Jurnal Akuakultur Indonesia 24 (2), 211–219 (2025)

Original article DOI: 10.19027/jai.24.2.211-219

The effectiveness of inulin on health status, digestive activity, and production of common carp *Cyprinus carpio*

Efektivitas Inulin terhadap status kesehatan, aktivitas pencernaan dan produksi ikan mas *Cyprinus carpio*

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(Received October 26, 2024; Revised December 19, 2024; Accepted April 21, 2025)

ABSTRACT

The addition of inulin to fish diets has the potential to enhance health, growth performance, and feed utilization. This study aims to evaluate the role of inulin on the health status, digestive activity, and production levels of common carp (*Cyprinus carpio*) in farming. This study employed a completely randomized design with four treatments and three replications, consisting of diets with 0, 2, 4, and 6 g/kg of inulin diet. The study involved 120 fish, with each group containing 10 fish weighing 8.46 ± 0.42 g was reared in each 30 L aquarium for 30 days. Fish were fed according to the treatment, at satiation twice daily. Hematology parameters showed normal ranges and intestinal histology revealed an increase in intestinal villi size with the addition of inulin. The treatment with inulin at 4 g/kg diet showed highest number of lactic acid bacteria in the fish intestines (P<0.05). The activity of digestive enzymes, including α-amylase, lipase, and protease, increased the inulin dose increased (P<0.05). The best final weight, weight gain and feed conversion ratio were observed in the 4 g/kg inulin treatment (P<0.05). Meanwhile, the specific growth rate in the 2, 4, and 6 g inulin groups was not significantly different from the 0 g group (P>0.05). The survival rate was similar across all treatments (P>0.05). These results suggest inulin can be used as a prebiotic feed additive to enhance production in carp farming.

Keywords: common carp, feed additive, growth, inulin, prebiotic

ABSTRAK

Penambahan inulin dalam pakan berpotensi meningkatkan kesehatan, kinerja pertumbuhan dan pemanfaatan pakan ikan. Penelitian ini bertujuan mengevaluasi peran inulin terhadap status kesehatan, aktivitas pencernaan, dan tingkat produksi pada budidaya ikan mas (*Cyprinus carpio*). Penelitian ini menggunakan rancangan acak lengkap dengan empat perlakuan dan tiga ulangan, yang terdiri dari 0, 2, 4, dan 6 g/kg inulin pakan. Penelitian ini menggunakan ikan 120 ekor, masing-masing10 ekor dengan berat 8,46 ± 0,42 g dipelihara dalam tiap akuarium bervolume 30 L selama 30 hari. Ikan diberi pakan sesuai perlakuan, secara *at satiation* dua kali sehari. Parameter hematologi menunjukkan kisaran normal, dan histologi usus menunjukkan peningkatan ukuran vili usus dengan penambahan inulin. Jumlah bakteri asam laktat tertinggi dalam usus ikan ditunjukkan oleh perlakuan yang diberi inulin 4 g/kg pakan (P<0,05). Aktivitas enzim pencernaan, yaitu α-amilase, lipase, dan protease, meningkat seiring dengan peningkatan dosis inulin (P<0,05). Berat akhir, pertambahan berat dan rasio konversi pakan terbaik ditunjukkan pada perlakuan pakan inulin 4 g/kg pakan (P<0,05). Sementara itu, laju pertumbuhan spesifik pada perlakuan penambahan inulin 2, 4, dan 6 g tidak berbeda nyata dengan perlakuan 0 g (P>0,05). Tingkat kelangsungan hidup tidak berbeda nyata pada semua perlakuan (P>0,05). Hasil ini menunjukkan bahwa dalam budidaya ikan mas, inulin dapat digunakan sebagai imbuhan pakan dalam bentuk prebiotik untuk meningkatkan produksi.

Kata kunci: ikan mas, imbuhan pakan, inulin, pertumbuhan, prebiotik

INTRODUCTION

Common carp is a kind of freshwater fish widely raised in East Kalimantan Province, Indonesia. This fish farming activity is takes place in ponds and cages along the Mahakam River. Production of common carp in East Kalimantan was 16,776 tons in 2021 and 15,762 tons in 2022 (BPS, 2022). The growing demand for commercial feed, which is not always matched by increased production, presents a problem local common carp farmers, along with health issues caused by lower, water quality during certain seasons (Agustina *et al.,* 2014).

Efforts continue to increase fish production, including the use of feed additives. Inulin is one of the additives used in aquaculture. It is an indigestible dietary fiber in the form of a Fructan compound. However, bacteria in the digestive tract can ferment dietary fiber, helping improve digestive, health and enhance food absorption (Holscher, 2017). The addition of certain doses of inulin to cultured fish feed has been shown to improve health by reducing stress responses, boosting immunity, increasing growth performance and survival, and aiding enzymatic activity in the digestive tract (Ghafarifarsani *et al.,* 2021; Yones *et al.,* 2020; Zhang *et al.,* 2023).

The role of inulin as a prebiotic in various fish species shows inconsistent results. Ajdari *et al*. (2022) found that adding 2 g/kg of inulin could boost growth, innate immunity and digestive enzymes in common carp. The prebiotic inulin at 2-2.5 g/kg was able to enhance digestive enzyme activity in tropical gar larvae (*Atractosteus tropicus*) however, the best growth was observed in the control group (De La Cruz-Marín *et al.,* 2023). In rainbow trout (*Oncorhynchus mykiss*), a 1% dose of inulin promoted optimal growth by increasing digestive enzyme activity and functioning as an antioxidant (Hunt *et al.,* 2019). Agustina and Susanto (2024) found that red tilapia (*Oreochromis* sp.) fed with 4 g/kg of inulin experienced improvements in body weight, feed conversion ratio, protein efficiency ratio, digestive enzyme activity, and the number of lactic acid bacteria compared to other treatments.

This research aims to gather information about using inulin as a feed additive certain dose. It is very important to consider the impact on the health of common carp, so monitoring hematological parameters is crucial. Additionally, the addition of inulin affects the structure of the fish's intestines, and this parameter can provide an overview of the health status of the fish's digestive system. The number of lactic acid bacteria in the intestine with inulin relates to inulin's role as a prebiotic for common carp, which in turn influences enzymatic activity in the digestive tract and its production levels.

MATERIAL AND METHODS

Fish preparation

The common carp used in this study came from a local hatchery. The fish were healthy, they showed no abnormalities in swimming behavior, body, or appetite, with an average weight of 8.46 ± 0.42 g. The fish were first acclimated for five days in a 1 m diameter plastic container with water had been previously settled and aerated. During acclimation, the fish were fed twice daily, at 08.00 and 18.00 to satiety using a commercial feed containing 38% protein. The fish were fasting for one day before being treated with inulin added to their diet.

Experimental design

This research is a laboratory study that uses a completely randomized design with four treatments the addition of inulin at doses 0, 2, 4, and 6 g/kg, each with three replications. Ten fish were kept in each aquarium, which had a water volume of 30 L, and were aerated for 30 days and fed according to the treatment. During rearing, the water quality remained within the normal range, with temperature between 27.1-28.0°C, pH levels from 7.1-7.5, dissolved oxygen from 7.3-7.9 mg/L, and total ammonia nitrogen from 0.18-0.20 mg/L. Siphoning was performed daily, and the water was replaced with fresh water equal to the amount removed during siphoning.

Experimental diets preparation

The feed used consists of commercial pellets with the following nutritional composition: 38% protein, 6.53% lipids, 10.29% ash, 3.03 % crude fiber, and 29.81% carbohydrates. The inulin used is Now Brand Inulin Prebiotic Pure Powder (NOW Foods Inulin Powder, Organic-227g). The pellets are mixed with inulin according to the treatment dose, then combined with 20% water and stirred until smooth. The mixture of feed and inulin is then pelletized using a pellet machine and dried at a temperature of 50 ± 5°C for two to three hours or until the pellets are dry. The finished pellets are then stored in a closed container for use in further tests.

Hematological parameters and histology of fish intestines

The hematological parameters of the fish were checked at the start of rearing, on days 15 and 30. The first step was to collect a blood sample from the caudal vein using a syringe. Total red blood cells and total white blood cells based on the Blaxhall and Daisley (1973) procedure. Additionally, the Sahli method with a haemometer (Wedemeyer & Yasutake, 1977) was used to evaluate hemoglobin levels, and hematocrit was measured according to the Anderson and Siwicki method (1995).

Histological examination of fish intestines was conducted at the end of the experiment. Intestinal samples were fixed in a neutral buffered formalin solution (10%). Specimens were prepared following the method by Roberts (2001). Tissue sections were stained with hematoxylin-eosin (HE), observed under an Olympus CX23 microscope, and photographed using an Olympus SZ-14 camera to analyze pathological changes.

Number of lactic acid bacteria (LAB) in fish intestines

The parameter for the number of lactic acid bacteria in the common carp intestines was calculated based on the total number of bacterial colonies on MRS Agar media, following the total plate count method by Mousavi *et al*. (2016). Sampling was conducted at the start of maintenance, specifically on the 15^{th} and 30^{th} days.

Digestive enzyme activity

Enzyme activity parameters include α-amylase, protease, and lipase activities, measured at the beginning of maintenance and on day 30. Measurement of α-amylase and protease enzyme activity was determined based on the method Chamaiporn *et al*. (2021). Meanwhile, lipase enzyme activity was measured using the method by Nolasco-Soria *et al*. (2018).

Growth, feed utilization, and survival rate of fish

Growth performance and feed utilization parameters including final weight, weight gain, specific growth rate, feed conversion ratio and survival rate of common carp were measured using the following formulas by Gabriel *et al*. (2019):

$$\text{WG (g)} = \text{W2} - \text{W1}$$

$$\text{SGR (\%/day)} = \left(\frac{\text{Ln W2} - \text{Ln W1}}{\text{t}}\right) \times 100$$

$$\text{SR (\%)} = \frac{\text{Number of survived fish}}{\text{Initial number of fish}} \times 100$$

Note:
W2 = Final body weight
W1 = Initial body weight
t = Trial period
WG = Weight gain
SGR = Specific growth rate
FCR = Feed conversion ratio
FI = Feed intake
SR = Survival rate

Statistical analysis

Observational data such as the number of lactic acid bacteria in fish intestines, digestive enzyme activity, growth performance, feed utilization, and survival were analyzed for diversity using ANOVA (analysis of variance) in the SPSS application at a 95% confidence level. Additionally, Duncan's follow-up test was conducted to identify any differences between the treatments. The hematological parameters and histological features of the fish intestine were analyzed descriptively in tables and figures.

RESULTS AND DISCUSSION

Hematological parameters and histology of common carp intestines

During the observations, the hematological parameters of common carp showed fluctuations (Table 1). Hemoglobin increased from the beginning to the end of rearing in all treatments, ranging from 5.10 to 8.20 g/dL, with the highest level observed in the treatment added with 4 g/kg inulin. Meanwhile, the hematocrit level of common carp fluctuated, ranging from 30.99% to 43.58% on the 15^{th} day then decreased by the 30^{th} day, except for the treatment with 6 g/kg inulin. Additionally, the red blood cell counts in common carp fluctuated during the study, ranging from 1.04 to 2.13×10^6 cells/mm³. The control treatment showed the highest number of red blood cells on the final rearing day compared to other treatments. On the 15^{th} day, the treatment with 2 g/kg inulin showed the highest white blood cell count, at 2.62×10^4 cells/mm³. Overall, white blood cell counts increased by the 15^{th} day of rearing and then decreased by the 30th day across all treatments.

Figure 1 shows that the intestinal histology of common carp varies with different treatments involving inulin in the diet. The control treatment (0 g/kg inulin) displays a normal structure where the spaces between the villi appear sparse (a). In Figure (b), the intestine of common carp treated with 2 g/kg inulin shows an increase in villus size resulting in a wider intestinal surface (lumen) and hyperplasia of mucus cells in the lamina propria. Figure (c) demonstrates that the tips of the microvilli are fused when treated with 4 g/kg inulin. Meanwhile Figure (d), which depicts fish intestines 6 g/kg inulin, shows swelling of the intestinal villi.

Number of lactic acid bacteria in common carp intestines

The number of lactic acid bacteria in the intestines of common carp increased across all treatments over the 30 days of rearing (Figure 2). During the observation period, the counts range

Table 1. Values of hematological parameters of common carp with the addition of inulin in the diet during observation.

Parameters	Day	Addition of inulin in the diet (g/kg)			
		0	2	4	6
Hemoglobin (g/dL)	0	5.10 ± 0.70	5.10 ± 0.70	5.10 ± 0.70	5.10 ± 0.70
	15	5.87 ± 1.64	6.43 ± 0.48	7.33 ± 0.76	5.47 ± 0.50
	30	7.70 ± 1.54	7.87 ± 0.32	8.20 ± 0.75	7.10 ±1.05
Hematocrit (%)	0	30.99 ± 8.48	30.99 ± 8.48	30.99 ± 8.48	30.99 ± 8.48
	15	41.91 ± 4.27	43.59 ± 14.50	35.53 ± 10.36	30.48 ± 9.93
	30	41.59 ± 4.12	35.79 ± 2.83	34.53 ± 1.04	34.00 ± 3.69
Red blood cells (×10^6 cell/mm^{-3})	0	1.12 ± 0.04	1.12 ± 0.04	1.12 ± 0.04	1.12 ± 0.04
	15	1.62 ± 0.37	1.52 ± 0.20	1.05 ± 0.19	1.04 ± 0.13
	30	2.13 ± 0.25	1.91 ± 0.08	1.83 ± 0.22	1.81 ± 0.06
White blood cells (×10^4 cell/mm^{-3})	0	2.01 ± 0.07	2.01 ± 0.07	2.01 ± 0.07	2.01 ± 0.07
	15	2.05 ± 0.42	2.62 ± 0.98	2.20 ± 0.35	2.33 ± 0.32
	30	2.01 ± 0.18	2.40 ± 0.06	2.19 ± 0.20	2.31 ± 0.26

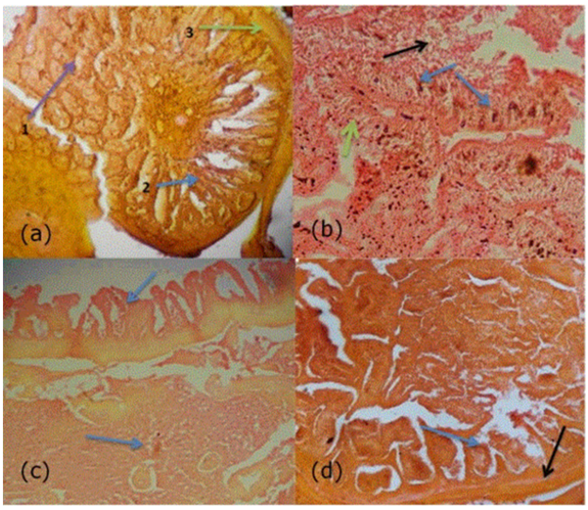

Figure 1. (a) Fish intestines with 0 g/kg inulin showed that the spaces between the villi (HE stain 100×), (b) Fish intestines with 2 g/kg inulin exhibited hyperplasia of mucus cells in the lamina propria, goblet cells with enlarged cell nuclei, and larger villi that increase the width of the intestine surface (lumen) (HE stain 400×), (c) Fish intestines with 4 g/kg inulin displayed fused microvilli at the top (HE stain 100×); (d) Fish intestines with 6 g/kg inulin showed swollen villi (HE stain 100×).

from about 2.02-3.28 log cfu/mg. On the 30th day, it was observed that the highest number of lactic acid bacteria in the intestines occurred when 4 g/kg inulin was included in the diet, which was significantly different from the other treatments (P<0.05).

Digestive enzyme activity

The activity of digestive enzymes in the intestines of common carp increased over 30 days of rearing. This rise was in line with the increasing inulin dose used in this study. The enzyme activities of α-amylase, lipase, and protease varied significantly among treatments (P<0.05). The activity of α-amylase ranged from 2.50 to 4.03 IU/mL, lipase from 0.10 to 0.17 IU/mL, and protease from 0.23 to 0.40 IU/mL (Figure 3).

Growth performance, feed utilization, and survival rate of common carp

The production performance of common carp in this study was evaluated based on growth, feed utilization, and survival rates over a 30-day rearing period (Table 2). The highest final weight

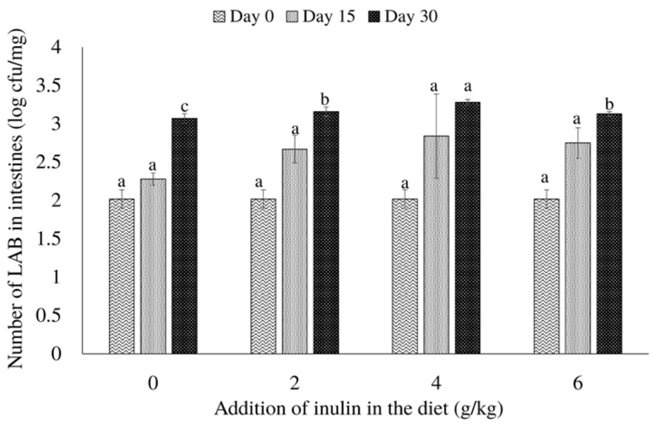

Figure 2. Number of lactic acid bacteria in the intestines of common carp with the addition of inulin in the diet. The average number followed by the same letter on the same day is not significantly different (P> 0.05).

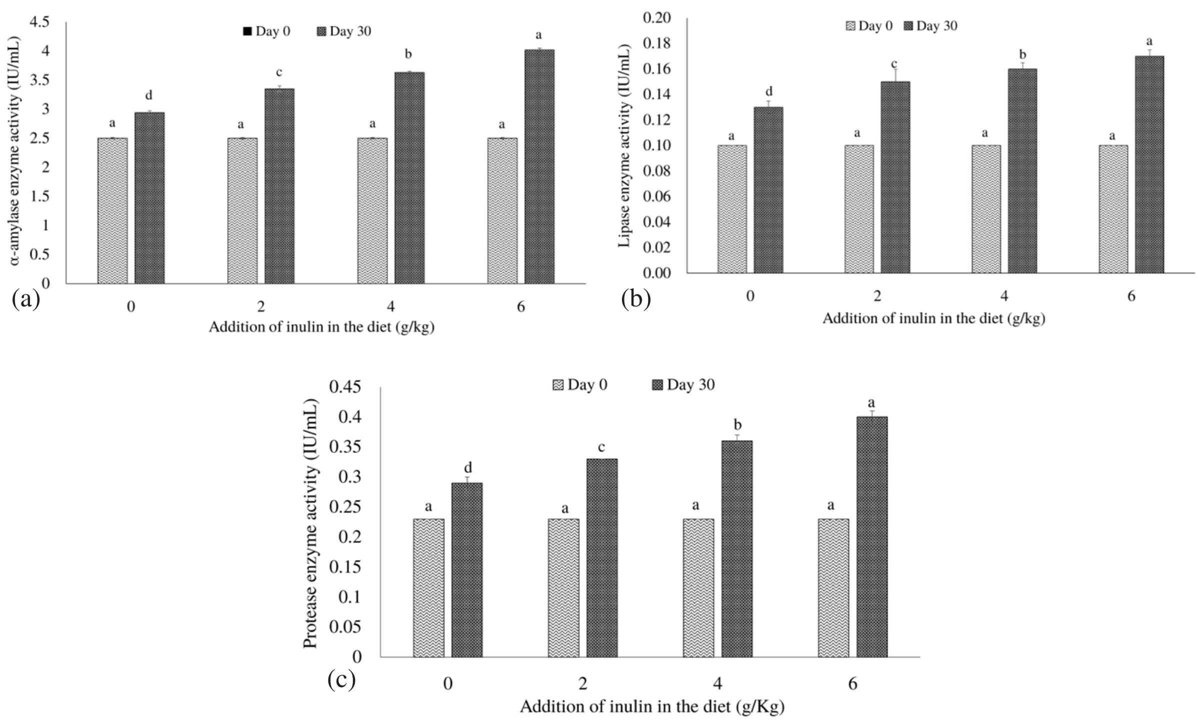

Figure 3. Activity of digestive enzymes in common carp with the addition of inulin to the diet (a) α-Amylase, (b) Lipase, (c) Protease. The average number followed by the same letter on the same day is not significantly different (P>0.05).

and weight gain were observed in the 4 g/kg inulin treatment, with values of 15.47 g and 6.65 g, respectively, which were significantly higher than those in other treatments (P<0.05). The highest specific growth rate was also observed in the 4 g/kg inulin group, at 1.65%/day, however, this difference was not statistically significant, as the 0 g/kg inulin group had a rate of 1.36 %/day (P>0.05). The lowest feed conversion ratio was recorded in the 4 g/kg inulin treatment at 1.28, significantly lower than the 1.65 ratio observed in the 0 g/kg inulin group (P<0.05). The survival rate ranged from 76.67% to 86.67% and did not differ significantly among the treatments (P>0.05).

Discussion

The effect of prebiotics on fish health, growth, and feed utilization depends on the type of prebiotic, species, dose, and duration of use (De La Cruz-Marín *et al.,* 2023). Hematological parameters are useful indicators for assessing fish health. Fish with good health are more likely to grow better in culture conditions. Common hematological parameters include hemoglobin, hematocrit, red blood cells, white blood cells, and total protein (Esmaeili, 2021). In this study, the hematological parameters of common carp remained within the normal range. This indicates that adding up to 6 g/kg of inulin to the diet does not negatively affect the fish's health. Akrami *et al*. (2015) found that adding 0-1.5 g/kg of inulin to the diet of juvenile gibel carp (*Carassius auratus gibelio*) did not have a significant effect on hematological parameters. White blood cells in common carp tended to increase with the addition of inulin, especially at a dose of 2 g/kg. This aligns with research by Agustina and Susanto (2024), which showed that adding inulin to tilapia affects white blood cells. An increase in white blood cells suggests that inulin could potentially enhance the immune system of common carp. The ability of inulin to boost fish immunity was also observed in Asian seabass (*Lates calcarifer*), where adding 5 g/kg of inulin to the diet significantly increased white blood cells compared to the control 8.2×10^3 mm^{-3} (Ali *et al.,* 2018). However, more comprehensive observations of immune parameters are still needed to confirm inulin's ability to improve the immune system in common carp.

Up to a dose of 6 g/kg, inulin added to the common carp diet affected intestinal histology. Figure 1 illustrates that increasing the dose of inulin affects the growth of intestinal villi over a 30 rearing period. There is a tendency for hyperplasia, or an increase in goblet cells, followed by the fusion of intestinal microvilli and swelling. In juvenile Nile tilapia, the addition of 5 g/kg of inulin to the diet enhanced villus length and the number of intestinal goblet cells (Tiengtam *et al.,* 2015). Additionally, Ali *et al*. (2018) foundobserved an increase in the density of Asian Seabass intestinal microvilli with 2% inulin supplementation. The impact of prebiotics in feed on villi height is related to increased fermentation in the intestinal tract. Fermentation of prebiotics produces several short-chain fatty acids, which help increase villi height (Boonanuntanasarn *et al.,* 2017). An increase in the length of the intestinal villi results in greater surface area, which enhances the absorption of available nutrients.

The number of lactic acid bacteria in the intestines of common carp increased over 30 days of cultivation across all treatments. The addition of inulin at a dose of 4 g/kg showed the highest amount of lactic acid compared to other treatments. Mousavi *et al*. (2016) found that

Table 2. Growth performance, feed utilization, and survival rate of common carp with the addition of inulin in the diet.

Parameters	Addition of inulin in the diet (g/kg)			
	0	2	4	6
Initial weight (g)	8.11 ± 0.37[a]	8.46 ± 0.57[a]	8.82 ± 0.27[a]	8.47 ± 0.16[a]
Final weight (g)	13.22 ± 0.92[b]	12.09 ± 0.28[b]	15.47 ± 0.62[a]	12.85 ± 0.49[b]
Weight gain (g)	5.11 ± 0.62[b]	3.63 ± 0.77[c]	6.65 ± 0.86[a]	4.37 ± 0.46[bc]
SGR (%/day)	1.36 ± 0.14[ab]	0.91 ± 0.29[c]	1.65 ± 0.24[a]	1.12 ± 0.13[bc]
FCR	1.65 ± 0.21[a]	1.75 ± 0.18[a]	1.28 ± 0.15[b]	1.75 ± 0.27[a]
Survival rate (%)	76.67 ± 5.77[a]	86.67 ± 5.77[a]	83.33 ± 5.77[a]	76.67 ± 5.77[a]

Note: SGR = specific growth rate; FCR = feed conversion ratio. The average number followed by the same letter in the same row is not significantly different (P>0.05).

adding 1% inulin ~~in~~to the diet of juvenile carp *C. carpio* boosted the number of *Lactobacillus*, and lactic acid bacteria in the gut as well as increased immune response. This aligns with Hunt *et al*. (2019), who stated that inulin can increase the number of lactic acid bacteria by promoting more fermentation products in fish digestion.

An increase in lactic acid and short-chain fatty acids from inulin fermentation in the intestine lowers pH, which optimizes the growth of lactic acid bacteria. This creates competition for nutrients between lactic acid bacteria and various pathogenic bacteria, thereby indirectly inhibiting the growth of pathogens in the fish intestine. Fish digest nutrients in feed with the help of digestive enzymes, which can further improve feed efficiency (Widanarni *et al.,* 2015). The activity of digestive enzymes and changes related to adding prebiotics to feed vary depending on the fish species. The results of this study align with research that adds 2 and 4 g/kg of mannan-oligosaccharides (MOS) to the of *Astroststeus tropicus* larvae, increasing lipase and α-amylase activity (Maytorena-Verdugo *et al.,* 2022). Adding inulin in the appropriate amount produces various vitamins and enzymes that enhance digestive enzyme activity (De La Cruz-Marín *et al.,* 2023). When included in feed, inulin increases the production of short-chain fatty acids via intestinal microbiota, which lowers intestinal pH, and stimulates or improves fish digestive enzymes (Mehrgan *et al.,* 2022).

The best growth performance and feed efficiency in this study were observed with a treatment of 4 g/kg of inulin. These results suggest that adding inulin to common carp feed can improve digestion by increasing lactic acid bacteria and their enzyme activity. Proper nutrition can then be used more effectively for growth. These effects are related to the microbiome's ability to ferment inulin as a prebiotic and to changes in gut structure, which develop with the fish (Hoseinifar *et al.,* 2017). In accordance with this result, in red tilapia, the addition of 4 g/kg inulin shows the best growth performance compared to other treatments, with an increase in the number of lactic acid bacteria in the intestine and enhanced activity of the fish's digestive enzymes (Agustina & Susanto, 2024). The addition of 1.5% inulin into the diet can improve the survival and growth performance of juvenile golden pompano (*Trachinotus ovatus*). This is linked to fish fed inulin having higher intestinal villi than the control, and the inclusion of inulin increases the abundance of beneficial such as *Achromobacter* and *Prevotella* (Lan *et al.,* 2022). Several factors, including prebiotic doses, intestinal bacterial communities, intestinal structure of fish species, and developmental stages, can influence prebiotic fermentation, which subsequently affects fish growth performance (Hoseinifar *et al.,* 2016).

The survival rate of common carp was not significantly different between treatments. Fish health conditions remain within the normal range during the rearing period. The temperature varies from 27.1-28.0°C, pH from 7.1-7.5, dissolved oxygen from 7.3-7.9 mg/L, and total ammonia nitrogen from 0.18-0.20 mg/L. The water quality conditions in this study can are still tolerable for carp according to Goran *et al*. (2016). Water quality remains within the limits of fish tolerance. Supplementing the diet with 6 g/kg of inulin does not cause health issues, so the survival rate remains relatively high. However, the fish mortality rate in inulin treatments of 2 and 4 g/kg is lower than in the control. Group adding Mannan oligosaccharide (MOS) at a dose of 2 g/kg to African catfish (*Clarias gariepinus*) in a recirculating aquaculture system (Genç *et al.,* 2020) showed similar results, with better survival rates than the control.

CONCLUSION

This study successfully evaluates the effect of inulin as a feed additive on common carp. It shows that 4 g/kg of inulin is the optimal dose for enhancing health, digestive activity, and production of these fish. Since inulin has the potential to act as a prebiotic, it is recommended to use it at the start of fish rearing.

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