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Research Article
Growth Performance and Health Status of Common Carp (*Cyprinus carpio*) Supplemented with Prebiotic from Sweet Potato (*Ipomoea batatas* L.) Extract

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Abstract

Objective: This study aimed to evaluate the effect of prebiotic from sweet potato (*Ipomoea batatas* L.) extract on the growth performance and health status of common carp (*Cyprinus carpio*) before and after being infected by *Aeromonas hydrophila*. **Materials and Methods:** Prebiotic was supplemented through feed (0.5, 1 and 2% v/w) for 30 days. The parameters observed during prebiotic supplementation included the populations of the dominant bacterial species in the intestines, digestive enzymes activity, growth performance and immune responses of the fish. On day 32, a challenge test using the pathogenic bacteria *A. hydrophila* was carried out and fish resistance was determined by counting the survival rate of the fish at day 45. **Results:** Feed containing prebiotic supplementation at a dose of 2% showed a significant effect ($p<0.05$) on Total Viable Bacterial Count (TVBC) in the fish intestines and on protease activity and resulted in the best value for daily growth rate and feed conversion ratio ($p<0.05$) when compared with the control and other treatment groups. The predominant bacteria growing in the fish intestines were identified as *Bacillus pumilus*, *Staphylococcus kloosii*, *Staphylococcus hominis* and *Aeromonas veronii*. At the end of the feeding trial with prebiotic supplementation, the values of total leukocytes and phagocytic activities in the fish receiving 2% prebiotic were higher than controls. The survival rates of common carp after the challenge test in the 1 and 2% prebiotic treatments groups were 87.5 and 100%, respectively, while that of fish without prebiotic supplementation was only 50%. **Conclusion:** These results indicate that prebiotic supplementation for common carp has positive effects on the growth performance and health status of common carp infected by *A. hydrophila* and the best result was obtained with a 2% prebiotic supplementation.

Key words: Prebiotic, sweet potato, growth, health status, *Cyprinus carpio*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The common carp (*Cyprinus carpio*) is a major freshwater fish species that is cultured in Indonesia. However, changes in the natural environment and the increasing intensity of its production have led to the emergence of diseases that could harm the cultivation of this species. Disease outbreaks in common carp are often caused by the pathogenic bacteria *Aeromonas hydrophila*, which has proven to be a major limiting factor in the production and profitability of common carp with an overall negative impact on the aquaculture industry and economic development. This pathogen is a motile, short-rod-shaped, Gram-negative bacteria. It is a facultative anaerobe able to grow at 11.2-40.5°C and it is commonly found in fish intestines[1]. The characteristics of this pathogen allow it to easily adapt to different environments because it can tolerate a wide range of turbidity, pH, salinity and temperature conditions. The optimum temperature for the growth of *A. hydrophila* is 28°C, which is similar to the optimum growth temperature of common carp and several other freshwater fish. This pathogen has a maximum adaptive temperature tolerance of up to 41°C[2]. This pathogen is opportunistic and widespread, it also causes a disease called Motile Aeromonad Septicemia (MAS), which can result in high mortality in common carp but it generally occurs when the fish are exposed to long-term infections in association with sub-acute stress factors or to short- term infections with acute stress factors[2,3].

Antibiotics and chemical drugs have commonly been used to inhibit and prevent pathogen growth. These treatments are also used for growth stimulation and chemotherapy[4]. However, the use of these materials has a high risk of increasing the prevalence of resistant bacterial strains, disrupting the normal stability and balance of the intestinal microflora and these residues can accumulate in fish carcasses and the aquatic environment[5]. Prebiotics are phyto-products containing a variety of carbohydrate components that cannot be digested by fish but prebiotics can be a source of organic carbons which are very important for the microflora in the digestive tract of fish, such as fructo-oligosaccharides (FOSs), galacto-oligosaccharides (GOSs), mannan-oligosaccharides (MOSs) and inulin. Prebiotics are widely studied and applied to the culture of various fish species. These compounds include inulin, FOSs, short-chain FOSs (scFOSs), MOSs, trans-galacto-oligosaccharides (TOSs), Bio-MOS® (MOSs derived from yeast), GOSs, xylo-oligosaccharides (XOSs), arabino-xylo-oligosaccharides (AXOSs), iso-malto-oligosaccharides (IMOs) and GroBiotic®-A (GBA)[6]. Prebiotics are a necessary substrate for growth in all groups of beneficial bacteria that live in the digestive tracts of fish. The use of 454 pyrosequencing has confirmed the positive effects of AXOSs on the intestinal microflora of juvenile Siberian sturgeon (*Acipenser baeri*)[7]. Prebiotics used for animals must meet several important criteria, such as having a resistance to gastric acidity, digestive enzymes hydrolysis and the absorption process in the gastrointestinal tract and they must be fermentable by intestinal microflora and selectively stimulate the growth and activity of beneficial bacteria. Important products derived from prebiotic fermentation by the intestinal microflora are short chain fatty acids (SCFAs)[8], which are the main energy source for the bacterial cells in the intestinal epithelium and they maintain the cells of the intestinal epithelium, stimulate the immune system of the fish to provide resistance against pathogens[9] and modulate fat synthesis[10].

Most of the studies on prebiotics have focused on fructans that serve as carbohydrates reserve in the plant species of the family Asteraceae and include compounds such as inulin, FOSs and GOSs[11,12]. These types of prebiotics are relatively easy to be processed and are inexpensive because they can be extracted from plants or produced via enzymatic synthesis[13,14]. The Sukuh sweet potato (*Ipomoea batatas* L.) variety is an alternative crop that contains carbohydrates that have potential as a source of prebiotics. The types of oligosaccharides contained in extract of raw and steamed Sukuh sweet potatoes variety include maltose, maltotriose, sucrose and raffinose. The raffinose content of raw and steamed Sukuh sweet potatoes variety are 48.04 and 39.50 ppm, respectively, corresponding to a raffinose concentration of 2.97%[15]. Because of the oligosaccharide content of Sukuh sweet potatoes variety, study is needed to evaluate the prebiotic effect of this material via *in vivo* tests on experimental animals. The aim of this study was to evaluate the growth performance and health status of common carp (*Cyprinus carpio*) supplemented by prebiotic from sweet potato (*Lpomoea batatas* L.) extract before and after being infected by *A. hydrophila*.

MATERIALS AND METHODS

Preparation of the prebiotic: Preparation of the prebiotic was carried out in several steps according to the method of Marlis[16]. Prebiotic production began with the production of sweet potato flour, oligosaccharide extraction using 70% ethanol and the measurement of total dissolved solids. Subsequently, the oligosaccharide extract, with a total dissolved solids concentration of 5% was analyzed for the

type and content of its oligosaccharides using High Performance Liquid Chromatography (HPLC). The oligosaccharides obtained in prebiotic from sweet potato extract included inulin (1.115%), fructo-oligosaccharides (FOSs) (1.015%) and galacto-oligosaccharides (GOSs) (1.488%).

Preparation of the experimental feed: Preparation of the experimental feed was carried out by adding three different doses of prebiotic (0.5, 1 and 2% v/w) to a commercial feed containing 31% protein. Mixing the feed with the prebiotic was performed by adding 2% egg white as a binder. For the control diet, only 2% egg white was added. After mixing, the feed was air-dried for 10-15 min to reduce the moisture content.

Rearing of fish and the experimental design: This study was conducted over 45 days, that consisted of 30 days for the rearing period or feeding trial with prebiotic supplementation, one day for the challenge test preparation and 14 days for observations after the challenge test. The experimental animals used were common carp with an average weight of 4.75±0.22 g, which were allowed to adapt to the experimental environment for 2 weeks. This study used a completely randomized design consisting of five treatments, each with three replications, namely, the control (-) (feed without prebiotic supplementation and without pathogen infection), control (+) (feed without prebiotic supplementation and with pathogen infection), A (feed supplemented with prebiotic at a dose of 0.5% v/w and with pathogen infection), B (feed supplemented with prebiotic at a dose of 1% v/w and with pathogen infection) and C (feed supplemented with prebiotic at a dose of 2% v/w and with pathogen infection). Experimental fish were stocked randomly into each aquarium (65×40×40 cm^3) with a stocking density of 15 fish/aquarium. Feeding was conducted using at satiation method three times per day (08:00, 12:00 and 16:00, Western Indonesia Time). Water quality was maintained by removing feces and replacing water in the rearing media (25% of water volume) every day. Water quality was monitored during the rearing period and the parameters were maintained within the following ranges: Temperature at 28-29°C, Dissolved Oxygen (DO) at 7.2-8.1 mg L^{-1}, pH at 7.4-8.04 and Total Ammonia Nitrogen (TAN) at 0.0032-0.0065 mg L^{-1}.

Challenge test: After the 30th day feeding trial, the fish were fasted for one day and then challenged with *A. hydrophila*. Infection was performed via an intramuscular injection with *A. hydrophila* at a concentration of 10^7 CFU mL^{-1} (0.1 mL individual^{-1}) using a 1 mL syringe and negative control fish were injected with Phosphate Buffered Saline (PBS). The clinical symptoms and mortality rates of the fish were observed daily for 14 days after injection.

Experimental parameters: The experimental parameters observed included growth performance, immune response, survival rate, total viable bacterial count, an identification of which bacterial species was dominant in the fish intestines and digestive enzymes activity. The growth performance parameters observed were Daily Growth Rate (DGR) and Feed Conversion Ratio (FCR). Growth performance was evaluated after 30 days of prebiotic treatment, while survival rate was calculated at the end of the prebiotic treatment and the challenge test. The digestive enzymes activities for amylase[17], protease[18] and lipase[19] were observed after 30 days of prebiotic treatment.

The bacterial populations in the intestines of the fish were observed in an intestinal sample (0.1 g), which was homogenized in 0.9 mL PBS. Serial dilutions were made and the suspension in each dilution tube (0.05 mL) was spread onto Trypticase Soy Agar (TSA) media. The number of bacterial cells in the sample was determined by counting the number of colonies that grew on the medium and multiplying this by the dilution factor. These data are expressed as CFU g^{-1} [20].

The predominant bacteria found to be growing in the fish intestines (bacteria which grew at the lowest dilution) were identified based on 16S rRNA gene sequences using the 63f primer (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and the 1387r primer (5′-GGG CGG WGT GTA CAA GGC-3′)[21]. The PCR master mix in each tube consisted of 25 µL Go *Taq* (Promega), 4 µL 63f primer, 4 µL 1387r primer, 17 µL ddH_2O and a DNA template that was taken directly from the sample isolates using a toothpick. The PCR conditions were as follows: predenaturation at 95°C for 5 min, denaturation at 95°C for 1 min, primer annealing at 55°C for 1 min, elongation at 72°C for 1 min and primer extension at 72°C for 5 min. The PCR amplification process consisted of 30 cycles and was performed using a PCR machine (MultiGene OptiMax, Labnet International, Inc., Calfornia, USA). The termination reaction was carried out with the temperature dropping to 4°C. The PCR products were electrophoresed with 1% agarose gel in 1x TAE buffer at 80 volts for 45 min, followed by visualization using a UV transilluminator. The DNA samples resulting from the amplification were sent for analysis to a company that provides sequencing services. Sequence results were aligned

with data in the GenBank database using BLAST-N online software program (www.ncbi.nlm.nih.gov).

The immune response parameters measured included total leukocytes, phagocytic activity[22] differential leukocyte levels (lymphocytes, monocytes and neutrophils), hematocrit, hemoglobin content and total erythrocytes. The immune responses and survival rates of the fish were measured twice, once after 30 days of the feeding trial with prebiotic supplementation (before the challenge test) and again 14 days after the challenge test.

Statistical analysis: The data obtained were analyzed using SPSS Statistics 17.0 software and Duncan's test was used to determine significant differences ($p<0.05$)[23].

RESULTS

Growth performance: The survival rate of the fish after the 30 day rearing period was 100% and there were no differences among the prebiotic treatments and the control. The daily growth rates of fish receiving the prebiotic treatments were higher ($p<0.05$) than control, with the highest value obtained with a 2% prebiotic treatment. Feed conversion ratios at all prebiotic treatment levels were lower ($p<0.05$) than control and the lowest feed conversion ratio value was obtained with a 2% prebiotic treatment. Feed supplementation with a prebiotic for 30 days had a significant effect ($p<0.05$) on the bacterial populations in the intestines of common carp. In this study, Total Viable Bacterial Count (TVBC) values in the intestines of fish treated with prebiotic were higher ($p<0.05$) than control and the highest value for this parameter was observed in the 2% prebiotic treatment group (Table 1).

Digestive enzymes activity: The protease activity in the digestive tract of common carp supplemented with 2% prebiotic was significantly higher ($p<0.05$) than control. The amylase and lipase activities in the digestive tracts of fish treated with 2% prebiotic were not significantly different ($p>0.05$) than control (Table 2).

Dominant bacterial species in the fish intestines: There were four bacterial isolates that predominantly grew in the intestines of fish treated with 2% prebiotic. The results of the analysis based on 16S rRNA gene sequences of these four bacterial isolates show that they correlated to *Bacillus pumilus* strain FR1-11 (similarity index of 99%), *Staphylococcus kloosii* strain 68 (similarity index of 99%), *Staphylococcus hominis* strain HN-3 (similarity index of 99%) and *Aeromonas veronii* strain BB1 (similarity index of 96%) (Table 3).

Immune responses and fish resistance: The values for total leukocytes and total erythrocytes of common carp after the 30 day feeding trial with 2% prebiotic were higher ($p<0.05$) than for those treated with 0.5 and 1% prebiotic and controls (positive and negative control). The survival rate of common carp after the challenge test in the 2% prebiotic treatment group was higher ($p<0.05$) than that of the positive control. The values for total leukocytes and the phagocytic activity

Table 1: Growth performance of common carp (*Cyprinus carpio*) after a 30 days rearing period with prebiotic supplementation at different doses

Parameters/prebiotic treatments	Control	0.50%	1.00%	2%
Survival rate (%)	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
Daily growth rate (% per day)	1.72±0.31^{a}	1.86±0.10^{a}	1.93±0.13^{a}	2.81±0.10^{b}
Feed conversion ratio				
Total viable bacterial count	2.34±0.13^{a}	2.03±0.07^{b}	1.72±0.06^{c}	1.34±0.05^{d}
Fish intestines (log CFU g^{-1})	7.44±0.23^{a}	8.66±0.22^{b}	8.99±0.23^{bc}	9.30±0.20^{c}

Different letters in the same row indicate significant differences[23] ($p<0.05$). Values shown are means and standard deviations

Table 2: Digestive enzymes activity (U.mg per protein) in common carp (*Cyprinus carpio*) supplemented with 2% prebiotic

Parameters/treatments	Prebiotic (2%)	Control
Amylase	0.5430±0.18^{a}	0.4950±0.12^{a}
Protease	0.0767±0.00^{b}	0.0373±0.01^{a}
Lipase	0.6090±0.07^{ab}	0.4670±0.01^{a}

Different letters in the same row indicate significant differences[23] ($p<0.05$). Values shown are means and standard deviations

Table 3: Results of a BLAST-N analysis of 16S-rRNA gene sequences of bacterial isolates in the intestines of common carp (*Cyprinus carpio*) supplemented with 2% prebiotic

Species	Homology (%)	Acc Number
Bacillus pumilus strain FR1-11	99	EU373536.1
Staphylococcus kloosii strain 68	99	JX102547.1
Staphylococcus hominis strain HN-3	99	KT003249.1
Aeromonas veronii strain BB1	96	KF446249.1

Table 4: Immune responses of common carp (*Cyprinus carpio*) after a 30-day rearing period with prebiotic supplementation

Parameters/prebiotic treatments	0.5%	1%	2%	Positive control	Negative control
Total leukocytes ($\times 10^5$ cells mm^{-3})	0.75±0.07^b	0.66±0.08^b	0.98±0.05^c	0.80±0.05^a	0.88±0.04^a
Total erythrocytes ($\times 10^6$ cells mm^{-3})	1.09±0.16^a	1.00±0.13^a	1.61±0.10^b	1.28±0.19^a	1.35±0.21^a
Hematocrit (%)	41.00±0.31^b	46.00±0.35^b	50.50±0.32^b	18.50±0.43^a	33.50±0.45^a
Hemoglobin (g%)	6.60±0.21^a	7.25±0.24^{ab}	7.45±0.19^b	6.60±0.21^a	6.80±0.20^a
Lymphocytes (%)	90.00±0.12^a	92.00±0.10^a	91.00±0.15^a	91.00±0.17^a	90.00±0.14^a
Monocytes (%)	1.00±0.12^a	1.00±0.09^a	1.00±0.11^a	1.00±0.1^a	1.00±0.09^a
Neutrophils (%)	9.00±0.12^a	8.00±0.11^a	9.00±0.14^a	8.00±0.12^a	9.00±0.13^a
Phagocytic activity (%)	25.00±0.11^a	23.00±0.14^a	48.00±0.12^b	26.00±0.15^a	27.00±0.18^a

Different letters in the same row indicate significant differences[23] ($p<0.05$). Values shown are means and standard deviations

Table 5: Survival Rate (SR) and immune responses of common carp (*Cyprinus carpio*) after the challenge test with *Aeromonas hydrophila*

Parameters/prebiotic treatments	0.5%	1%	2%	Positive control	Negative control
SR (%)	62.50±14.43^a	87.50±14.43^b	100.00±0.00^b	50.00±0.00^a	93.75±7.22^b
Total leukocytes ($\times 10^5$ cells mm^{-3})	1.29±0.18^a	1.68±0.15^b	2.07±0.12^c	1.38±0.14^a	1.26±0.11^a
Total erythrocytes ($\times 10^6$ cells mm^{-3})	1.04±0.16^a	0.99±0.19^a	0.73±0.20^a	1.02±0.18^a	1.17±0.14^a
Hematocrit (%)	23.00±0.29^a	28.00±0.27^a	31.00±0.30^a	22.00±0.32^a	32.00±0.26^a
Hemoglobin (g%)	4.90±0.18^a	5.60±0.15^a	5.70±0.19^a	5.00±0.16^a	6.10±0.20^a
Lymphocytes (%)	82.00±0.14^a	84.00±0.11^a	85.00±0.10^a	82.00±0.16^a	87.00±0.12^a
Monocytes (%)	1.00±0.10^a	1.00±0.08^a	2.00±0.13^a	1.00±0.11^a	1.00±0.10^a
Neutrophils (%)	17.00±0.13^a	15.00±0.11^a	13.00±0.14^a	17.00±0.11^a	15.00±0.10^a
Phagocytic activity (%)	44.00±0.32^b	54.00±0.30^{bc}	62.00±0.26^c	36.00±0.24^a	29.00±0.23^a

Different letters in the same row indicate significant differences[23] ($p<0.05$). Values shown are means and standard deviations

after the challenge test in the 2% prebiotic treatment group were higher ($p<0.05$) than those for the positive control. The total erythrocytes, hematocrit level and hemoglobin content of fish decreased after the challenge test (Table 4, 5).

DISCUSSION

Prebiotics are carbohydrates that are not digested by fish, most of which are short chain monosaccharides commonly known as oligosaccharides. They can change the composition of the intestinal microflora by changing the types of substrates available to bacteria to those that are more appropriate to the micro-ecophysiological conditions of the resident intestinal microflora, allowing them to grow rapidly. Therefore, prebiotics only promote the growth of beneficial microflora and/or reduce the growth of pathogenic microflora in the intestines of the host. They can also reduce the pH of the intestinal fluid through the production of Short Chain Fatty Acids (SCFAs) and change the concentations of extracellular enzymes produced by probiotics[24-26].

Various types of carbohydrate components that have prebiotic characteristics have been widely applied in aquaculture systems, including mannan-oligosaccharides (MOSs), fructo-oligosaccharides (FOSs), galacto-oligosaccharides (GOSs), inulin, trans-galacto-oligosaccharides (TOSs) and lactose[27]. The results of the present study show that supplementation with prebiotic derived from a sweet potato extract containing several types of oligosaccharides, such as FOSs (1.015%), GOSs (1.488%) and inulin (1.115%) has positive effects on the bacterial populations in the intestines of experimental fish. The degree of the positive effects on the bacterial populations in the intestines was affected by the prebiotic dose. Total Viable Bacterial Count (TVBC) increased with increasing prebiotic doses. This indicates that the doses of prebiotic provided a substrate for the growth of bacteria in the intestine, which led to larger bacterial populations in the treated groups than in the control. Growth performances and feed conversion ratios were better in common carp consuming feed containing prebiotic with the best results obtained using a dose of 2%. The improvement of growth performance was caused by the increases in digestive enzymes activity, improvement of microvilli structure on the surface of enterocytes, which increased the surface area for nutrient absorption and then increasing feed efficiency and the production of Short Chain Fatty Acids (SCFAs) as a result of prebiotic fermentation by intestinal endocellular microflora. Supplementation with MOSs at a dose of 0.2% has been shown to lengthen the microvilli length of cobia (*Rachycentron canadum*) larvae[28] and increased the microvilli density of the foregut and hindgut of gilthead sea bream (*Sparus aurata*)[29]. Oligosaccharides are also able to improve intestinal morphology along with their potent ability to control oxidative stress. This can increase the efficiency of intestinal absorption through the expansion of intestinal microvilli, which has a positive impact on growth[30,31].

An improvement in digestive enzymes activity due to prebiotic supplementation in common carp was demonstrated by the data from the present study;

supplementation with 2% prebiotic resulted in a protease activity that was significantly higher than that of the control. This was caused by the integration of increases in the endocellular enzyme activity in fish and the extracellular enzyme activity of the microflora modulated by the prebiotic supplementation. Similar results were obtained from a study on crucian carp supplemented with prebiotic xylo-oligosaccharides (XOSs)[32] and on freshwater crayfish supplemented with MOSs (*Cherax destructor*)[33].

An improvement in intestinal microfloral diversity due to prebiotic supplementation was shown in this study based on the identification of bacteria that were predominant in the intestines of common carp that received prebiotic supplementation. In fish receiving 2% prebiotic supplementation, several predominant bacterial species were identified, such as *Bacillus pumilus* strain FR1-11, *Staphylococcus kloosii* strain 68, *Staphylococcus hominis* strain HN-3 and *Aeromonas veronii* strain BB1. The existence of these bacteria in the intestines of common carp was expected to improve the growth performance and health status of fish. The presence of *B. pumilus* strain FR1-11, *S. kloosii* strain 68, *S. hominis* strain HN-3 and *A. veronii* are safe for common carp because no clinical symptoms or mortality occurred after these bacteria were injected into the fish. In addition, several previous studies have reported that these bacteria are beneficial. The results of previous study by Sun *et al.*[34] showed that *B. pumilus* and *Bacillus clausii* improved the growth performance and immune response of grouper (*Epinephelus coioides*). The *B. pumilus* and many other members of the *Bacillus* genus have been widely evaluated as probiotics in aquaculture systems, both for improving water quality and for reducing the incidence of disease outbreaks in various aquaculture species[35]. The *B. pumilus* isolated from the midgut of *Penaeus monodon* shows a strong inhibition against the pathogenic bacteria *Vibrio alginolyticus*, *Vibrio mimicus* and *Vibrio harveyi*, which was observed through *in vitro* assays[36]. The application of probiotic *B. pumilus*, *Bacillus licheniformis* and *Bacillus subtilis* derived from sea water and from soil has also been shown to reduce TAN levels, promoting the growth and survival of shrimp (post-larvae) without water replacement[37].

Blaxhall and Daisley[38] stated that hematological parameters such as hematocrit levels, hemoglobin content and total erythrocytes describe the health status of fish. Prebiotic supplementation in common carp for 30 days did not negatively impact the total erythrocytes, which were still within the normal range after treatment. Kumar *et al.*[39] stated that the number of red blood cells varies within the range of 1.05-3.0×106 cells mm^{-3}.The results of this study also showed that supplementation with prebiotic had a positive effect on the total leukocytes of common carp before the challenge test, at which point the total leukocytes in fish receiving prebiotic treatments were higher than those of control fish.

The infection with aeromonas hydrophila that occurred after the challenge test induced several changes in the hematological parameters of the fish, including a decrease in hematocrit, hemoglobin and total leukocytes. The cytotoxicity of *A. hydrophila* and the accumulation of its extracellular products (α and β hemolysin, aerolysin, enterotoxins ACT, ALT and AST, protease and RNase) cause necrosis and hemolysis in erythrocytes and a decrease in the levels of iron ions[40], thus, causing decreases in the number of red blood cells in the experimental fish. Prebiotic supplementation in common carp in the present study had a positive effect on their resistance to *A. hydrophila* as demonstrated by the survival rates after the challenge test, which were higher in the fish receiving prebiotic treatments than in the positive control fish. This could be due to differences in the health status of the fish before the challenge test, which may have been better as a result of prebiotic supplementation, suggestion that supplementation could provide common carp a better defense system against pathogenic infection.

The mechanism of action of prebiotics is similar to that of antibodies, which have the ability to bind with antigens and to remove pathogens from the intestinal tissue, making them act as a stimulant that can react to produce cellular and humoral immune responses. Pathogenic bacteria are generally attached to enterocytes by type 1 fimbriae. This adhesion is a key factor in the pathogen's ability to cause disease. The FOSs, GOSs and inulin (prebiotics contained in oligosaccharides from sweet potato extract), as well as MOSs are unique oligosaccharides because they have receptors for type 1 fimbriae. The function of the main components of these prebiotics is in acting as competitors for the adhesion sites on enterocytes where pathogenic bacteria attach. This brings the bacteria out of the intestinal tissue before the bacteria can bind to the enterocytes. The positive effect of prebiotics is not determined by whether the pathogens are able to ferment the prebiotics, but it is determined based on whether the pathogens are prevented from adhering to and colonizing the intestinal cells and if the prebiotics can reduce the ability of the pathogens to compete with the beneficial microflora that live in the intestines of fish. Naturally, prebiotics have the ability to stimulate the cellular immune system through indirect actions, which are mediated by their involvement in stimulating the growth of beneficial microflora, such as lactic

acid bacteria and *Bacillus* spp.[41,33]. An increase in the growth of beneficial microflora has an impact on the synergistic fermentation of undigested oligosaccharides, energy reserve surplus, synthesis of vitamin B and K, production of SCFAs, improvement of the structure and function of the digestive tract, reduction of cholesterol and stimulation of the local immune system, in which approximately 60% of total active lymphocytes in the fish immune system are facilitated by Gastrointestinal Associated Lymphoid Tissue (GALT) containing lamina propria and intra-epithelial lymphocytes[42-44]. Immune response improvements are also mediated through the production of SCFAs (e.g., acetate and lactic acid) by beneficial bacteria. These SCFAs stimulate the immune system, resulting in an increase in the protection and resistance against pathogenic infection in fish. This is in line with the results of this study, prebiotic supplementation at a dose of 0.5-2% showed high values for total leukocytes and phagocytic activity, followed by no mortality on the 2% prebiotic treatment group. These results indicate that the cellular immune responses and health status of common carp consuming feed containing prebiotic were better than those of positive control fish. These positive effects on the immune parameters that occurred as a result of prebiotic supplementation have also been reported by He *et al*.[45], MOS supplementation at a dose of 0.6% or FOS supplementation in hybrid tilapia was able to improve the survival rate and activity of cellular defense mechanisms, especially the activity of lysozymes.

The prebiotic used in this study created ideal conditions for the proliferation of several bacterial strains that are thought to be potent probiotics, which could potentially produce substances that stimulated the immune system and improved the protection of common carp against *A. hydrophila* infection. Several studies have reported that *B. pumilus* (Dominant bacteria in the intestines of common carp supplemented with prebiotic) can suppress and reduce the pathogenic bacteria to protect the intestines of this fish from infection. This is in line with the antagonistic and suppressive effect of *B. pumilus* against several pathogenic bacteria, such as *Staphylococcus aureus*, *V. harveyi*, *Vibrio parahaemolyticus* and *Staphylococcus saprophyticus*[46,47]. These findings suggest that the stimulation of *B. pumilus* strain FR1-11, *S. kloosii* strain 68, *S. hominis* strain HN-3 and *A. veronii* strain BB1 by prebiotic from sweet potato extract also have a positive impact on the immune responses of the common carp. The *A. hydrophila* is a normal member of the intestinal microflora of fish, as is *A. veronii* strain BB1. Further studies are needed to evaluate this possibility that this prebiotic stimulates these beneficial bacteria, because the fish digestive tract is one of the main access routes for most pathogenic bacteria. The manipulation of intestinal microflora to achieve beneficial effects for the host, such as increase in the growth, digestion, immunity and disease resistance, needs to be developed[48,49]. Manipulation of the intestinal microflora can be performed through prebiotic supplementation.

However, the members of the intestinal microflora of aquatic organisms vary and are more dependent on environmental factors than are terrestrial species and prebiotic treatment alone is not sufficient to regulate the intestinal microflora. Therefore, additional studies are needed to evaluate the synergistic role of the combination of probiotic and prebiotic treatments.

CONCLUSION

In conclusion, the supplementation with prebiotic derived from sweet potato extract in common carp was shown to be capable of providing positive effects on the growth performance and health status of the fish before and after being infected by *A. hydrophila*. The best results were obtained using a 2% prebiotic supplementation.

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