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Original Article

Effects of apple cider vinegar on growth performance and non-specific immune parameters of skin mucus in common carp (*Cyprinus carpio*) fingerlings

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Abstract: This study was undertaken to evaluate the effects of apple cider vinegar on growth performance and non-specific immune parameters of skin mucus (alkaline phosphatase, lysozyme and total protein) in common carp fingerlings. For this purpose, a total of 240 fish were stocked in twelve tanks for four treatment with three replications and fed by diets supplemented with 0, 1, 2 and 4 % apple cider vinegar for 60 days. The result showed that there was no significant difference in body weight increase (BWI), feed conversion ratio (FCR), specific growth rate (SGR) and condition factor (CF) among the treatments (P>0.05). The results revealed that there is no significant difference among skin mucus alkaline phosphatase of fish in all treatments. Although skin mucus lysozyme activity and total protein significantly increased by apple cider vinegar in comparison with the control group. The highest and lowest skin mucus lysozyme activity and total protein in common carp fingerlings were observed at 2% apple cider vinegar and control group, respectively. Therefore, apple cider vinegar improves skin mucus lysozyme activity and total protein and can be a good candidate for antibiotic replacement in common carp fingerlings.

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Introduction

According to the UN Food and Agriculture Organization, aquaculture is growing more rapidly than all other animal food-production sectors (www.fao.org). Aquaculture is becoming a more concentrated industry, with fewer, but much larger, farms (Yousefi et al., 2019). Infectious diseases are always a hazard and may cause significant economic losses and problems with animal welfare. Intensive aquaculture led to growing problems with bacterial diseases (Taheri Mirghaed et al., 2018; Hoseini et al., 2020a, b). To solve this problem, farmers frequently use antibiotic compounds to treat bacterial diseases (Alderman and Hastings, 1998); therefore it has resulted in the development and spread of antibiotic resistance bacteria (Yonar, 2012; Hoseini and Yousefi, 2019), so there is a need to use alternative therapies for bacterial pathogens in fish (Chakraborty and Hancz, 2011; Chakraborty et al., 2014). In this case, various immunestimulants, including certain feed additives are being used to increase the resistance of aquatic organisms to infectious agents (Lee et al., 2015). Recently, some scientists proved the positive effects of medicinal plants or herbs as feed additives. These herbs improved the growth and feed utilization of the fish and also reduced diseases by regulating pathogens in gastrointestinal tract (Fazelan et al., 2020; Rajabiesterabadi et al., 2020; Yousefi et al., 2020).

One of feed additives is organic acid components which are known to have stimulatory potential effect on the immune system and have been proven efficient for disease prevention (Taheri Mirghaed et al., 2019). Various types of organic acids, such as acetic acid, butyric acid, citric acid, lactic acid, malic acid, sorbic acid, propionic acid, as well as their salts, are used to improve the health of fish (Hossain et al. 2007; Ng et al., 2009; Safari et al., 2017) and shrimp (Pourmozaffar et al. 2017). Apple cider vinegar is an acidic solution, which contains organic acids such as acetic acid and malic acid, vitamin B and C, and minerals (Iman et al., 2015). Previous study

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demonstrated that using organic acid in diet resulted in improve blood indexes and healthy in rainbow trout fingerling (Taheri Mirghaed et al., 2019). Also, in another studies positive effect of apple cider vinegar in fish (Beheshti et al., 2012; Safari et al., 2017) and shrimp (Pourmozaffar et al., 2017) healthy and immunology are demonstrated.

Therefore, in this study we used apple cider vinegar to determine whether this solution has an effect on growth performance including and skin mucus non-specific immune parameters in common carp fingerlings.

Materials and Methods

Two hundred forty common carp, *Cyprinus carpio*, fingerlings (10 ±0.56 g) were purchased from a private sector farm and transferred to the Aquaculture Laboratory of Gorgan University of Agricultural Sciences and Natural Resources (Iran). In this study, we used 12 glass tanks (40×60×40 cm), and each tank (40-L) was aerated with an air pump. After 7 days acclimation, fish were distributed into three experimental groups and one control group, each with 3 replicates.

Preparing the experimental diet: Apple cider vinegar (5% acetic acid) was supplied from 1&1 Co. (Fars, Iran). 1, 2 and 4 percentage of apple cider vinegar were mixed with basal diet (8.8% moisture, 11.36% ash, 34.5% crud protein and 10.7% fat), and then pelleted by a meat grinder (Hoseinifar et al., 2017). Experimental diets were kept at 4°C until used. We chose the doses based on the literature (Safari et al., 2017). Fish were fed on experimental diet at 5% of body weight and 3 times per day for 60 days (NRC, 2011).

Sampling: At the first and the end of experiment, 10 specimens were randomly selected from each treatment and were anesthetized in clove oil solution (150 ppm) for 40-50 s (Yousefi et al., 2018), dried with tissue paper, and then weight and length were measured. In addition, skin mucus was obtained following the method described before (Ross et al., 2000, Subramanian et al., 2007) for non-specific immune parameters assay, at the end of the experiment. The mucus samples were transferred to 15 ml sterile tube, centrifuged at 1500 g (4ºC) for 10 min and the supernatants were poured in 2-ml tubes and stored at -80 ºC until analysis.

Growth Performance: In this study growth indices were calculated by following formula (Hoseini et al., 2016):

$BWI\% = (Wt- W0)/W0\times100$

FCR (Feed conversion ratio) = $F/(Bt – B0)$

SGR % (Specific growth rate) = $(Ln\ Wt - Ln\ W0) \times100/t$

$CF = 100 \times (Wt/TL^3)$

Where Wt and W0 are final and initial body weight (g), respectively, F is relative food intake (g), Bt and B0 are final and initial fish biomass (g), respectively, t = time of rearing (days) and TL = total length (cm).

Non-specific immune parameters of skin mucus: Alkaline phosphatase in skin mucus was determined using commercial competitive kits (Bio-chemistry kit, Iran). The skin mucus lysozyme activity was measured based on lysis of *Micrococcus luteus* using turbidimetric assay following (Hoseini et al., 2018). Total protein content of samples was determined according to Lowry et al. (1951).

Data analysis: All data are shown as mean±SEM. The SPSS 16 software was used for statistical analyses. One-way analysis of variance (ANOVA) followed by LSD tests was used to assess the significant effects of apple cider vinegar concentrations on growth performance indices and skin mucus non-specific immune parameters. A value of $P<0.05$ was considered statistically significant for all statistical tests.

Results

The result showed that there was no significant difference in BWI, FCR, SGR and CF among the treatments ($P> 0.05$) (Table 1).

Skin mucus alkaline phosphatase, lysozyme activity and total protein: The effects of apple cider vinegar on non-specific immune parameters of common carp skin mucus are showed in Figures 1 to 3. The results revealed that there is no significant difference among skin mucus alkaline phosphatase activity of fish in all

Table 1. Effect of different concentrations of apple cider vinegar on growth performance in common carp fingerlings after 60 days.

Growth indices	Concentrations of apple cider vinegar			
	Control	1%	2%	4%
BWI (%)	98.89 ± 48.83	101.68 ± 21.45	99.02± 59.42	90.47± 42.17
FCR	2.53± 0.24	2.84± 0.44	3.12± 0.09	2.4± 0.18
SGR (%)	1.64± 0.81	1.75± 0.35	1.65± 0.99	1.50± 0.70
CF (%)	1.24± 0.38	1.23± 0.08	1.30± 0.09	1.23± 0.95

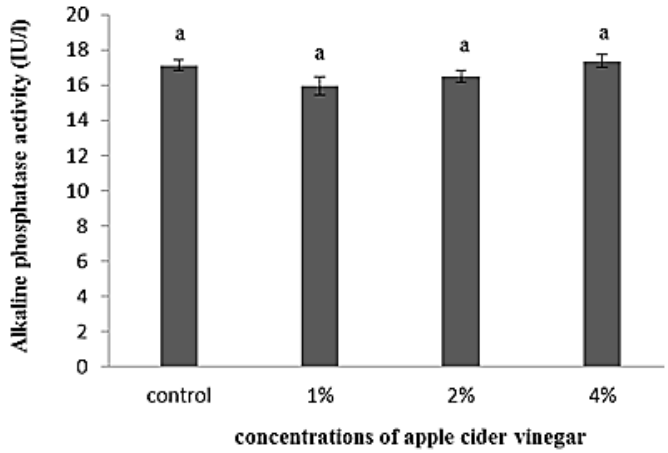

Figure 1. Effect of different concentrations of apple cider vinegar on skin mucus alkaline phosphatase in common carp fingerlings after 60 days. Data are presented as relative gene expression and mean±SEM. Different lowercase letter indicates significant differences among treatments.

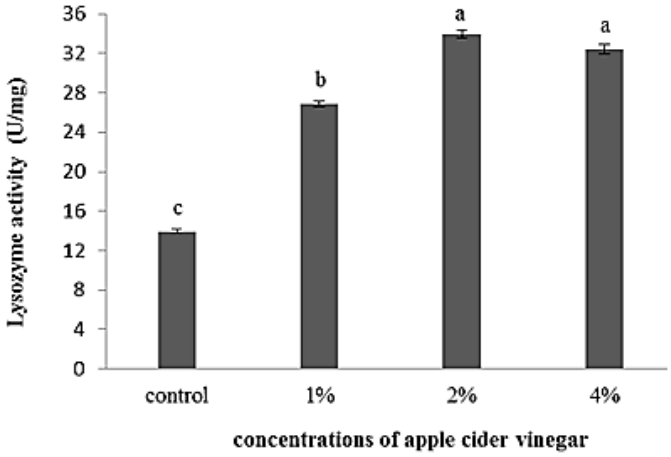

Figure 2. Effect of different concentrations of apple cider vinegar on skin mucus lysozyme activity in common carp fingerlings after 60 days. Data are presented as relative gene expression and mean±SEM. Different lowercase letter indicates significant differences among treatments.

treatments and control group (P>0.05) (Fig. 1). Common carp fingerlings showed a dose-dependent increase in lysozyme activity with apple cider vinegar.

Lysozyme activity was significantly enhanced by

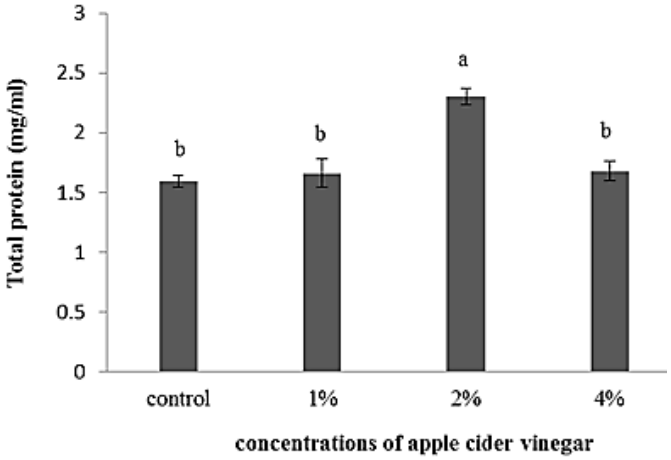

Figure 3. Effect of different concentrations of apple cider vinegar on skin mucus total protein in common carp fingerlings after 60 days. Data are presented as relative gene expression and mean±SEM. Different lowercase letter indicates significant differences among treatments.

apple cider vinegar relative to controls (Fig. 2) (P<0.05). According to Figure 3, there was significant difference detected among different treatment groups in skin mucus total protein (P<0.05). The highest skin mucus total protein in common carp fingerlings was observed in 2% apple cider vinegar and lowest skin mucus total protein was detected in the control group.

Discussions

In this study, apple cider vinegar used as dietary supplements to assess the potential growth rates and immunological effects on common carp fingerlings. Our results showed that using 1, 2 and 4% apple cider vinegar as a supplementary in diet had no effect on BWI, FCR, SGR and CF after 60 days in fish. Similarly, using of organic acid in diet had not significant effect on growth performance in *Oreochromis* sp. (Ng et al., 2009) and *Pagrus majo* (Hossein et al., 2007).

Also, this study assessed the ability of the apple

cider vinegar in skin mucus non-specific immune parameters of common carp fingerlings. Mucus is an important barrier in fish, because it provides the substrate in which antibacterial mechanisms may act, and in most fish species the mucus covers most of the external surfaces, and mainly the skin (Tort et al., 2003). Fish skin mucus contains many humoral non-specific defense factors for example, lysozyme, complement, interferon, C-reactive protein, and lectin, transferrin (Saurabh and Sahoo, 2008). These innate immune molecules are especially important for each species, because each live in a water medium rich in pathogens (Ingram, 1980) and thus play a key role in maintaining homoeostasis in the animal (Saurabh and Sahoo, 2008).

We demonstrated that 60 days of feeding with apple cider vinegar altered the skin mucus levels of lysozyme activity and total protein in common carp fingerlings that are related to non-specific immunity in fish. Previous researches demonstrated that some immune-stimulant such as organic acids can enhance some of specific and non-specific immune responses (Hossain et al. 2007; Ng et al., 2009; Beheshti et al., 2012; Safari et al., 2017), and can be a good candidate as an alternative to the drugs, chemicals and antibiotics currently being used to control fish diseases in fish culture (Bairwa et al., 2012). In addition, lysozyme activity were significantly higher in fish Japanese flounder (*Paralichthys olivaceus*) fed a fermented vegetable product supplemented diet (Ashida and Okimasu, 2005). It has been reported that apple cider vinegar has multiple antimicrobial properties on different microbial species (Yagnik et al., 2018). Aapple cider vinegar consists of acetic acid, flavonoids such as gallic acid, tyrosol catechin, epicatechin, benzoic acid, vaninilin, caftaric acid, coutaric acid, caffeic acid, acid and ferrulic acid that can effect on immune defense and oxidative responses (Budak et al., 2011; Nazıroğlu et al. 2014).

In general, the results of this study showed that the addition of apple cider vinegar in diet of common carp has beneficial effects on the activity of lysozyme and mucus protein that related to non-specific immune. Therefore, our data suggest that feeding with apple cider vinegar may positively affect non-specific immune in fish. Also, 2% vinegar in the diet showed better results than other levels. Therefore, apple vinegar can be used as immune-stimulant in diet of common carp fingerlings.

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