Ann. Anim. Sci., Vol. 23, No. 2 (2023) 505–517 *DOI: 10.2478/aoas-2022-0074*

sciendo

DIETARY POWDER AND MOLECULAR IMPRINTED POLYMER NANOENCAPSULATED SODIUM PROPIONATE TO ENHANCE GROWTH PERFORMANCE, DIGESTIVE ENZYMES ACTIVITY, ANTIOXIDANT DEFENSE, AND MUCOSAL IMMUNE RESPONSE IN AFRICAN CICHLID (*LABIDOCHROMIS LIVIDUS*) FINGERLINGS

Mehrdad Sarkheil[1]♦, Omid Safari[1], Davood Kordestani[2]

[1]Department of Fisheries, Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad, Mashhad, P.B. 91773-1363, Iran
[2]Department of Chemistry, Amirkabir University of Technology, Tehran, Iran
♦Corresponding author: sarkheil@um.ac.ir
ORCID: Mehrdad Sarkheil, http://orcid.org/0000-0003-4799-4658

Abstract

This study was conducted to examine the effects of powder sodium propionate (P-SP) and SP- loaded molecular imprinted polymer (MIP) nanoparticles (MIP-SP NPs) on the growth, skin mucosal immune parameters, and digestive and liver enzymes activities of African cichlid (*Labidochromis lividus*) fingerlings. Fish with an average weight of 500±2 mg were stocked into 12 experimental units and fed on experimental diets prepared by supplementing the basal diet (control) with MIP NPs, P-SP (5 g SP kg^{-1} of dry diet), and MIP-SP NPs for 8 weeks. The findings demonstrated that growth indices improved in the MIP-SP NPs followed by the P-SP dietary group compared to the control groups (P<0.05). The activity of digestive enzymes of lipase, trypsin, protease, and alkaline phosphatase was higher in the fish fed SP-supplemented diets than in the controls (P<0.05). The protease and lipase activities in the MIP-SP NPs dietary group increased by 29.33% and 48.81% compared to the control, respectively (P<0.05). In addition, the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels of liver tissue decreased in the SP dietary groups, while the catalase (CAT), superoxide dismutase (SOD), and alkaline phosphatase (ALP) levels increased compared to the control groups (P<0.05). The highest SOD and ALP levels were observed in the fish fed on the MIP-SP NPs-supplemented diet (P<0.05). Furthermore, the skin mucosal immune indices, including alternative hemolytic complement activity (ACH50), lysozyme, and total immunoglobulin (Ig) levels increased in the MIP-SP NPs and P-SP dietary groups compared to the controls (P<0.05). The findings indicated that sodium propionate encapsulated in molecularly imprinted polymer nanoparticles could enhance the efficiency of dietary SP in African cichlid fish.

Key words: organic salt, acidifier, nanoencapsulation, drug delivery, antioxidant enzyme, ornamental fish

Nowadays, the outbreak of various bacterial, fungal, and viral diseases is among the main constraints to the productivity of the aquaculture industry. Traditionally, antibiotics have been administered as growth promoters and disease controllers in animal feed (Liu et al., 2014; Wongsasak et al., 2014; He et al., 2017). However, their usage in aquaculture is restricted due to the emergence of antibiotic-resistant pathogenic strains (Cabello et al., 2013; Safari et al., 2016; Chow et al., 2017), various adverse effects on the environment (Rico et al., 2012; Romano et al., 2014), and the threat to human consumers through the accumulation of antibiotic residues in aquatic animals (Defoirdt et al., 2009; Marshall and Levy, 2011). Over the past decades, various immunostimulants (Sajeevan et al., 2009; Safari and Sarkheil, 2018; Shekarabi et al., 2021; Rashmeei et al., 2020), probiotics (Cámara-Ruiz et al., 2020; Wuertz et al., 2021; Shekarabi et al., 2022), and prebiotics (Zhou et al., 2007; Safari and Paolucci, 2018) have been identified as environmentally-friendly antibiotic alternatives. Recently, several studies have been conducted on the effects of dietary organic acids and their salts on growth performance, enhancement of immune status, and disease resistance in various aquaculture species (Hoseinifar et al., 2016; Safari et al., 2017; He et al., 2017; Kakavand et al., 2021).

Organic acids and their salts composed of short-chain fatty acids (C1–C7) and carboxylic acids with one or more carboxyl groups (-COOH) in their structure are known as acidifiers. These compounds are often used in livestock feed for controlling infectious diseases (Defoirdt et al., 2009; Ng and Koh, 2016). Typically, organic acids apply their antibacterial effect by altering the pH of bacterial cells, thereby inhibiting the growth of bacteria. Consequently, they reduce pathogenic bacteria within the host animal's gastrointestinal tract (Booth and Stratford, 2003; Baruah et al., 2008). Propionic, lactic, and formic acids and their salts are the most common organic acids examined in aquaculture (Hoseinifar, et al., 2016; Ng and Koh, 2016). There are reports that organic acids and their salts effectively enhance the growth, nutrient utilization, and health status of aquatic animals (Agouz et al., 2015; Safari et al., 2016; Chow et al., 2017). These improve-

ments are attributed to a significant decrease in pH of the gut and upper intestinal tract (Baruah et al., 2005; Abu Elala and Ragaa, 2015), an increase in the digestive enzyme activities (Su et al., 2014; Castillo et al., 2014), stimulating the growth of intestinal epithelial cells (Topping and Clifton, 2001; Gao et al, 2011; Kakavand et al., 2021), improvement in the digestibility of major nutrients (Morken et al., 2011), and altering the gut microbial community (Silva et al., 2016). Van der Wielen (2002) reported that butyrate is less ideal as a feed additive for animals because of its quick absorption in the upper digestive tract. It has been shown that the efficiency of butyric acid can be increased when administered in encapsulation due to the slow release and effective delivery of the butyric acid throughout the gastrointestinal tract (Chow et al., 2017). In this regard, leaching of organic acids and their salts from feed into water body is considered a fundamental challenge in their administration in aquafeeds (Ng and Koh, 2016). Also, encapsulation or coating of organic acids is beneficial in preventing leaching.

Drug delivery is a useful approach for the slow and controlled release of pharmaceutical compounds to obtain the maximum therapeutic effects in humans or animals (Zaidi, 2016). Micro and nano-encapsulation technologies can be used for the entrapment, protection, and controlled release of active ingredients, thereby improving their bioavailability (Poncelet, 2006; Paramera et al., 2011). Biopolymers and synthetic ones are considered very exciting and useful drug delivery devices in the pharmaceutical industry (Luliski, 2013; Zaidi, 2016). Molecularly imprinted polymers (MIP) are synthetic materials in drug dosage forms that provide great potential in drug delivery (Cunliffe et al., 2005; Piletsky and Turner, 2006). In drug delivery, MIPs are usually synthesized by a polymerization process in the presence of a template (target molecule), functional monomer, and cross-linker (Jaiswal et al., 2015). MIPs exhibit outstanding advantages such as the slow and controlled release of drugs, efficient drug loading, stability and resistance to harsh conditions (e.g., pH, organic solvents, temperature, and pressure), biocompatibility, and ease of preparation (Puoci et al., 2011; Gao et al., 2014; Asadi et al., 2016; Zaidi, 2016). Asadi et al. (2016) synthesized nanostructured molecularly imprinted polymer for controllable sustained release of olanzapine as an antipsychotic drug to the brain tissue of rats under an external magnetic field. Zhu et al. (2017) showed that vinblastine (VBL)-loaded molecular imprinted nanoparticles (MIPNPs) had a sustained-release behavior. Also, they showed that level of VBL-loaded MIPNPs in the tissues and serum of rats was higher than that of commercially available injections. To the best of our knowledge, information on the use of MIPs as drug or active ingredient carriers is limited in aquatic animals.

Therefore, in the present study, a MIP was synthesized as a template carrier for sodium propionate (SP). Next, we examined the potential effect of dietary SP-loaded MIP nanoparticles versus powder SP on the growth indices, activity of digestive and antioxidant enzymes, and the immune status of African cichlid (*Labidochromis lividus*) fingerlings. Cichlid fish are among the world's most diverse and popular ornamental fish. The African cichlid, one of the most common species in aquarium fish farms, is produced commercially for the ornamental fish market (Smith, 2000). Regarding the rapid increase in the ornamental fish trade worldwide (Raja et al., 2019), it is necessary to enhance the growth, health status, and disease resistance of the fish for further development of the ornamental fish industry.

Material and methods

Chemicals

Methacrylic acid, ethylene glycol dimethacrylate, potassium persulfate, acetone and ethanol were purchased from Merck Co. Also, sodium propionate ($C_3H_5NaO_2$) was purchased from Sigma-Aldrich Co.

Synthesis of molecular imprinted polymer-sodium propionate (MIP-SP) particles

First, 1 mM methacrylic acid and 1 mM sodium propionate (SP) were poured into 50 mL of water/ethanol solution (50:50 v/v) and mechanically stirred at room temperature for 1 h. Then, 4 mM ethylene glycol dimethacrylate and 0.1 M potassium persulfate were added to the solution, in the order of their appearance. The mixture was stirred for 10 min and sonicated for 5 min. Then, the reaction was purged using N_2 for 10 min, and an N_2-filled balloon was placed over the balloon reaction to deoxygenate the solution. The reaction balloon was placed in a bain-marie bath (water bath) at 60°C for 20 h. In the following, the reaction balloon was cooled to room temperature. The sediment was collected from the balloon bottom and washed several times with ethanol and acetone, in the order of their appearance. The product (MIP-SP) was dried at room temperature. Finally, the synthesized MIP particles without loading SP were considered as control.

Characterization of synthesized MIP-SP particles

Field emission scanning electron microscopy (FE-SEM; MIRA3 TESCAN, Brno, Czech Republic) coupled with X-ray energy dispersive spectroscopy (EDS) was used to determine synthesized particles' shape, size, and elemental composition. Transmission electron microscopy (TEM; ZEISS LEO 912 AB) was also applied to determine the shape and size of particles. The average diameter and size distribution of particles were determined by measuring the diameter of 310 individual particles on TEM images using AxioVision digital image processing software (Release 4.8.2.0, Carl Zeiss Micro Imaging GmbH, Germany). The functional groups in MIP-SP particles were identified using Fourier transform infrared (FT-IR) spectroscopy (Bruker Alpha, Ettlingen, Germany) over the range of 400 to 4000 cm^{-1}.

The SP content of the synthesized MIP-SP nanoparticles was measured in triplicate according to the method described by Sarkheil et al. (2021). Briefly, 0.5 g of MIP-SP powder was poured into a 50 mL round bottom balloon containing 9 mL of HCL, 3 mL of HNO_3 and, 2 mL of H_2O_2. The powder sample was digested by maintaining the mixture at room temperature for 24 h and then, heating at 95°C for 2 h. Afterward, the digested sample was filtered using a Whatman filter paper (0.45 µm) and diluted to 25 mL using double distilled water. Finally, the concentration of Na^+ ions in the solution was measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

Preparation of experimental diets

In this study, four experimental diets were prepared by supplementing a basal diet with two forms of powder SP (P-SP) and MIP-SP nanoparticles (MIP-SP NPs) at 5 g kg^{-1} of dry diet according to the SP inclusion level of diets for various fish species at different life stage (Hoseinifar et al., 2016; Wassef et al., 2019; Sarkheil et al., 2021). The basal diet without SP and supplemented with MIP nanoparticles (MIP NPs) was considered as control. The ingredients and proximate chemical analysis of the experimental diets are given in Table 1. The experimental diets were prepared by grinding the basal diet into powder form using a miller. Next, water was added to the mixture and mixed well to obtain a uniform paste, to which different forms of SP were added. The dough was pelletized using a meat grinder with a mesh diameter of 1 mm to match the size of commercial feeds. Afterward, the pellets were dried at 30°C for 24 h and stored at –20°C until use in the feeding trial. The pH of prepared diets was determined using a pH meter (Crison Basic 20 model) according to the procedure explained by Boland et al. (1981).

Table 1. Ingredients and proximate composition of experimental diets

Ingredients (g kg^{-1} dry-weight basis)	Control	MIP NPs	P-SP	MIP-SP NPs
Fish meal[1]	205	205	205	205
Wheat flour[1]	220	220	220	220
Soybean meal[1]	250	250	250	250
Corn gluten[1]	125	125	125	125
Soybean oil[1]	25	25	25	25
Fish oil[1]	25	25	25	25
Mineral premix[2]	35	35	35	35
Vitamin premix[3]	35	35	35	35
Carboxymethyl cellulose[4]	59	5.18	54	0.18
Anti-fungi[5]	15	15	15	15
BHT[6]	1	1	1	1
Vit. C[7]	5	5	5	5
Sodium propionate[8]	0	0	5	0
Molecular imprinting polymer nanoparticles	0	53.82	0	0
Molecular imprinting polymer-sodium propionate nanoparticles	0	0	0	58.82
Proximate composition (g kg^{-1} dry-weight basis)				
dry matter	958.6	958.6	958.6	958.6
crude protein	405	405	405	405
crude fat	62	62	62	62
crude fiber	341	341	341	341
ash	71	71	71	71
gross energy (Mj/Kg)	16.15	16.15	16.15	16.15
pH	5.91	5.87	5.79	5.77

MIP-NPs: molecular imprinted polymer nanoparticles; P-SP: powder sodium propionate; MIP-SP NPs: molecular imprinted polymer-sodium propionate nanoparticles.

[1]Behparvar Aquafeed Co, Iran.

[2]Mineral premix contains (mg kg^{-1}): Mg, 100; P, 150; Zn, 60; Fe, 40; Cu, 5; Co, 0.1 and I, 1 (Kimia Roshd Co, Gorgan, Iran).

[3]Vitamin premix contains (mg kg^{-1}): E, 30; K, 3; thiamine, 2; riboflavin, 7; pyridoxine, 3; pantothenic acid, 18; niacin, 40; folacin, 1.5; choline, 600; biotin, 0.7 and cyanocobalamin, 0.02 (Kimia Roshd Co, Gorgan, Iran).

[4]Sigma-Aldrich Co, Germany.

[5]Kimia Roshd Co, Gorgan, Iran.

[6]Kimia Roshd Co, Gorgan, Iran.

[7]Kimia Roshd Co, Gorgan, Iran.

[8]Sigma-Aldrich Co, Germany.

Proximate composition of the diet

The chemical composition of the experimental diets was analyzed in triplicate based on standard methods (AOAC, 2005). The dry matter was measured by oven drying at 105°C for 24 h. Also, the crude protein (N×6.25) was determined by the Kjeldahl system (Buchi Labortechnik AG, Flawil, Switzerland) after acid digestion. In addition, the crude lipid was measured by the Soxtec system HT 1043 (Foss Tecator, AB). The ash content of diets was determined by placing the samples in a muffle furnace (Exciton Co., EX.1200-2 L, model) at 550°C for 12 h. The crude fiber of the experimental diets was analyzed using NaOH (Merck Co.) and H_2SO_4 (Merck Co.) solutions to digest the diet samples. Then, the digested samples were placed in crucibles and dried at 120°C for 12 h. The crucibles were transferred into a muffle furnace at 550°C. Finally, the weight of the crucibles was measured.

Fish and experimental conditions

The African cichlid (*Labidochromis lividus*) fingerlings (average weight 0.5±0.002 g and average length 3.79±0.05 cm, n=250) were purchased from a local supplier of ornamental fish in Mashhad, Razavi Khorasan province. The fish were maintained in two 250 L fiberglass tanks filled with dechlorinated tap water under continuous aeration and 12:12 light: dark photoperiod for two weeks. During the acclimation period, the fish were fed the basal diet twice a day. In the following, the fish were randomly divided into four groups in three replicates (n=18 each replicate) and stocked in a 12-glass aquarium (150 L) filled with 130 L of dechlorinated tap water. Each glass aquarium was equipped with an air stone for continuous aeration using a central air pump (Hailea ACO 318, India), an aquarium heater, and a white fluorescent lamp to adjust the water temperature to 25°C and 12:12 light: dark cycle, respectively. The fish were fed with the experimental diets three times daily at 8:00 a.m., 12:00 p.m., and 4:00 p.m. to apparent satiation for eight weeks. During the feeding period, 25% of the water in each aquarium was replaced with freshwater every day. The feces were also siphoned after 1 hour of feeding. During the eight weeks of the experiment, we recorded the temperature, dissolved oxygen, and pH of water in each aquarium using a portable multi-meter (AZ-8603, model) as 25±2.3°C, 6.25±0.65 mg L^{-1}, and 7.30±0.6, respectively.

Assessment of growth performance

In the present study, we analyzed the effects of two forms of dietary SP on the growth performance and survival of fish through measurement of the final length and weight of fish in each aquarium at the end of the feeding trial. The parameters of growth performance and the survival rate of fish were determined using the following formula:

Weight gain (g) = [final body weight (g) − initial body weight (g)]

Specific growth rate (%) = (SGR; % Body weight day^{-1}) = [(Ln final body weight (g)) - Ln initial body weight (g))/ Time (day)] × 100

Daily growth index (DGI) (g) = [(final body weight (g) − initial body weight (g)) / Time (day)]

Feed conversion ratio (FCR) = (Feed consumed (g) / Weight gain (g))

Condition factor (CF) (g cm^{-3}) = [final weight (g) / final length $(cm)^3$] × 100

Survival rate (%) = (final number of fish / initial number of fish) × 100

Digestive enzymes assessment

At the end of the feeding experiment, the fish were starved for 24 h. Then, three fish were randomly selected from each aquarium and anesthetized individually using ground clove oil (80 mg L^{-1}). Afterward, intestine tissue was removed from each fish's body and rinsed with cold distilled water (4°C) (Huang et al., 1999). Tissue homogenization was performed by a homogenizer (IKA T25 model) in 0.2 M NaCl (1:5; w/v) (Gawlicka et al., 2000). Homogenates were centrifuged (15000×g, 4°C) for 15 min and supernatants were kept at –80°C and used to measure digestive enzymes' activities by an ultraviolet-visible (UV-vis) spectrophotometer (DR 5000™ model, HACH Co., USA). Results were reported as U mg^{-1} protein min^{-1}.

Protease activity was assayed according to the casein-hydrolysis method (Hidalgo et al., 1999). Trypsin activity in the intestine homogenate was measured according to the method by Erlanger et al. (1961). The N-α-benzoyl-L-arginine-p nitroanilide (BAPNA) was used as a substrate. The α-amylase activity was determined based on the method of the 3, 5-dinitrosalicylic acid (Worthington, 1991). Also, lipase activity was assayed based on the method detailed by Gawlicka et al. (2000) using p-nitrophenylmyristate as a substrate. Finally, the activity of the alkaline phosphatase (ALP) enzyme was determined using a commercial kit (Pars Azmoon Company, Iran) at an optical density (OD) of 405 nm.

Immunological assessment

Collection of skin mucus was performed according to the protocol described by Subramanian et al. (2007). Briefly, fish were starved for 24 h at the end of the feeding trial, and three specimens were randomly sampled from each glass aquarium. The sampled fish were anesthetized using clove oil (80 mg L^{-1}) and then transferred individually into a polyethylene bag containing NaCl solution (50 mM; 5 mL g^{-1} fish; Merck, Germany). Fish were rubbed slowly inside the plastic bag for 1–2 min to collect the skin mucus. Subsequently, the collected mucus was immediately poured into a test tube (15 mL) and centrifuged (1500×g for 10 min at 4°C). Eventually, the obtained supernatant was stored at –80°C for future analysis.

The skin mucus total immunoglobulin (Ig) was determined according to the procedure proposed by Siwicki et al. (1994). First, the total protein content of each skin mucus sample was measured based on the standard

method described by Lowry et al. (1951). Subsequently, Ig molecules were precipitated using a 12% polyethylene glycol solution, and each sample's total protein was re-measured. The difference in the two measured protein contents was calculated as the total Ig content. The lysozyme activity of samples was determined by the lysis of the lysozyme-sensitive Gram-positive *Micrococcus luteus* bacterium, as explained by Hoseinifar et al. (2016). Alternative complement pathway hemolytic activity (ACH50) of samples was measured following the method explained by Yano (1992). Briefly, diluted skin mucus samples from 50 to 250 µL were poured in tube tests. Next, barbitone buffer in the presence of ethyleneglycol-bis (2-aminoethoxy)-tetraacetic acid (EGTA) and Mg^{2+} was added to increase the total volume of each test tube to 250 µL. Afterward, 100 µL of New Zealand Rabbit Red Blood Cells (RaRBC) was allotted into each tube test and incubated for 90 min at 20°C. In the following, 3.15 mL of NaCl was added to each tube test and centrifuged for 5 min at 1600×g. The OD of obtained supernatant was read at 414 nm. Finally, the number of ACH50 unit mL^{-1} was determined based on the skin mucus volume producing 50% ACH.

Liver enzymatic analysis

The liver enzymes activities were measured in the sampled fish at the end of the feeding experiment. The liver tissues of the fish were removed, washed with *ice*-cold 0.95% saline and homogenized in the presence of ice-cold 0.1 M Tris HCL buffer (pH=7.1) using a glass homogenizer. Next, the homogenized tissues were centrifuged (1000 rpm, 4°C for 10 min), and the obtained supernatants were stored at -80°C to measure the liver enzymes in the future (Jindal et al., 2018).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined based on the colorimetric method described by the Frankel-Reitman method (Reitman and Frankel, 1957) using commercial kits (Ziest Chem Diagnostic Co., Tehran, Iran) at a wavelength of 505 nm. Superoxide dismutase (SOD) activity was measured according to the procedure explored by Marklund and Marklund (1974). Catalase (CAT) activity was measured according to the methodology of Aebi (1984) using hydrogen peroxide (H_2O_2) as a substrate. Alkaline phosphatase (ALP) activity was examined using a commercial kit (Pars Azmoon Company, Iran) at an OD of 405 nm.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). SPSS software (Version, 19) was applied for statistical analyses. Also, the normality assumption of data was assayed by performing the Kolmogorov-Smirnov test. Significant differences between means at $P<0.05$ were determined by subjecting the data to one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test.

Results

Characterization of synthesized MIP-SP particles

Synthesized MIP-SP particles with small spherical shape were shown in Fe-SEM and TEM micrographs (Figure 1 a, c). The loading of SP into MIP particles was confirmed by the presence of sodium (Na^+) ions in the EDS analysis (Figure 1 b). The measurement of particle sizes on TEM micrographs showed that the particles had a mean diameter of 61.22±13.90 nm with a size distribution of 27.87 to 98.97 nm (Figure 1 c, d). Based on the result of ICP-OES, the amount of SP loaded into MIP nanoparticles was 8.5%.

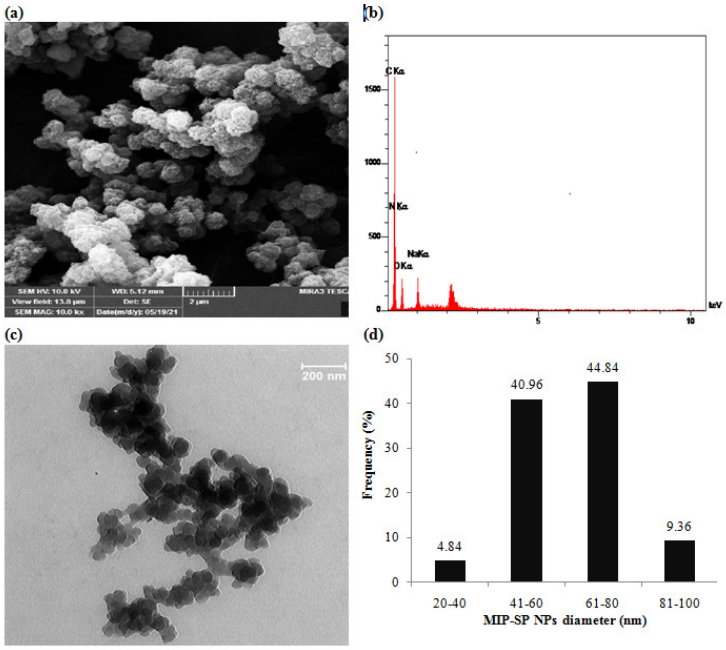

Figure 1. FE-SEM micrograph (a), EDS pattern (b), TEM micrograph (c) and histogram for particle size distribution of MIP-SP nanoparticles (d)

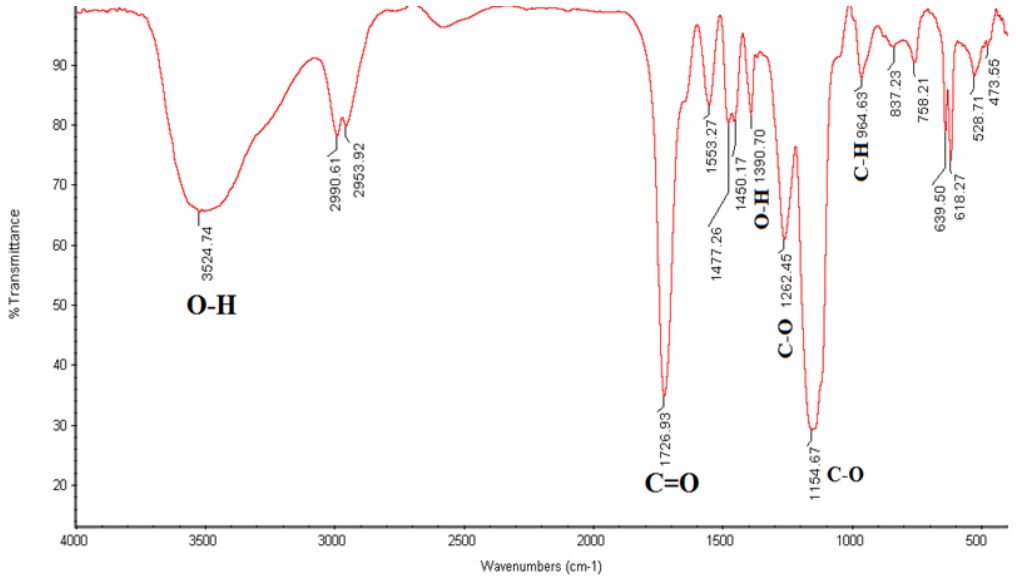

Figure 2. FT-IR spectra of MIP-SP NPs

Table 2. Growth performance, feed utilization indices and survival rate of African cichlid (*L. lividus*) fingerlings fed on diets supplemented with different forms of sodium propionate for 56 days (mean ± SD, n=3)

Parameters	Dietary sodium propionate groups			
	control	MIP NPs	P-SP	MIP-SP NPs
Initial weight (g)	0.51±0.017 a	0.50±0.006 a	0.50±0.002 a	0.50±0.009 a
Final weight (g)	5.12±0.32 a	5.24±0.09 a	6.11±0.17 b	6.83±0.64 c
Weight gain (g)	4.60±0.33 a	4.73±0.08 a	5.61±0.17 b	6.33±0.65 c
SGR (%BW day^{-1})	4.11±0.15 a	4.16±0.009 a	4.45±0.05 b	4.64±0.19 b
DGI (g)	0.082±0.005 a	0.084±0.001 a	0.10±0.003 b	0.12±0.010 c
CF (g cm^{-3})	1.56±0.14 a	1.57±0.13 a	1.83±0.12 a	1.87±0.19 a
FCR	2.18±0.043 c	2.19±0.09 c	1.80±0.12 b	1.52±0.166 a
Survival rate (%)	83.33±5.55 a	85.18±3.20 a	87.03±8.48 a	92.59±6.41 a

MIP-NPs: molecular imprinted polymer nanoparticles; P-SP: powder sodium propionate; MIP-SP NPs: molecular imprinted polymer-sodium propionate nanoparticles. SGR: specific growth rate; DGI: daily growth index; CF: condition factor; FCR: food conversion ratio. Data assigned with different letters in a row are significantly different (ANOVA, P<0.05).

Figure 2 presents the FT-IR spectra obtained for MIP-SP NPs. In this figure, the typical acrylic polymer bands are clearly observed in the FT-IR spectrum. The appeared signal at 3524 cm^{-1} is attributed to the presence of O-H stretching. The bending vibration of the hydroxyl group is also observed at 1390.7 cm^{-1}. The strong signal in the spectral region of 1726 cm^{-1} can be assigned to the C=O functional groups at propionate, methacrylic acid, and acrylic ester. Also, the signals that appeared at 1262.4 cm^{-1} and 1154.6 cm^{-1} are for the symmetric and asymmetric C-O stretch bands, respectively. Other important absorption peaks that were shown at 1640 cm^{-1} and 964.6 cm^{-1} are attributed to the presence of stretching and out-of-plane vibration of residual vinylic C=C and C-H bonds, respectively.

Growth performance

Table 2 shows the growth parameters and survival rate of African cichlid fingerlings fed on diets supplemented with two forms of sodium propionate for 8 weeks. Overall, no significant difference was observed between the initial weight of fish in different dietary groups (P>0.05). The final weight (FW), weight gain (WG), specific growth rate (SGR), and daily growth index (DGI) of fish increased in SP dietary groups compared to control groups (P>0.05). The highest FW, WG, and DGI indices were observed in the MIP-SP NPs dietary group (P<0.05). The FCR value decreased in the fish fed on SP-supplemented diets compared to the controls (P<0.05). In addition, fish fed on a MIP-SP NPs-supplemented diet showed the lowest FCR (P<0.05). The condition factor (CF) parameter

and survival rate (%) showed no significant differences between the dietary groups (P>0.05).

Digestive enzymes assays

Table 3 presents the effects of different SP-supplemented diets on the digestive enzymes activities. The protease, trypsin, lipase, and alkaline phosphatase activities were higher in the SP dietary groups than in the control groups (P<0.05). Also, the protease and lipase activities were significantly higher in the MIP-SP NPs dietary group than in the P-SP dietary group (P<0.05). No significant difference in the α-amylase activity was observed between different dietary groups (P>0.05).

Immunological analyses

Figure 3 (a–c) shows the changes in the skin mucus immune parameters in the fish fed SP-supplemented diets. The total immunoglobulin (Ig), lysozyme (LYZ), and alternative hemolytic complement (ACH50) activities were higher in the SP dietary groups than in the control groups (P<0.05). The highest levels of these indices were observed in the MIP-SP NPs dietary group (P<0.05).

Liver enzymatic assessment

Figure 4 (a–e) presents the liver enzyme levels in the fish fed on SP-supplemented diets. The alanine aminotransferase (ALT) and the aspartate aminotransferase (AST) levels decreased significantly in the SP-supplemented dietary groups compared to the control groups (P<0.05) (Figure 4 a–b). The SP-dietary groups showed higher superoxide dismutase (SOD), catalase (CAT), and alkaline phosphatase (ALP) levels than the controls (P<0.05). The SOD and ALP levels in the fish fed on a MIP-SP NPs-supplemented diet were higher than those fed on a P-SP-supplemented diet (P<0.05) (Figure 4 c, e).

Table 3. Digestive enzymes activity (U mg protein^{-1} min^{-1}) of African cichlid (*L. lividus*) fed on diets supplemented with different forms of sodium propionate for 56 days (mean ± SD, n=3)

Enzyme	Dietary sodium propionate groups			
	control	MIP NPs	P-SP	MIP-SP NPs
Protease	1.50±0.065 a	1.52±0.015 a	1.79±0.020 b	1.94±0.01 c
Trypsin	1.22±0.086 a	1.25±0.049 a	1.47±0.020 b	1.56±0.021 b
Lipase	1.27±0.045 a	1.28±0.070 a	1.47±0.045 b	1.89±0.025 c
Amylase	0.55±0.015 a	0.56±0.25 a	0.57±0.30 a	0.58±0.20 a
Alkaline phosphatase	0.93±0.060 a	0.90±0.035 a	1.26±0.065 b	1.41±0.17 b

MIP-NPs: molecular imprinted polymer nanoparticles; P-SP: powder sodium propionate; MIP-SP NPs: molecular imprinted polymer-sodium propionate nanoparticles. Data assigned with different letters in a row are significantly different (ANOVA, P<0.05).

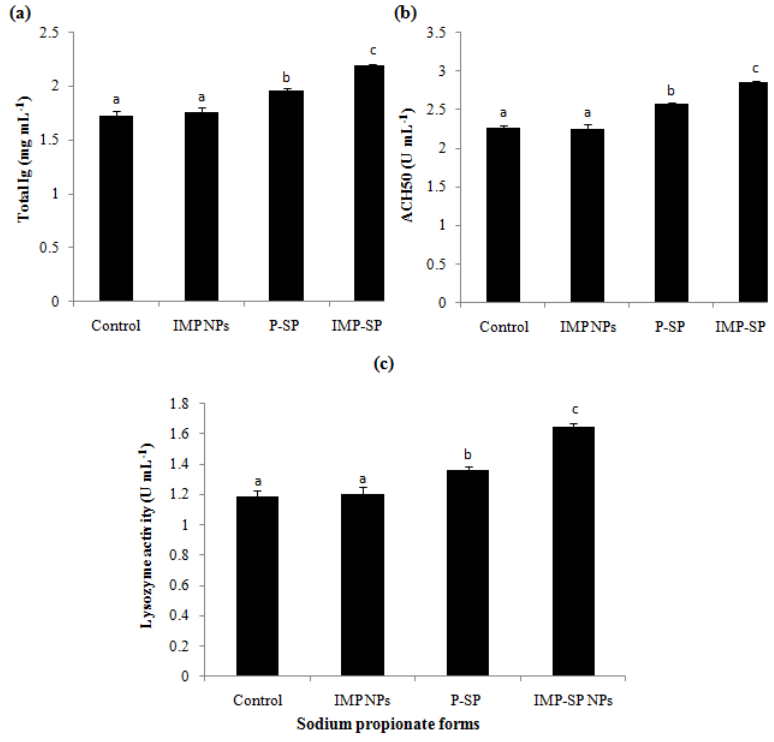

Figure 3. Total immunoglobulin (Ig) (a), alternative hemolytic complement activity (ACH50) (b) and lysozyme activity (c) levels in skin mucus of African cichlid (*L. lividus*) fingerlings fed on diets supplemented with different forms of sodium propionate for 56 days. Bars assigned with different letters are significantly different (ANOVA, P<0.05). MIP-NPs: molecular imprinted polymer nanoparticles; P-SP: powder sodium propionate; MIP-SP NPs: molecular imprinted polymer-sodium propionate nanoparticles

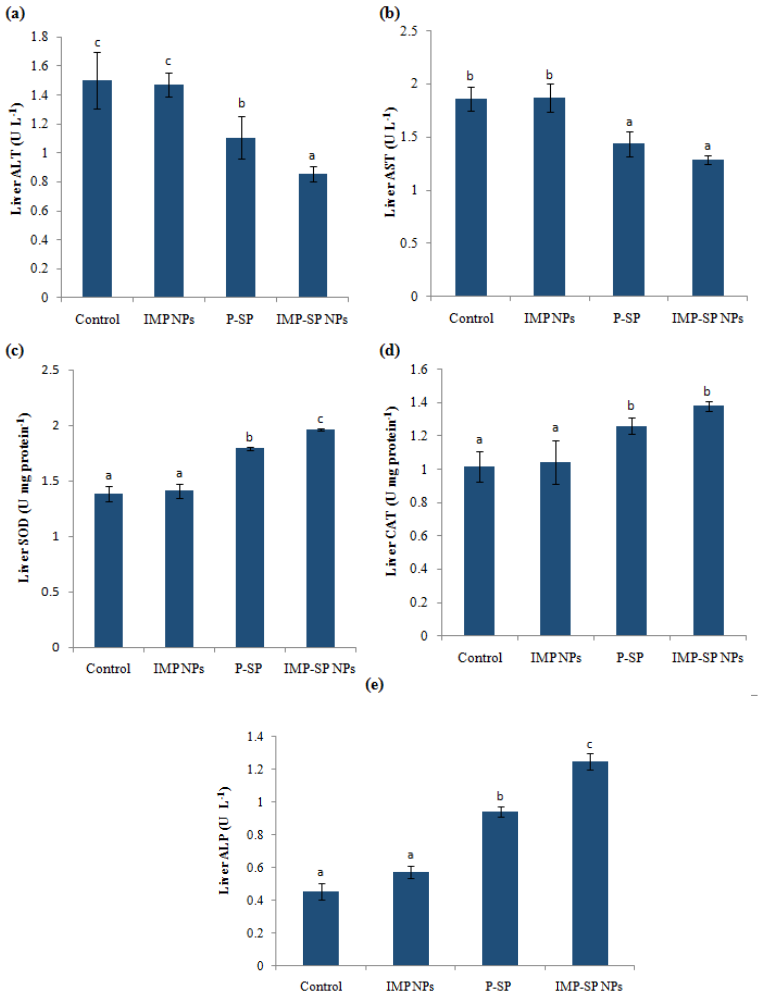

Figure 4. Alanine aminotransferase (ALT) (a), aspartate aminotransferase (AST) (b), superoxide dismutase (SOD) (c), catalase (CAT) (d), and alkaline phosphatase (ALP) (e) levels in liver of African cichlid (*L. lividus*) fingerlings fed on diets supplemented with different forms of sodium propionate for 56 days. Bars assigned with different letters are significantly different (ANOVA, $P<0.05$). MIP-NPs: molecular imprinted polymer nanoparticles; P-SP: powder sodium propionate; MIP-SP NPs: molecular imprinted polymer-sodium propionate nanoparticles

Discussion

Encapsulating pharmaceutical agents in a carrier can be considered as a suitable approach to improve their efficiency for aquatic animals by slow and controlled release and reducing leaching. Inorganic and organic carriers (e.g., synthetic polymers) are two major types of targeted or non-targeted drug deliveries (Senapati et al., 2018). In the current study, sodium propionate (SP) was encapsulated in molecular imprinted polymer (MIP) nanoparticles as a drug delivery vehicle. The characterization of synthesized particles showed that the MIP particles were in nanoscale with a mean diameter of 61.22±13.0 nm and loaded with SP (8.5%). In this study, we investigated the effects of dietary MIP-SP NPs on the survival, growth, digestive, and antioxidant enzymes' activity and skin mucus indices of African cichlid (*L. lividus*) fingerlings compared to the powder form of sodium propionate (P-SP).

Feeding the African cichlid fingerlings with SP-supplemented diets at 5 g kg^{-1} of dry diet for 56 days improved growth performance, including the final weight, WG, SGR (%), DGI, and FCR. Several studies have attributed the improvement of nutrient utilization and growth performance of aquatic animals fed on diets containing organic acids and their salts. These compounds also lower digestion pH (Chowdhury et al., 2021), change the microbial population of the intestinal tract (Ng and Koh, 2016), stimulate digestive enzyme secretion (Chowdhury et al., 2021), and increase feed intake (Da Silva et al., 2015; Omosowone et al., 2018). Omosowone et al. (2018) showed that *Clarias gariepinus* and *Oreochromis niloticus* fingerlings fed a 2% butyric acid-supplemented diet for 12 weeks had better feed utilization and growth performance. Feeding red sea bream *Pagrus major* with 1% citric acid improved weight gain, FCR values, and phosphorus absorption from dietary components (Hossain et al., 2007). Fabay et al. (2022)

also reported that supplementing the Nile tilapia diet with 0.2% formic and propionic acid/salt increased protein and fat retention rates.

The administration of encapsulated form of SP had more significant effect on the final weight, WG, DGI, and FCR than the powder form of SP. Nanoencapsulation of bioactive ingredients improves their bioavailability by increasing the surface-to-volume ratio of nanocarrier, enhancing their interaction with metabolism and enzyme factors and allowing them to pass through cell walls (McClements and Jafari, 2018). Nanocarriers protect bioactive ingredients from premature degradation in the biological environment, increase cellular uptake, and prolong their presence in the blood (Kumari et al., 2010). Safari et al. (2021) reported that dietary administration of encapsulated organic salts (Na-acetate, Na-butyrate, Na-lactate and Na-propionate) at 20 g kg^{-1} improved the growth performance and survival of crawfish (*Astacus leptodactylus leptodactylus*). Chow et al. (2017) attributed the improved growth performance of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fed on encapsulated butyric acid (ButiPEARL)-supplemented diet to the slow release of the butyric acid in the gastrointestinal tract. They reported the better growth of villi in the small intestine due to more accessibility of butyric acid and thus better digestibility of nutrients. Kalantarian et al. (2020) reported that feeding *Salmo trutta caspius* juveniles with diets supplemented with 5, 10 and 15 g sodium diformate kg^{-1} diet for 60 days significantly increased villi height in the proximal area of intestine.

One possible reason for improving the growth performance of African cichlid fingerlings fed on SP-supplemented diets might be the increased activity of digestive enzymes, including the trypsin, protease, alkaline phosphatase, and lipase. Results of a research also showed an increase in the trypsin, protease, and lipase activities in *S. trutta caspius* fed sodium diformate-supplemented diets for 60 days (Kalantarian et al., 2020). The increase in digestive enzyme activity might be due to the secretion of secretin resulting from acidification of the gastrointestinal tract (Ng and Koh, 2016). The current study indicated the more positive effect of encapsulated sodium propionate on the activities of protease and lipase enzymes than its powder form. The nanoparticle delivery system enhances the absorption of encapsulated bioactive compounds in the gastrointestinal tract through active endocytosis (Rieux et al., 2006). Internalized nanoparticles in epithelial cells may be translocated to endo/lysosome and degraded in lysosome to release their contents. Alternatively, it may remain intact in endolysosome and enter blood circulation by exocytosis (Qian et al., 2009; Li et al., 2014). Encapsulation of bioactive compounds is a practical approach for slow release and delivering them at the intended location of the gastrointestinal tract (Piva et al., 2007; Chen et al., 2017). Tian et al. (2017) found that microencapsulated sodium butyrate (MSB) had a superior effect on the improvement of the trypsin, lipase, amylase, and chymotrypsin activities compared to powder sodium butyrate (PSB) in grass carp (*Ctenopharyngodon idella*).

Disease outbreaks are considered among the major challenges to expand the aquaculture industry due to causing significant economic losses (Opiyo et al., 2018). Fish have developed several mechanisms to fight pathogenic microorganisms that inhabit aquatic environments (Cámara-Ruiz et al., 2021). The mucosal surfaces including gill, skin, gut, and olfactory organs, are coated by a mucosal layer, thereby providing the first line of defense mechanism in aquatic organisms (Rombout et al., 2011; Benhamed et al., 2014). The mucosal layer contains potent bioactive molecules such as antimicrobial peptides, lysozyme, complement proteins, immunoglobulins, lectins, and hemolysins (Palaksha et al., 2008; Nigam et al., 2012). Several studies have evaluated the effects of different feed additives such as organic acids and their salts on enhancing the innate immune system in aquatic organisms (Safari et al., 2016; Busti et al., 2020; Chowdhury et al., 2021). Organic acids like propionate and acetate can act as ligands for G protein-coupled receptor 43 and modulate fish immunity (Maslowski and Mackay, 2011). Sotoudeh et al. (2020) reported that administration of diets supplemented with blends of organic acids (sodium propionate and sodium acetate) for eight weeks increased plasma lysozyme and ACH50 of yellowfin seabream (*Acanthopagrus latus*) juveniles. Total immunoglobulin and lysozyme activity increased in the serum and skin mucus of sterlet sturgeon (*Acipenser ruthenus*) with increasing the dietary levels of potassium diformate (KDF) (Kakavand et al., 2021). In another study, Safari et al. (2017) showed up-regulation of immune-related genes expression with increasing lysozyme activity and Ig level in the skin mucus of common carp (*C. carpio*) fed SP-supplemented diets for eight weeks. Likewise, the present study showed that the inclusion of two different forms of SP into the diet significantly increased the lysozyme and total Ig levels, and ACH50 activity in the skin mucus. The SP administration in nanoencapsulated form showed greater incremental effects on the mucosal immunity of African cichlid. In this respect, Busti et al. (2020) stated that encapsulated organic acids and identical natural compounds are protected from degradation in the stomach and arrive intact in the intestine tract, where they exert their effects on the gut microbiota of European sea bass juveniles. Some studies have reported that gut microbiota is critical in regulating fish's innate immunity (Gómez and Balcázar, 2008; Nie et al., 2017). Abu Elala and Ragaa (2014) reported the modification of beneficial intestinal flora in the gut of tilapia (*Oreochromis niloticus*) after feeding with 0.3% potassium diformate (KDF), which resulted in the activation of humeral and cellular innate immune responses.

AST and ALT enzymes are found in the kidney, heart, muscles, and mainly in liver cells (Rastiannasab et al., 2016). These enzymes metabolize protein and break down food to produce energy and their tissue activities are markers for liver function (Metón et al., 2015; Ras-

tiannasab et al., 2016). Increased AST and ALT levels in the liver and blood indicate damage to liver cells (Huang et al., 2006; Rastiannasab et al., 2016). The current study showed that feeding the African cichlid fingerlings with diets supplemented with MIP NPs and different forms of SP did not increase AST and ALT levels in the liver tissue. These results indicated that the supplemented diets had no detrimental effect on liver tissue function. Agouz et al. (2015) also found that ALT and AST levels did not increase significantly in the blood of Nile tilapia *O. niloticus* fed on diets containing 1% and 1.5% of two organic acid salts (calcium lactate + sodium acetate, 1:1) blend compared to the control group.

Alkaline phosphatase (ALP) is a poly-functional enzyme that plays a vital role in the membrane transport activities, mineralization of the skeleton of aquatic organisms (Zikic et al., 2001), growth, and protein synthesis (Ram and Sathyanesan, 1985). Administration of dietary encapsulated organic acid significantly increased ALP level in the serum of Pacific white shrimp (*Litopenaeus vannamei*) compared to the control group (Chowdhury et al., 2021). In the present study, the liver ALP level increased significantly in the SP dietary groups.

Factors such as feeding behavior and environmental parameters can either enhance or weaken the antioxidant defense response in fish and shellfish (Martínez-Álvarez et al., 2005; Hoseinifar et al., 2020). Some studies have reported the beneficial effects of feed additives such as probiotics, prebiotics, and synbiotics on promoting antioxidant enzyme activity in different fish species (Dawood et al., 2018; Van Doan et al., 2020). Up-regulation in genes of glutathione peroxidase (GPx), glutathione-disulfide reductase (GSR), and glutathione S-transferase (GSTA) as antioxidant enzymes was noticed in the liver of common carp (*C. carpio*) fed on diets containing 1% and 2% of SP (Safari et al., 2017). Chiu et al. (2008) also found an increase in SOD activity in grouper juveniles (*Epinephelus fuscoguttatus*) fed on diets containing 1.0 and 2.0 g kg^{-1} of sodium alginate. The present study showed that administration of different forms of SP in the diets at 5 g kg^{-1} significantly increased the activity of SOD and CAT liver enzymes. In contrast to these results, Safari et al. (2016) showed down-regulation of CAT and SOD genes in the liver of zebra fish (*Danio rerio*) fed diets supplemented with 5, 10, and 20 g SP/kg of diet.

The bioavailability of antioxidant molecules is limited due to the difficulty of passing through the cell membranes and degradation during tissue delivery. Nano-encapsulated antioxidant molecules provide advantages such as increased bioavailability and controlled release of antioxidants (Khalil et al., 2020). The findings of our study revealed that the liver SOD activity increased in the fish fed on a MIP-SP NPs-supplemented diet compared to those fed with a P-SP-supplemented diet.

Conclusion

This study was conducted to compare the effects of two forms of sodium propionate on the growth performance, mucosal immune response, and digestive and liver enzymes activities of African cichlid (*L. lividus*) fingerlings. In conclusion, sodium propionate encapsulated in MIP-nanoparticles showed higher efficiency than powder sodium propionate in improving African cichlid fingerlings' growth performance and mucosal immune response. It is recommended to use modified MIP nanoparticles as an effective strategy for the active delivery of pharmaceutical compounds to the target sites in fish and shellfish species.

Acknowledgments

The authors thank the staff of the Faculty of Natural Resources and Environment of Ferdowsi University of Mashhad (FUM) for their cooperation in conducting this research. This study was financially supported by a grant from the Ferdowsi University of Mashhad (No. 2/47528).

Conflict of interest

The authors declare that there are no conflicts of interest.

Ethics approval

All fish experiments were performed according to Ferdowsi University of Mashhad (FUM) animal ethic right and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals.

Funding

This work was supported by a grant from Ferdowsi University of Mashhad (No. 2/47528).

Authors' contributions

Mehrdad Sarkheil: supervision, conceptualization, methodology, data collection, project administration, writing – original draft preparation; Omid Safari: writing – review and editing; Davood Kordestani: conceptualization, methodology, writing – review and editing. All authors read and approved the final version.

References

Abu Elala N.M., Ragaa N.M. (2015). Eubiotic effect of a dietary acidifier (potassium diformate) on the health status of cultured *Oreochromis niloticus*. J. Adv. Res., 6: 621–629.

Aebi H. (1984). Catalase *in vitro*. Meth. Enzymol., 105: 121–126.

Agouz H.M., Soltan M.A., Meshrf R.N. (2015). Effect of some organic acids and organic salt blends on growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*). Egypt. J. Nutr. Feeds, 18: 443–450.

AOAC (2005). Official Methods of Analysis, 18th ed. AOAC International, Maryland, USA.

Asadi E., Abdouss M., Leblanc R.M., Ezzati N., Wilson J.N., Kordestani D. (2016). Synthesis, characterization and *in vivo* drug delivery study of a biodegradable nano-structured molecularly imprinted polymer based on cross-linker of fructose. Polymer, 97: 226–237.

Baruah K., Pal A.K., Sahu N.P., Jain K.K., Mukherjee S.C., Debnath D. (2005). Dietary protein level, microbial phytase, citric acid and

their interactions on bone mineralization of *Labeo rohita* (Hamilton) juveniles. Aquac. Res., 36: 803–812.
Baruah K., Norouzitallab P., Debnath D., Pal A.K., Sahu N.P. (2008). Organic acids as non-antibiotic nutraceuticals in fish and prawn feed. Aquacult. Health Int., 12: 4–6.
Benhamed S., Guardiola F.A., Mars M., Esteban M.Á. (2014). Pathogen bacteria adhesion to skin mucus of fishes. Vet. Microbiol., 171: 1–12.
Boland F.E., Lin R.C., Mulvaney T.R., Mcclure F.D., Johnston M.R. (1981). pH determination in acidified foods: collaborative study. J. Assoc. Off. Anal. Chem., 46: 332–336.
Booth I.R., Stratford M. (2003). Acidulants and low pH. In: Food preservatives, Russel N.J., Gould G.W. (eds). Kluwer Academic/Plenum Publishers, New York, pp. 25–47.
Busti S., Rossi B., Volpe E., Ciulli S., Piva A., Amico F.D., Soverini M., Candela M., Gatta P.P., Bonaldo A., Grilli E., Parma L. (2020). Effects of dietary organic acids and nature identical compounds on growth, immune parameters and gut microbiota of European seabass. Sci. Rep., 10: 21321.
Cabello F.C., Godfrey H.P., Tomova A. (2013). Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. Environ. Microbial., 15: 1917–1942.
Cámara-Ruiz M., Carmen Balebona B., Moriñigo M.A., Esteban M.A. (2020). *Probiotic Shewanella putrefaciens* (SpPdp11) as a fish health modulator: a review. Microorganisms, 8: 1990.
Cámara-Ruiz M., Cerezo I.M., Guardiola F.A., García-Beltrán J.M., Balebona M.C., Moriñigo M.Á., Esteban M.Á. (2021). Alteration of the immune response and the microbiota of the skin during a natural infection by *Vibrio harveyi* in European Seabass (*Dicentrarchus labrax*). Microorganisms, 9: 964.
Castillo S., Rosales M., Pohlenz C., Gatlin D.M. (2014). Effects of organic acids on growth performance and digestive enzyme activities of juvenile red drum *Sciaenops ocellatus*. Aquaculture, 433: 6–12.
Chen J., Wang Q., Liu C.M., Gong J. (2017). Issues deserve attention in encapsulating probiotics: critical review of existing literatures. Crit. Rev. Food Sci. Nut., 57: 1228–1238.
Chiu S.T., Tsai R.T., Hsu J.P., Liu C.H., Cheng W. (2008). Dietary sodium alginate administration to enhance the non-specific immune responses, and disease resistance of the juvenile grouper *Epinephelus fuscoguttatus*. Aquaculture, 27: 66–72.
Chow E.P.Y., Liong K.H., Schoeters E. (2017). Dietary encapsulated butyric acid (Butipearl™) and microemulsified carotenoids (Quantum GLO™ Y) on the growth, immune parameters and their synergistic effect on pigmentation of hybrid Catfish (*Clarias macrocephalus* × *Clarias gariepinus*). J. Fish. Aquac., 8: 2.
Chowdhury M.A.K., Song H., Liu Y., Bunod J.D., Dong X.H. (2021). Effects of microencapsulated organic acid and their salts on growth performance, immunity and disease resistance of Pacific White Shrimp *Litopenaeus vannamei*. Sustainability, 13: 7791.
Cunliffe D., Kirby A., Alexander C. (2005). Molecularly imprinted drug delivery systems. Adv. Drug. Deliv., 57: 1836–1853.
Da Silva B.C., Vieira F.N., Mourino J.L.P., Bolivar N., Seiffert W.Q. (2015). Butyrate and propionate improve the growth performance of *Litopenaeus vannamei*. Aquac. Res., 4: 7.
Dawood M.A.O., Koshio S., Esteban M.A. (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev. Aquacult., 10: 950–974.
Defoirdt T., Boon N., Sorgeloos P., Verstraete W., Bossier P. (2009). Short-chain fatty acids and poly-β-hydroxyalkanoates: (New) Biocontrol agents for a sustainable animal production. Biotech. Advan., 27: 680–685.
Erlanger B.F., Kokowsky N., Cohen W. (1961). The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem. Biophys., 95: 271–278.
Fabay R.V., Serrano A.E. Jr, Alejos M.S., Fabay J.V. (2022). Effects of dietary acidification and acid source on fish growth and feed efficiency (Review). World Acad. Sci. J., 4: 21.
Gao L., Wang J., Li X., Yan Y., Li C., Pan J. (2014). A core-shell surface magnetic molecularly imprinted polymers with fluorescence for λ-cyhalothrin selective recognition. Anal. Bioanal. Chem., 406: 7213–7220.
Gao Y., Storebakken T., Shearer K.D., Penn M., Øverland M. (2011). Supplementation of fishmeal and plant-protein based diets for rainbow trout with a mixture of sodium formate and butyrate. Aquaculture, 211: 233–240.
Gawlicka A., Parent B., Horn M.H., Ross N., Opstad I., Torrissen O.J. (2000). Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): indication of readiness for first feeding. Aquaculture, 184: 303–314.
Gómez G.D., Balcázar J.L. (2008). A review on the interactions between gut microbiota and innate immunity of fish. FEMS Microbiol. Immunol., 52: 145–154.
He W., Rahimnejad S., Wang L., Song K., Lu K., Zhang C. (2017). Effects of organic acids and essential oils blend on growth, gut microbiota, immune response and disease resistance of Pacific white shrimp (*Litopenaeus vannamei*) against *Vibrio parahaemolyticus*. Fish Shellfish Immunol., 70: 164–173.
Hidalgo M.C., Urea E., Sanz A. (1999). Comparative study of digestive enzymes in fish with different nutritional habits: Proteolytic and amylase activities. Aquaculture, 170: 267–283.
Hoseinifar S.H., Zoheiri F., Caipang C.M. (2016). Dietary sodium propionate improved performance, mucosal and humoral immune responses in Caspian white fish (*Rutilus frisii kutum*) fry. Fish Shellfish Immunol., 55: 523–528.
Hoseinifar S.H., Yousefi S., Van Doan H., Ashouri G., Gioacchini G., Maradonna F., Carnevali O. (2020). Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. Rev. Fish. Sci. Aquac., 29: 20.
Hossain M.A., Pandey A., Satoh S. (2007). Effects of organic acids on growth and phosphorus utilization in red sea bream *Pagrus major*. Fish Sci., 73: 1309–1317.
Huang F., Yan A., Mu S., Wang X. (1999). The protease and amylase of *Hypophthalmichthy molitrix* and *Aristichthys nobilis*. J. Fish. Sci. China, 6: 14–17.
Huang X.J., Choi Y.K., Im H.S., Yarimaga O., Yoon E., Kim H.S. (2006). Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. Sensors, 6: 756–782.
Jaiswal L., Rakkit S., Pochin K., Jaisamut P., Tanthana C., Tanmanee N. (2015). A thalidomide templated molecularly imprinted polymer that promotes a biologically active chiral entity tagged in colon carcinoma cells and protein-related immune activation. Process Biochem., 50: 2035–2050.
Jindal R., Sinha R., Brar P. (2018). Evaluating the protective efficacy of *Silybum marianum* against deltamethrin induced hepatotoxicity in piscine model. Environ. Toxicol. Pharmacol., 66: 62–68.
Kakavand M., Shekarabi S.P.H., Mehrgan M.S., Islami H.R. (2021). Potassium diformate in the diet of sterlet sturgeon (*Acipenser ruthenus*): Zootechnical performance, humoral and skin mucosal immune responses, growth-related gene expression and intestine morphology. Aquac. Nutr., 27: 2392–2404.
Kalantarian S.H., Mirzargar S.S., Rahmati-Holasoo H., Sadeghinezhad J., Mohammadian T. (2020). Effects of oral administration of acidifier and probiotic on growth performance, digestive enzymes activities and intestinal histomorphology in *Salmo trutta caspius* (Kessler, 1877). Iran. J. Fish. Sci., 19: 1532–1555.
Khalil I., Yehye W.A., Etxeberria A.E., Alhadi A.A., Dezfooli S.M., Julkapli N.B.M., Basirun W.J., Seyfoddin A. (2020). Nanoantioxidants: recent trends in antioxidant delivery applications. Antioxidants, 9: 24.
Kumari A., Yadav S.K., Pakade Y.B., Singh B., Yadav S.C. (2010). Development of biodegradable nanoparticles for delivery of quercetin. Colloids Surf. B., 80: 184–192.
Li Z., Jiang H., Xu C., Gu L. (2014). A review: Using nanoparticles to enhance absorption and bioavailability of phenolic phytochemicals. Food Hydrocoll., 1–12.
Liu W., Yang Y., Zhang J., Gatlin D.M., Ringo E., Zhou Z. (2014). Effects of dietary microencapsulated sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (*Cyprinus carpio*) pre-fed with or without oxidised oil. Br. J. Nutr., 112: 15–29.
Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265–275.

Luliski P. (2013). Molecular imprinted polymers as the future drug delivery devices. Acta Pol. Pharm., 70: 601–609.
Marklund S., Marklund G. (1974). Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay of superoxide dismutase. Eur. J. Biochem., 46: 469–474.
Marshall B.M., Levy S.B. (2011). Food animals and antimicrobials: impacts on human health. Clin. Microbiol. Rev., 24: 718–733.
Martínez-Álvarez R.M., Morales A.E., Sanz A. (2005). Antioxidant defenses in fish: Biotic and abiotic factors. Rev. Fish Biol. Fish., 15: 75–88.
Maslowski K.M., Mackay C.R. (2011). Diet, gut microbiota and immune responses. Nat. Immunol., 12: 5–9.
McClements D.J., Jafari S.M. (2018). General aspects of nanoemulsions and their formulation. In: Nanoemulsions: formulation, applications, and characterization, Jafari S.M., McClements D.J. (eds). Academic Press, pp. 3–20.
Metón I., Salgado M.C., Anemaet I.G., González J.D., Fernández F., Baanante I.V. (2015). Alanine aminotransferase: A target to improve utilisation of dietary nutrients in aquaculture. Int. J. Recent Adv., pp. 133–148.
Morken T., Kraugerud O.F., Barrows F.T., Sørensen M., Storebakken T., Øverland M. (2011). Sodium diformate and extrusion temperature affect nutrient digestibility and physical quality of diets with fish meal and barley protein concentrate for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 317: 138–148.
Ng W.K., Koh C.B. (2016). The utilization and mode of action of organic acids in the feeds of cultured aquatic animals. Rev. Aquac., 9: 342–368.
Nie L., Zhou Q.J., Qiao Y., Chen J. (2017). Interplay between the gut microbiota and immune responses of ayu (*Plecoglossus altivelis*) during *Vibrio anguillarum* infection. Fish Shellfish Immunol., 68: 479–487.
Nigam A.K., Kumari U., Mittal A.K. (2012). Comparative analysis of innate immune parameters of the skin mucous secretions from certain freshwater teleosts, inhabiting different ecological niches. Fish Physiol. Biochem., 38: 1245–1256.
Omosowone O.O., Dada A.A., Adeparusi E.O. (2018). Comparison of dietary butyric acid supplementation effect on growth performance and body composition of *Clarias gariepinus* and *Oreochromis niloticus* fingerlings. Iran. J. Fish. Sci., 17: 403–412.
Opiyo M.A., Marijani E., Muendo P., Odede R., Leschen W., Charo-Karisa H.A. (2018). Review of aquaculture production and health management practices of farmed fish in Kenya. Int. J. Vet. Sci. Med., 6: 141–148.
Palaksha K.J., Shin G.W., Kim Y.R., Jung T.S. (2008). Evaluation of non-specific immune components from the skin mucus of olive flounder (*Paralichthys olivaceus*). Fish Shellfish Immunol., 24: 479–488.
Paramera E.I., Konteles S.J., Karathanos V.T. (2011). Stability and release properties of curcumin encapsulated in *Saccharomyces cerevisiae*, ß-cyclodextrin and modified starch. Food Chem., 125: 913–922.
Piletsky S., Turner A. (2006). A new generation of chemical sensors based on MIPs. In: Molecular imprinting of polymers, Piletsky S.A., Turner A.P.F. (eds). Landes Bioscience, Georgetown, TX, USA, pp. 64–79.
Piva A., Pizzamiglio V., Mauro M., Tedeshchi M., Piva G. (2007). Lipid microencapsulation allows slow release of organic acids and natural identical flavors along the swine intestine. Anim. Sci., 85: 486–493.
Poncelet D. (2006). Microencapsulation: fundamentals, methods and applications. Surf. Chem. Biomed. Environ. Sci., 228: 23–34.
Puoci F., Cirillo G., Curcio M., Parisi O.I., Iemma F., Picci N. (2011). Molecularly imprinted polymers in drug delivery: state of art and future perspectives. Expert Opin. Drug Deliv., 8: 1379–1393.
Qian M., Cai D., Verhey K., Tsai B. (2009). A lipid receptor sorts polyomavirus from the endolysosome to the endoplasmic reticulum to cause infection. PLoS Pathog., 5: e1000465.
Raja K., Aanand P., Padmavathy S., Sampathkumar J.S. (2019). Present and future market trends of Indian ornamental fish sector. Int. J. Fish. Aquat. Sci., 7: 06–15.
Ram R.N., Sathyanesan A.G. (1985). Mercuric chloride, cythion and ammonium sulfate induced changes in the brain, liver and ovarian alkaline phosphatase content in the fish *Channa punctatus*. Envir. Ecol., 3: 263–268.
Rashmeei M., Shekarabi S.P.H., Mehrgan M.S., Paknejad H. (2020). Stimulatory effect of dietary chasteberry (*Vitex agnus-castus*) extract on immunity, some immune-related gene expression, and resistance against *Aeromonas hydrophila* infection in goldfish (*Carassius auratus*). Fish Shellfish Immunol., 107: 129–136.
Rastiannasab A., Afsharmanesh S., Rahimi R., Sharifian I. (2016). Alternations in the liver enzymatic activity of common carp, *Cyprinus carpio* in response to parasites, *Dactylogyrus* spp. and *Gyrodactylus* spp. J. Parasit. Dis., 40: 1146–1149.
Reitman S., Frankel S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56–63.
Rico A., Satapornvanit K., Haque M.M., Min J., Nguyen P.T., Telfer T.C., van den Brink P.J. (2012). Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: a critical review. Rev Aquac., 4: 75–93.
Rieux A.D., Fievez V., Garinot M., Schneider Y.-J., Preat V. (2006). Nanoparticles s potential oral delivery systems of proteins and vaccines: a mechanistic approach. J. Control Rel., 116: 1–27.
Romano N., Koh C.B., Ng W.K. (2014). Dietary microencapsulated organic acids blend enhances growth, phosphorus utilization, immune response, hepatopancreatic integrity and resistance against *Vibrio harveyi* in white shrimp, *Litopenaeus vannamei*. Aquaculture, doi: 10.1016/j.aquaculture.2014.09.037
Rombout J.H.W.M., Abelli L., Picchietti S., Scapigliati G., Kiron V. (2011). Teleost intestinal immunology. Fish Shellfish Immunol., 31: 616–626.
Safari O., Paolucci M. (2018). Effect of in vitro selected synbiotics (galactooligosaccharide and mannanoligosaccharide with or without *Enterococcus faecalis*) on growth performance, immune responses and intestinal microbiota of juvenile narrow clawed crayfish, *Astacus leptodactylus leptodactylus* Eschscholtz, 1823. Aquac. Nutr., 24: 247–259.
Safari O., Sarkheil M. (2018). Dietary administration of eryngii mushroom (*Pleurotus eryngii*) powder on haemato-immunological responses, bactericidal activity of skin mucus and growth performance of koi carp fingerlings (*Cyprinus carpio* koi). Fish Shellfish Immunol., 80: 505–513.
Safari R., Hoseinifar S.H., Kavandi M. (2016). Modulation of antioxidant defence and immune response in zebra fish (*Danio rerio*) using dietary sodium propionate. Fish Physiol. Biochem., 42: 1733–1739.
Safari R., Hoseinifar S.H., Nejadmoghadam S., Khalili M. (2017). Non-specific immune parameters, immune, antioxidant and growth-related genes expression of common carp (*Cyprinus carpio* L.) fed sodium propionate. Aquac. Res., 48: 4470–4478.
Safari O., Paolucci M., Motlagh H.M. (2021). Effect of dietary encapsulated organic salts (Na-acetate, Na-butyrate, Na-lactate and Na-propionate) on growth performance, haemolymph, antioxidant and digestive enzyme activities and gut microbiota of juvenile narrow clawed crayfish, *Astacus leptodactylus* Eschscholtz, 1823. Aquac. Nutr., 27: 91–104.
Sajeevan T., Philip R., Singh I.B. (2009). Dose/frequency: a critical factor in the administration of glucan as immunostimulant to Indian white shrimp *Fenneropenaeus indicus*. Aquaculture, 287: 248–252.
Sarkheil M., Kordestani D., Safari O. (2021). Dietary supplementation with powder and gelatine micro and nanoencapsulated sodium propionate: Influence on growth performance, digestive and antioxidant enzymes and mucosal immunity of koi carp (*Cyprinus carpio* koi). Aquac. Nutr., 27: 2251–2266.
Senapati S., Mahanta A.K., Kumar S., Maiti P. (2018). Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduct Target Ther., 3: 7.
Shekarabi S.P.H., Mostafavi Z.S., Mehrgan M.S., Islami H.R. (2021). Dietary supplementation with dandelion (*Taraxacum officinale*) flower extract provides immunostimulation and resistance against *Streptococcus iniae* infection in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol., 118: 180–187.

Shekarabi S.P.H., Ghodrati M., Dawood M.A.O., Masouleh A.S., Roudbaraki A.F. (2022). The multi-enzymes and probiotics mixture improves the growth performance, digestibility, intestinal health, and immune response of Siberian sturgeon (*Acipenser baerii*). Ann. Anim. Sci., 22: 1063–1072.

Silva B.C., Vieira F.N., Mouriño J.L.P., Bolivar N., Seiffert W.Q. (2016). Butyrate and propionate improve the growth performance of *Litopenaeus vannamei*. Aquac. Res., 47: 612–623.

Siwicki A.K., Anderson D.P., Rumsey G.L. (1994). Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Vet. Immunol. Immunopathol., 41: 125–139.

Smith M.P. (2000). Lake Malawi Cichlids. Barron's Educational Series, Inc., New York, USA.

Sotoudeh E., Sangari M., Bagheri D., Morammazi S., Torfi Mozanzadeh M. (2020). Dietary organic acid salts mitigate plant protein induced inflammatory response and improve humoral immunity, antioxidative status and digestive enzyme activities in yellowfin seabream, *Acanthopagrus latus*. Aquac. Nutr., 26: 1669–1680.

Su X., Li X., Leng X., Tan C., Liu B., Chai X. (2014). The improvement of growth, digestive enzyme activity and disease resistance of white shrimp by the dietary citric acid. Aquac. Int., 22: 1823–1835.

Subramanian S., MacKinnon S.L., Ross N.W. (2007). A comparative study on innate immune parameters in the epidermal mucus of various fish species. Comp. Biochem. Physiol. B, Biochem. Mol. Biol., 148: 256–263.

Tian L., Zhou X.Q., Jiang W.D., Liu Y., Wu P., Jiang J., Kuang S.Y., Tang L., Tang W.N., Zhang Y.A., Xie F., Feng L. (2017). Sodium butyrate improved intestinal immune function associated with NF-kB and p38MAPK signalling pathways in young grass carp (*Ctenopharyngodon idella*). Fish Shellfish Immunol., 66: 548–563.

Topping D.L., Clifton P.M. (2001). Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol. Rev., 81: 1031–1064.

Van der Wielen P. (2002). Dietary strategies to influence the gastrointestinal microflora of young animals and its potential to improve intestinal health. In: Nutrition and health of the gastrointestinal tract, Blok M.C., Vahl H.A., De Lange L., Van De Braak AE., Hemke G., Hessing M. (eds). Wageningen Academic Publishers, The Netherlands, pp. 37–60.

Van Doan H., Hoseinifar S.H., Ringø E., Esteban M.A., Dadar M., Dawood M.A.O., Faggio C. (2020). Host-associated probiotics: a key factor in sustainable aquaculture. Rev. Fish Sci. Aquac., 28: 16–42.

Wassef E.A., Saleh N.E., Abdel-Meguid N.E., Barakat K.M., Abdel-Mohsen H.H., El-Bermawy N.M. (2019). Sodium propionate as a dietary acidifier for European seabass (*Dicentrarchus labrax*) fry: immune competence, gut microbiome, and intestinal histology benefits. Aquac. Int., 28: 1–17.

Wongsasak U., Chaijamrus S., Kumkhong S., Boonanuntanasarn S. (2014). Effects of dietary supplementation with b-glucan and synbiotics on immune gene expression and immune parameters under ammonia stress in Pacific white shrimp. Aquaculture, 436: 179–187.

Worthington C. (1991). Worthington enzyme manual related biochemical, Freehold, New Jersey.

Wuertz S., Schroeder A., Wanka K.M. (2021). Probiotics in fish nutrition-long-standing household remedy or native nutraceuticals?. Water, 13: 1348.

Yano T. (1992). Assays of hemolytic complement activity. Techniq. Fish Immunol., pp. 131–141.

Zaidi S.B. (2016). Molecular imprinted polymers as drug delivery vehicles. Drug Deliv., 23: 2262–2271.

Zhou Z., Ding Z., Huiyuan L. (2007). Effects of dietary short-chain fructooligosaccharides on intestinal microflora, survival, and growth performance of juvenile white shrimp, *Litopenaeus vannamei*, J. World Aquac. Soc., 38: 296–301.

Zhu Y., Yang L., Huang D., Zhu Q. (2017). Molecularly imprinted nanoparticles and their releasing properties, bio-distribution as drug carriers. Asian J. Pharm. Sci., 12: 172–178.

Zikic R.V., Stajn S., Pavlovic Z., Ognjanovic B.I., Saicic S. (2001). Activities of superoxide dismutase and catalase in erythrocyte and plasma transaminases of goldfish (*Carassius auratus gibelio* Bloch.) exposed to cadmium. Physiol. Res., 50: 105–111.

Received: 13 IV 2022
Accepted: 21 IX 2022