through the tail ablation method. Tubes were kept at 0 °C for 3 h, until the blood was coagulated; then, serum was separated by centrifugation at 4 °C (at 10,000 rpm for 5 min). Skin mucus was collected using the method developed by Subramanian et al. (2007).

After anesthesia, the fish were packed individually inside a zipped bag containing 10 mL of 50 M sodium chloride. The mucus was collected from the bags and poured into the 15-mL centrifuge tubes. Then, the mucus-containing falcons were centrifuged at 1500 rpm for 10 min to separate the supernatant.

The whole intestine samples were mixed with a weight/volume ratio of 1 to 5 sodium chloride (0.2 M) (Gawlicka et al. 2000) then the mixture homogenized by a Disperser homogenizer model DI18. The resulting suspension was centrifuged (Hettich Refrigerator D-78532) at 5000 rpm for 30 min at 4 °C. The supernatant was removed and used for the enzymatic assay.

Non-specific immunity indicators of serum and skin mucus

Serum samples were diluted 100 times with sodium chloride (0.005%). The biuret method was used to determine the protein content. In other words, 0.1 mL of each serum sample was mixed with polyethylene glycol (a volume of 12%) and incubated for 2 h, which would allow the immunoglobulin molecules to be deposited. Then, centrifuge was performed at 5000 rpm at 4 °C. The supernatant was 50 times diluted with NaCl (0.85%). The difference between the amount of protein in the initial sample and after the addition of polyethylene glycol is called "the total immunoglobulin" and is expressed in mg ml^{-1} (Siwicki et al. 1994).

The capability of lysozyme to degrade the peptidoglycan layer of *Micrococcus lysodeikticus* was used in this experiment. First, the phosphate-buffered saline was added to the test tube, and then, some *M. lysodeikticus* (Sigma) was combined and shaken slowly. A 200 μl of the solution was removed and read at 450 nm. In the next step, 15 μl of serum or mucus was added to the tube, and after 20 min, absorption was read. Then, the adsorption was compared with the standard curve (egg white lysozyme (Sigma)), and lysozyme of the samples was calculated based on the spectrophotometry method in mg ml^{-1} (Sankaran and Gurnani 1972).

Alternative complement pathway activity was measured based on the hemolysis of red blood cells of rabbits described by Amar et al. (Amar et al. 2000). After the preparation of the rabbit's red blood cells by buffer (pH = 7, 0.01 M), it was added to the samples, and the mixture was incubated at 20 °C for 90 min. Finally, 3.15 ml of 0.85% NaCl solution was added to each of the tubes; then, the tubes were centrifuged at 1600 rpm for 10 min at 4 °C. The optical density of the supernatant was read at 414 nm. The volume of the serum or mucus, which causes 50% hemolysis, is the complement activity.

Growth performance

In order to monitor the effect of dietary ACV on the growth of *C. auratus*, individuals were weighed at the end of the trial with a digital balance (0.01 g). Feed conversion ratio and growth indicators included weight gain, daily growth, and specific growth rate, which were calculated as follows (Ahmadniaye Motlagh et al. 2019a): weight gain (g) = (final weight − initial weight), daily growth (g) = (weight gain (g)/experiment days), specific growth rate (SGR) = [(Ln final weight (g) – Ln initial weight (g))/experiment days] × 100, and feed conversion ratio (FCR) = (feed consumed (g)/weight gain (g)).

Digestive enzymes

For protease activity determination, casein hydrolysis method was used (pH = 8). The enzyme reaction

mixture of casein containing 0.1 μl of tris-hydrochloric acid and enzyme sample (0.1 ml) was incubated for an hour at 37 °C. The reaction was stopped by adding 0.6 ml of trichloroacetic acid, after keeping the samples for an hour in 2 °C; samples were centrifuged at 1800 rpm for 10 min. Supernatant absorption was read at 280 nm.

The specific activity of amylase enzyme was measured using the Bernfeld method (Bernfeld 1951). In this method, starch was used as a substrate. The starch was prepared in sodium phosphate buffer (0.02 M) containing sodium chloride (0.006 mol). Distilled water was used to dilute the sample. A unit of the specific activity of the α-amylase (U) enzyme is equal to 1 μmol of maltose released in 1 min per 1 mg of soluble protein at 25 °C.

The Worthington method was used to measure lipase activity (Worthington 1988). In this method, Arabic gum emulsion, olive oil was used as a substrate. Arabic gum was crushed with olive oil and some ice and then was mixed with distilled water. After blending, the mixture was filtered to obtain a clear solution. For sample dilution, calcium chloride (0.005 mol) was used. A specific activity unit for lipase (U) is equal to 1 μmol of fatty acid released in 1 min/mg of soluble protein at 25 °C. For all enzymes, activity units were expressed based on U mg protein^{-1} min^{-1}.

For alkaline phosphatase (ALP), a solution of 0.6 mg L^{-1} of p-nitrofenil-phosphate was used as a substrate. The solution decomposes under the action of the enzyme to phosphate and para nitrophenol yellow solution. Ringer's solution was used to dilute the tissue and substrate. Then, the diluted tissue was centrifuged for about 15 min with 5000 rpm. Incubation was carried out for 60 min at 25 °C, and the optical density was read using a spectrophotometer at 405 nm. The activity level of the alkaline phosphatase activity is based on the amount of product produced as a result of the effect of the enzyme on the substrate in 1 min and 1 g of tissue (Walter and Schütt 1974).

Data analysis

Normality and homogeneity of variance were tested using the Shapiro-Wilk and the Levene tests, respectively. One-way ANOVA and Duncan's tests were used to compare the averages at 5% confidence levels. Data analyzing was done by SPSS 24.

Results

Non-specific immunity indicators of serum and skin mucus

Table 1 shows the effect of the apple vinegar on skin mucus and serum non-specific immune parameters. Based on the results, there was a significant increase ($p < 0.05$) in the lysozyme activity, ACH50, and total immunoglobulin of skin mucus. The highest values of the mentioned indices were observed in T2. About blood non-specific immunity, the results showed that the activity of lysozyme in the serum of control fish was significantly lower than the experimental groups ($p < 0.05$); the highest value was observed in T2 (2.71 ± 0.10 U mg^{-1} protein). There was no significant difference between T1, T2, and control for ACH50, but the T3 showed the minimum activity (1.52 ± 0. 25 U ml^{-1}) among the treated fish and control ($p < 0.05$). Serum total immunoglobulin was significantly decreased in control fish ($p < 0.05$), and T2 showed the highest value (3.88 ± 0.45 mg ml^{-1}).

Growth performance

The results of 105 days feeding *C. auratus* with different levels of apple cider vinegar on growth indices including final weight, weight gain, daily growth, and SGR are shown in Table 2. Results showed that there was no significant difference between the groups supplemented with apple cider vinegar. However, animals supplemented with apple cider vinegar in the highest concentration (4%) showed improvements in respect with the final weight, weight gain, daily growth, and SGR values. FCR was not significantly influenced ($p < 0.05$) by ACV; however, it was higher in the control group.

Digestive enzymes

Digestive enzyme activities (protease, amylase, lipase, and ALP) of *C. auratus* fed different levels of ACV are summarized in Fig. 1. Results showed that there is no significant difference ($p > 0.05$) in protease-specific activity among fish fed with T1, T3, and control diets, but T2 represented a sharp and significant ($p < 0.05$) increase compared with the control. Lipase activity showed a negligible increase in treatments, but this increment was not significant ($p > 0.05$). Amylase activity decreased significantly ($p < 0.05$) in treated fish.

Table 1 The mean (± SD) initial weight (g), final weight (g), weight gain (g), daily growth (g), specific growth rate (SGR; % body weight day^{-1}), and feed conversion ratio (FCR) of *C. auratus* fed different levels of dietary apple cider vinegar for 105 days ($n = 3$)

	Dietary apple cider vinegar (ACV) levels (%)			
	0 (Control)	T1	T2	T3
Initial weight (g)	7.42 ± 0.04	7.33 ± 0.09	7.42 ± 0.01	7.35 ± 0.09
Final weight (g)	15.33 ± 0.61^{a}	15.61 ± 0.46^{ab}	15.65 ± 0.93^{ab}	17.35 ± 1.39^{b}
Weight gain (g)	7.91 ± 0.59^{a}	8.28 ± 0.37^{ab}	8.23 ± 0.92^{ab}	10.00 ± 1.40^{b}
Daily growth	0.08 ± .01^{a}	0.08 ± .00^{ab}	0.08 ± .01^{ab}	0.10 ± 0.01^{b}
SGR	1.97 ± 0.07^{a}	2.01 ± 0.04^{ab}	2.00 ± 0.11^{ab}	2.19 ± 0.14^{b}
FCR	3.88 ± 0.38	3.76 ± 0.34	3.71 ± 0.44	3.13 ± 0.47

Values are mean ± SE of 15 fish; different superscript letters in a row indicate significant differences between groups ($p < 0.05$). T1, T2, and T3, apple cider vinegar at 1%, 2%, and 4% of kg feed respectively; *SGR*, specific growth rate; *FCR*, feed conversion ratio

ALP activity was significantly ($p < 0.05$) enhanced in T2 and T3 compared with the control, and the highest ALP activity was detected in T2 (1.05 ± 0.14 U mg $protein^{-1}$ min^{-1}).

Discussion

Organic acids and their salts containing short-chain fatty acids (C1–C7) and one or more carboxyl groups with weak acid properties are lipophilic and can penetrate into the Gram-negative bacteria through the cell membrane based on their simple structure and small size. Right after their penetration, they make changes in the pH of the cytoplasm in the bacterium, resulting in a decrease in the intracellular pH and an increase in the removal of hydrogen ion in the cell. Therefore, it degrades acid-sensitive proteins and DNA, which in turn prevents activity of acid-sensitive enzymes and metabolic and anabolic processes of the cells (Salem and Amin 2012).

Due to severe restrictions on the use of antibiotics, the need for finding an appropriate alternative to protect aquaculture from pathogen bacteria and stimulate the immune system of fishes and crustaceans has always been addressed by the researchers (Ahmadniaye Motlagh et al. 2019b; Motlagh et al. 2020). Various studies have focused on the effects of organic acids on bacterial communities of the digestive system and have reported reduction of total bacteria, lactic acid bacteria, and viable bacteria count in the intestine (Da Silva et al. 2013; Chuchird et al. 2015). According to a review of

Table 2 Comparison of skin mucus and serum non-specific immune parameters of *C. auratus* fed different concentrations of apple cider vinegar for 105 days

	Treatments			
	Control	T_1	T_2	T_3
Skin mucus non-specific immune parameters				
Lysozyme (U mg^{-1} protein)	1.86 ± 0.36^{a}	2.38 ± 0.43^{b}	2.94 ± 0.33^{d}	2.52 ± 0.34^{b}
ACH50 [1] (U ml^{-1})	0.74 ± 0.18^{a}	1.13 ± 0.09^{b}	1.61 ± 0.22^{c}	1.19 ± 0.13^{b}
Total Ig [2] (mg ml^{-1})	1.50 ± 0.09^{a}	1.89 ± 0.10^{b}	2.71 ± 0.10^{c}	2.31 ± 0.11^{d}
Serum non-specific immune parameters				
Lysozyme (U mg^{-1} protein)	1.50 ± 0.09^{a}	1.89 ± 0.10^{b}	2.71 ± 0.10^{d}	2.31 ± 0.11^{c}
ACH50 (U ml^{-1})	2.33 ± 0.22^{b}	2.27 ± 0.26^{b}	2.24 ± 0.21^{b}	1.52 ± 0. 25^{a}
Total Ig (mg ml^{-1})	2.30 ± 0.45^{a}	3.04 ± 0.47^{b}	3.88 ± 0.45^{c}	3.21 ± 0.38^{b}

Values are mean ± SE of 3 fish; different superscript letters in a row indicate significant differences between groups ($p < 0.05$). T1, T2, and T3, apple cider vinegar at 1%, 2%, and 4% of kg feed, respectively; *ACH50*, alternative complement pathway activity; *Total Ig*, total immunoglobulin

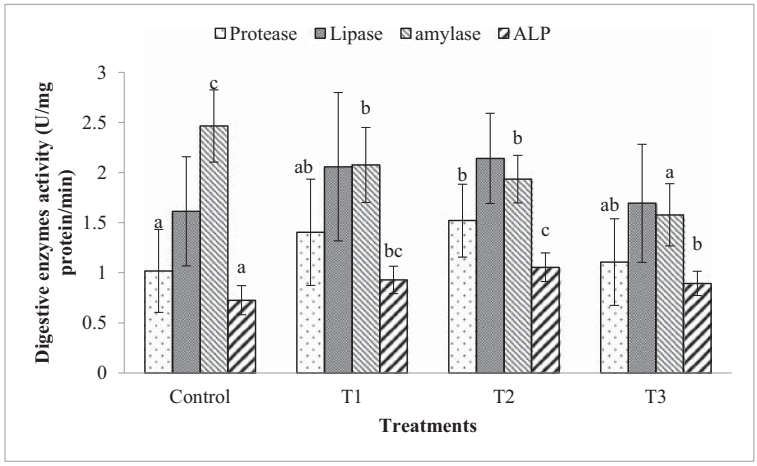

Fig. 1 Specific activities of protease, lipase, amylase, and ALP in the intestine of *C. auratus* fed different levels of dietary apple cider vinegar for 105 days (mean ± SD, $n = 3$). ALP, alkaline phosphatase. Bars with different letters are significantly different ($p < 0.05$)

the literature, while expression of immune-related genes has attracted attention of the researchers, quantitative studies have been conducted in aquaculture to investigate activity of lysozyme, complement, and total immunoglobulin as a result of organic acid consumption.

It has been reported that organic acids, as an oral additive, are able to stimulate systemic and mucosal immunity in *Danio rerio* (Safari et al. 2016), *Rutilus frisii kutum* (Hoseinifar et al. 2016), *Oreochromis niloticus* (Reda et al. 2016), and *Epinephelus coioides* (Chiu et al. 2008). Results of a study showed the increase in the activity of lysozyme, serum complement, total immunoglobulin, and expression of immune and antioxidant-related genes as a result of combined consumption of ACV with *Lactobacillus casei* in common carp (*C. carpio*) (Safari et al. 2017). Sodium butyrate dietary administration in Nile tilapia culture system only led to an increase in the number of lymphocytes, monocytes, and hematocrit; no significant difference was observed in the total number of white and red blood cells (Ali et al. 2018). In this experiment, results of biochemical analysis of serum and skin mucus in *C. auratus* showed the highest activity of lysozyme, complement, and total immunoglobulin in fish fed with 2% ACV diet, which is in agreement with the mentioned researches.

Results of this study showed that the use of 4% ACV in the diet caused a significant increase in the growth index of *C. auratus*. Previous studies have reported that organic acids can boost growth and improve nutritional performance in aquatic animals such as the administration of potassium diformate in the diet of *Oreochromis niloticus* (Elala and Ragaa 2015), ACV in the diet of *C. carpio* (Safari et al. 2017) and *Andinoacara rivulatus* (Motlagh et al. 2020), and a mixture of organic acids in the diet of *Litopenaeus vannamei* (Romano et al. 2015), while some studies reported contradictory results and showed that, for example, the application of dietary organic acids did not improve the growth of red tilapia (Ng et al. 2009), *Oncorhynchus mykiss* (Gao et al. 2011; Hernández et al. 2013), and *Clarias gariepinus* (Mdegela, et al., 2006), possibly attributing to the differences in species, type of organic acid, and the dosage (Luckstadt 2008). On the other hand, researchers have announced that acetic acid can increase fat metabolism in body cells and reduce fat storage (Li et al. 2018). Several studies have shown that the mice treated with acetic acid have lower liver triglyceride content, and the expression of the genes responsible for lipid oxidation has increased (Kondo et al. 2009).

An increase or decrease in the growth of aquatic organisms in response to feed additives occurs as a result of a series of different processes. For instance, researchers have declared that organic acids will be beneficial for the creatures through influencing the gut bacteria (Elala and Ragaa 2015; Silva et al. 2016), decreasing gastrointestinal pH and increasing intestinal discharge rates (Sugiura et al. 1998; Baruah et al. 2005), influencing activity of digestive enzymes (Castillo et al. 2014) and gut morphology (Hamer et al. 2008; Gao et al. 2011), increasing solubility of the minerals (such as phosphorus), amino acids, and fats (Sugiura et al. 1998), increasing expression of growth or immune-related genes (Pourmozaffar et al. 2017; Duan et al. 2018), and increasing tolerance to environmental

conditions (Duan et al. 2018). All these outcomes are manifested through the increase in growth. It can be said that the increment in growth indices observed in this study is likely associated with enhanced activity of digestive enzymes. Findings of this study are consistent with the study by other researchers who proved that the number of digestive enzymes-secreting cells increased in the intestinal tract of the shrimps treated with organic acid (Romano et al. 2015). Pepsin, trypsin, and lipase activity has also been shown to increase in response to the application of organic acids in the diet of *Sciaenops ocellatus* (Castillo et al. 2014).

Results of another study on the use of succinic acid for 56 days at the dosages of 0.25, 0.50, and 1.00% on secretion of digestive enzymes in *P. vannamei* showed that activity of amylase, lipase, and pepsin increased in hepatopancreas (Duan et al. 2018). Similar results have been reported for *Oreochromis niloticus* (Ali et al. 2018) and Siberian sturgeon (*Acipenser baerii*) (Najdegerami et al. 2015) fed with sodium butyrate and poly-beta-hydroxybutyrate, respectively. However, no significant effect has been found regarding the administration of organic acids on the activity of digestive enzymes in turbot (*Scoophthalmus maximus*) (Dai et al. 2018) and silver catfish (*Rhamdia quelen*) (Pereira et al. 2019). Researchers (Jun-sheng et al. 2006; Lückstädt 2008) believe that a small decrease in gastrointestinal pH will be effective in increasing activity of digestive enzymes. Besides, an increase in the number of beneficial gut bacteria such as lactic acid bacteria and their involvement in the production of digestive enzymes can also increase the activity of digestive enzymes (Ahmadniaye Motlagh et al. 2019a).

Conclusion

To date, no study has been carried out on *C. auratus*, as a popular ornamental fish to investigate the effect of ACV administration on the growth, immunity, and activity of digestive enzymes. Results of the present study confirmed that ACV could increase growth performance, skin and serum immunity, as well as activity of digestive enzymes. Since this organic acid is not expensive, it can be a good alternative to common antibiotics as growth promoters if applied at a dosage of 4%. Finally, it is suggested to investigate other possible aspects of inclusion of ACV in the diet such as its effects on reproductive physiology of fish and other aquatic species in the future studies.

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Compliance with ethical standards

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

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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