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Inulin supplementation in feed as a prebiotic for red tilapia *Oreochromis* sp.

Suplementasi inulin dalam pakan sebagai prebiotik pada ikan nila merah *Oreochromis* sp.

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ABSTRACT

The objective of this study was to assess the impact of inulin as a prebiotic in feed on red tilapia (*Oreochromis* sp.) growth performance, hematological parameters, feed utilization, digestive enzyme activity, and the quantity of lactic acid bacteria in the intestine. This study has a fully randomized design with four treatments and three replications, namely inulin at doses of 0, 1, 2, and 4 g/kg feed. One-way ANOVA was used to assess data on growth performance, feed utilization, survival rate, digestive enzyme activity, and the amount of lactic acid bacteria, while the hematological parameters were analyzed descriptively. Red tilapia fry weighing an average of 5.27 ± 0.45 g was raised for 30 days in plastic container containing 40 L of water at a density of 15 fish per container (375 fish/m^3). Fish were fed three times a day at satiation. Red tilapia-fed diet supplemented with 4 g/kg inulin of feed had higher body weight increase, protein efficiency ratio, digestive enzyme activity, and amount of lactic acid bacteria while food conversion ratio was lower compared to other treatments ($p<0.05$). Meanwhile, the specific growth rate and survival rate were not different from the control group. Hematological parameters were within the normal range for all treatments. Thus, the supplementation of inulin in feed of 4 g/kg has the potential as a prebiotic for red tilapia.

Keywords: digestive enzyme activity, inulin, lactic acid bacteria, prebiotic, red tilapia

ABSTRAK

Tujuan dari penelitian ini adalah untuk mengkaji pengaruh inulin sebagai prebiotik pada pakan terhadap kinerja pertumbuhan, parameter hematologi, pemanfaatan pakan, aktivitas enzim pencernaan, dan kuantitas bakteri asam laktat di usus ikan nila merah (*Oreochromis* sp.). Penelitian ini memiliki rancangan acak lengkap dengan empat perlakuan dan tiga ulangan yaitu inulin dengan dosis 0, 1, 2, dan 4 g inulin/kg pakan. One-way ANOVA digunakan untuk menilai data kinerja pertumbuhan, pemanfaatan pakan, kelangsungan hidup, aktivitas enzim pencernaan, dan jumlah bakteri asam laktat, sedangkan parameter hematologi dianalisis secara deskriptif. Benih ikan nila merah dengan berat rata-rata 5,27 ± 0,45 g dipelihara selama 30 hari dalam wadah plastik dengan volume air 40 L dan kepadatan 15 ekor per wadah (375 ekor/m^3). Ikan diberi makan tiga kali sehari secara *at satiation*. Ikan nila merah yang diberi suplemen inulin 4 g/kg pakan menunjukkan pertumbuhan bobot, rasio efisiensi protein, aktivitas enzim pencernaan, dan jumlah bakteri asam laktat yang lebih besar, sedangkan konversi pakan lebih rendah dibandingkan dengan perlakuan lainnya ($p<0,05$). Parameter laju pertumbuhan spesifik dan tingkat kelangsungan hidup tidak berbeda dengan kelompok kontrol Parameter hematologi berada dalam kisaran normal untuk semua perlakuan. Suplementasi inulin pada pakan sebanyak 4 g/kg berpotensi sebagai prebiotik ikan nila merah.

Kata kunci: aktivitas enzim pencernaan, bakteri asam laktat, ikan nila merah, inulin, prebiotik

INTRODUCTION

The production of tilapia (*Oreochromis niloticus*) cultivated in East Kalimantan Province has decreased. Referring to the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia, tilapia production in East Kalimantan in 2019 was 18,046.45 tons and decreased to 11,090.55 tons in 2020, including red tilapia (*Oreochromis* sp.). The pandemic has reduced aquaculture activity in this area. Another reason is the high cost of commercial feed, which has lowered farmer's interest in producing fish.

On the other hand, cultivation activities have changed from originally traditional to semi-intensive cultivation systems. This makes cultivators increase the density of seed stocking and feeding. This change in culture system will greatly affect feed utilization, growth performance, and fish health. If it continues in the long term and without good control, it is feared that production will continue to decline due to reduced growth and health of fish, while production costs will increase.

Increased fish farming activities lead to increased potential for the spread of disease in fish. On the other hand, disease control using antibiotics must be limited so that an alternative way of dealing with this problem is needed. One of the efforts to control the use of these materials is to use prebiotics in fish feed (Pietrzak *et al*., 2020). Prebiotics are non-digestible nutrients exhibiting a positive effect on the host organism by selectively activating the growth and activity of bacteria present in the digestive tract of fish (Kazuń *et al*., 2023).

Most prebiotics are ingredients capable of triggering the growth or activity of certain microbes, thereby improving host health (Davani-Davari *et al*., 2019). The activity of microorganisms in the digestive tract has a positive effect on immunity, disease resistance, and nutrient digestion (Guerreiro *et al*., 2018). Prebiotics that are widely used and proven to be able to increase production of aquaculture are fructooligosaccharides (FOS), trans-galactooligosaccharides (TOS), mannan-oligosaccharides (MOS), lactose, and inulin. Inulin is a class of carbohydrates that contain fructose polymers, terminal glucose sucrose, and small oligosaccharides (Hunt *et al*., 2019).

Inulin has been investigated for its role as a prebiotic in several types of fish, including carp (*Cyprinus carpio*) (Ajdari *et al*., 2022), gibel carp (*Carassius auratus gibelio*) (Akrami *et al*., 2015), grass carp (*Ctenopharyngodon idella*) (Mo *et al*., 2015), and rainbow trout (*Oncorhynchus mykiss*) (Hunt *et al*., 2019; Ghafarifarsani *et al*., 2021). Inulin has been shown to increase the growth and resistance of fish to disease. Therefore, this study was conducted to evaluate the effect of inulin on feed utilization, digestive enzyme activity, growth performance, survival rate, health conditions, and the number of lactic acid bacteria in the intestine of red tilapia.

MATERIALS AND METHODS

Fish preparation

The red tilapia, the test animal, has an average weight of 5.27 ± 0.45 g and length 3.5 ± 0.5 cm after adaptation. Red tilapia obtained from hatcheries in Samarinda City, East Kalimantan Province, were adapted for seven days in a tarpaulin tub with a diameter of 2 m and a volume of 1,500 L of water. The fish were fed three times a day during adaptation *at satiation* with commercial feed.

Feed preparation

By combining inulin in powder form, a commercial feed specific for tilapia with a particle size of 0.5-0.7 mm, a protein content of 39% and lipid content of 5% was produced. The feed was blended until smooth and then added inulin according to the research dose of inulin, namely 0, 1, 2 and 4 g/kg feed and mixed well. Mixing commercial feed with inulin without using a binder. The mixture of feed and inulin was then added with 10% water and molded. The molded feed was then dried in an oven at 40-50°C until dry.

Feeding and culture system

This research was an experimental laboratory with a completely randomized design using four treatments and three replications. The treatment given is namely 0, 1, 2 and 4 g/kg inulin feed. The weight of red tilapia was measured using an analytical balance with an accuracy of 0.01 g at the beginning of the study. The fish that has been weighed is put into a container with a volume of 40 L of water.

In this test 12 containers were used and each contained 15 fish. Fish were raised for 30 days while being fed three times daily *at satiation* with treatment feed. Siphoning was done daily and new water treated with aeration and settled for seven days before was added at the amount that was removed during siphoning. The average water quality parameters in this study were as follows: temperature 25.6-27.2°C, pH 6.4-6.7, dissolved oxygen 5.33-5.98 mg/L and total ammonia nitrogen 0.001-0.002 mg/L.

Parameters of growth performance, feed utilization and survival rate

Following the feeding trial, the following formulas were used to compute the body weight increase (BWI), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR):

$$\text{BMI} = W_t - W_0$$

$$\text{SGR (\%/ day)} = \frac{\ln W_t - \ln W_0}{t} \times 100$$

$$\text{FCR} = \frac{\text{total feed intake (g)}}{\text{weight growth of biomass of fish (g)}}$$

$$\text{PER (\%)} = \frac{\text{weight growth of biomass of fish (g)}}{\text{protein intake (g)}} \times 100$$

$$\text{SR} = \frac{N_t}{N_0} \times 100$$

Note:

W_t = Final weight (g)
W_0 = Initial weight (g)
t = Rearing time (days)
Protein intake = total feed consumption × feed protein content
N_t = the number of fish at time t
N_0 = the number of fish at time 0

Hematological assay

At the start and at the end of the feeding experiment, fish hematological parameters were recorded. A syringe was used to draw the first sample of blood from the caudal vein. The parameters of blood measured include the Sahli method using a hemometer was used to measure hemoglobin levels (Wedemeyer & Yasutake, 1977), the total red blood cells and white blood cells were measured based on the procedure of Blaxhall and Daisley (1973), the hematocrit level and the leukocyte differential were measured according to the Anderson and Siwicki (1995) method.

Digestive enzyme activity

The digestive enzyme activity test was carried out by taking fish intestine samples on the 0^{th} day and 30^{th} day. The intestines of the fish are removed from the fish's stomach and the outside is cleaned with distilled water. The inside of the fish intestine was not removed and immediately put in a sample container and then frozen. The frozen intestinal sample is ready to be analyzed for its enzyme activity. Parameters measured consisted of α-amylase, protease, and lipase activity. Measurement of α-amylase enzyme and protease enzyme activity using the Hidalgo *et al*. (1999) method and lipase enzyme activity using the Borlongan (1990) method.

Lactic acid bacteria in the intestine

Samples in the form of fish intestines were taken and the contents were removed and weighed as much as 0.5 g. The sample that has been weighed is put into a tube containing 4.5 mL of sterile distilled water (first dilution), then serial dilutions are made up to 10^{-4}. Each dilution was taken 1 mL then inoculated on MRSA media. These methods based on the total plate count method (Yunita *et al*., 2015).

Data analysis

Observational data including digestive enzyme activity, feed utilization, growth performance, survival rate and number of lactic acid bacteria (LAB) in fish intestines, were analyzed for diversity using SPSS Application through analysis of variance (ANOVA) at a 95% confidence level. Then, to assess changes across treatments, Duncan's additional test was performed. Finally, hematology parameters were descriptively analyzed in tabular form.

RESULTS AND DISCUSSION

Results

Growth performance, feed utilization and survival rate

The performance of growth and feed utilization of red tilapia in this study is shown in Table 1. Inulin supplementation in the feed given for 30 days on red tilapia has a significant effect on biomass gain (increase in body weight), final body weight (biomass), FCR, and PER. Red tilapia treated with inulin at a dose of 4 g/kg of feed showed the best biomass gain compared to other treatments, followed by inulin treatment 2 g/kg feed, 1 g/kg feed, and control without inulin administration ($p<0.05$), while the specific growth rate parameter was not significantly different. In terms of feed utilization parameters, inulin administration at 4 g/kg feed showed the best FCR and PER values compared to other treatments ($p<0.05$). The survival rate of red tilapia during the study did not show a significant difference between treatments.

Hematological parameters

The hematological parameters of red tilapia-fed diet supplemented with inulin were still within the normal range (Table 2). Increasing the dose of inulin given in the feed has an effect on increasing several hematological parameters of red tilapia compared to the control. Hematological parameters that experienced an increase were hematocrit, hemoglobin, erythrocytes, leukocytes and lymphocytes. In contrast, the neutrophils and monocytes of red tilapia in this study tended to be more varied.

Digestive enzyme activity

The addition of inulin in the feed significantly affected the digestive enzyme activity of red tilapia during the 30 days of the study. The amylase and protease enzymes with highest activities were in the treatment of 4 g/kg inulin in feed, then the treatments of 2 and 1 g/kg inulin in feed and the control ($p<0.05$). Administration of inulin at doses of 1, 2, and 4 g/kg of feed did not show any difference in lipase activity, but it differed greatly from the control ($p<0.05$), as shown in Figure 1.

Lactic acid bacteria in the intestine

Total lactic acid bacteria in the intestines of red tilapia after being treated with the addition of inulin to the feed for 30 days increased significantly (Figure 2). At the beginning of maintenance the number of lactic acid bacteria in the intestine of red tilapia was 0.15×10^4 CFU/mL, this number increased with increasing doses of inulin given in feed for 30 days of rearing. On the 30th day, the highest number of lactic acid bacteria was in the treatment dose of 4 g inulin kg of feed which was 0.20×10^4 CFU/mL, the lowest in the control treatment which was 0.14×10^4 CFU/mL. In the 2 and 4 g/kg inulin treatments,

Table 1. Feed utilization and growth performance of red tilapia fingerling fed with different levels of inulin.

Parameters	IInulin dose treatmens			
	Control	1 g/kg	2 g/kg	4 g/kg
Initial weight (g)	5.28 ± 0.66^{a}	5.36 ± 0.28^{a}	5.20 ± 0.28^{a}	5.23 ± 0.71^{a}
Final weight (g)	11.83 ± 0.67^{a}	12.65 ± 0.80^{a}	12.89 ± 0.48^{ab}	14.05 ± 0.16^{b}
BWI (g)	6.55 ± 0.74^{a}	7.30 ± 1.04^{b}	7.70 ± 0.75^{b}	8.82 ± 0.81^{b}
SGR (%/day)	2.70 ± 0.40^{a}	2.73 ± 0.25^{a}	2.91 ± 0.89^{a}	3.27 ± 0.39^{a}
Total feed intake (g)	136.00 ± 10.58^{a}	132.77 ± 6.34^{a}	128.00 ± 5.57^{a}	139.33 ± 4.04^{a}
FCR	1.39 ± 0.06^{b}	1.31 ± 0.14^{b}	1.18 ± 0.06^{ab}	1.08 ± 0.07^{a}
PER (%)	1.89 ± 0.07^{a}	2.02 ± 0.22^{a}	2.23 ± 0.12^{ab}	2.45 ± 0.16^{b}
SR (%)	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}

Note: SGR: specific growth rate; FCR: feed conversion ratio; PER: protein efficiency ratio; BWI: body weight increase: SR: survival rate. Different superscripts letters in the same row indicate significant difference at 5% confidence level ($p<0.05$).

Table 2. The average range of red tilapia hematological parameter values during observation.

Parameters	Inulin dose treatments			
	Control	1 g/kg	2 g/kg	4 g/kg
Hct (%)	15.31-20.97	15.31-25.74	15.31-28.42	15.31-24.38
Hb (g/%)	3.00-3.14	3.00-4.40	3.00-4.60	3.00-4.67
RBC ($\times10^6$ cell/mm^3)	2.90-3.82	2.90-4.36	2.90-5.83	2.90-4.80
WBC ($\times10^4$ cell/mm^3)	1.94-2.93	1.94-3.47	1.94-3.22	1.94-4.06
Lymphocytes (%)	80.84-81,67	80,84-82,54	80,84-82,35	80,84-83,52
Neutrophils (%)	4.40-5,35	4.40-4,68	4.40-4.83	4.40-5.41
Monocytes (%)	9.71-11,13	9.71-12,09	9.71-12,72	9.71-11,05

Note: WBC: white blood cell; Hb: hemoglobin; Hct: hematocrit; RBC: red Blood Cell. The sign (-) shows the lowest to the highest value.

total lactic acid bacteria in the intestine were not significantly different, but were higher than the other treatments, especially the control ($p<0.05$).

Discussion

This study shows that giving inulin to red tilapia feed for 30 days can improve body weight and feed utilization. In contrast, Ghafarifarsani *et al*. (2021) found that giving inulin at a dose of 20 g/kgof feed resulted in the best growth performance for rainbow trout (*Oncorhynchus mykiss*) larvae. Fish growth is improved with the addition of inulin in the feed, which is the result of increased fish appetite and intestinal microflora (Lan *et al*., 2022). Krishnaveni *et al*. (2021) stated that the efficient use of nutrients from feed is related to the increased activity of digestive enzymes and the availability of materials to be absorbed in the intestine with the degradation of nutrients from these indigestible materials.

Prebiotics in the diet can increase the number of intestinal villi, thereby expanding the area of nutrient absorption and improving digestion (Boonanuntanasarn *et al*., 2017). Dobrianska *et al*. (2021) noted that when the intestine is well-developed, nutrient absorption is improved, which has an impact on growth and decreases FCR. There is variation in growth, feed utilization, and

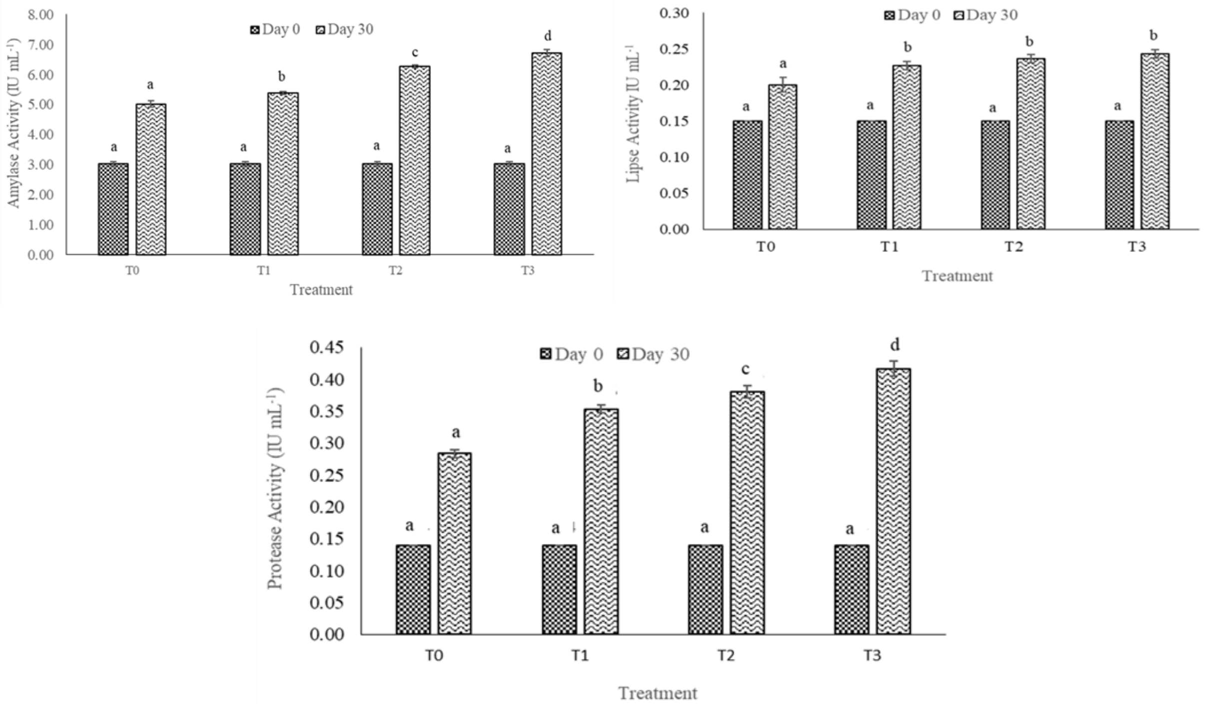

Figure 1. Digestive enzyme activity of red tilapia with the addition of inulin in diets. (T0: Control; T1: 1 g/kg; T2: 2 g/kg; T3: 4 g/kg inulin).

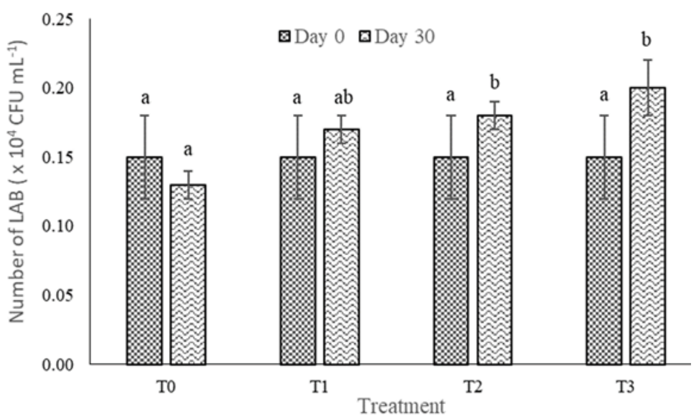

Figure 2. The number of lactic acid bacteria in red tilapia intestine. (T0: Control; T1: 1 g/kg; T2: 2 g/kg; T3: 4 g/kg inulin).

health benefits when the use of prebiotics depends primarily on the species, time of application, and dosage of the supplement, as well as the type of prebiotic (De La Cruz-Marín *et al*., 2023). Red tilapia reared for 30 days did not experience death or the survival rate reached 100% in all treatments. This showed that the administration of inulin in feed up to a dose of 4 g/kg of feed does not interfere with fish health.

This finding was in line with Yones *et al*. (2020), which showed that, similarly to other studies (Yones *et al*., 2019) conducted before, the hematological parameters of *O. niloticus* in their study remained within normal levels. The range of values of normal hematological parameters indicates that this type of fish did not experience any issues with health. Moreover, when inulin was included in the feed of tilapia (Yones *et al*., 2020) and Asian white snapper (Ali *et al*., 2018), it caused an increase in its total WBC count as well as higher levels of monocytes and neutrophils. Thus, we can conclude that nutrition, environmental stress, and infection are all influential factors when evaluating a fish's physiological and immunological conditions based on hematological parameters. Intestinal morphology can be improved by adding prebiotics to feed (Dobrianska *et al*., 2021).

This condition allows more components and nutrients to be digested and degraded, the condition of the intestine is better. Extracellular enzymes like proteases and lipases, as well as vitamins, fatty acids, and vital amino acids, are all produced by the digestive tract's epithelium. Prebiotics can improve substrate hydrolysis and increase the number of metabolites produced by the intestinal flora (Ziółkowska *et al*., 2021), improving fish absorption and digestion (Kumar *et al*., 2018). The study results are supported by De La Cruz-Marín *et al*. (2023), who found that the activity of protease and amylase in tropical gar (*Atractosteus tropicus*) larvae increased with inulin supplements in feed. Enzyme activity is stimulated by adding inulin in the right ratio so that the nutrients from the feed can be easily digested and the molecules can be absorbed, thereby achieving better digestion (Hunt *et al*., 2019).

Zhou *et al*. (2020) said that shrimp fed with 4 g/kg inulin changed the gut microbiota by multiplying the bacteria from the phylum Firmicutes and the genus *Bacillus*. Inulin and FOS stimulate the growth of certain microorganisms in the gut, such as Bifidobacteria and Lactobacilli. Inulin is an indigestible fiber that promotes lactic acid bacteria and increases fermentation products (Hunt *et al*., 2019). The increasing total lactic acid bacteria indicates that inulin has the potential to serve as a food supply for the lactic acid bacteria in red tilapia's intestines. The addition of inulin to red tilapia at a dose of 4 g/kg of feed for 30 days of rearing was able to support growth and utilization of feed through enzymatic activity in the digestive tract. The increase in the number of lactic acid bacteria in the intestine at the end of rearing is an indication that inulin has the potential as a prebiotic in red tilapia.

CONCLUSION

Inulin has the potential as a prebiotic in red tilapia with increased body weight, feed utilization, digestive enzyme activity, and total lactic acid bacteria in the intestine. The addition of inulin in the feed did not affect the health status and survival rate of the red tilapia but increases of WBC on final study indicates the ability of inulin to stimulate the red tilapia immune system, and a dose of inulin 4 g/kg of feed is the best dose as a prebiotic for red tilapia at 30 days reared.

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