Intestinal Enzymes and Lactid Acid Bacteria of Red Tilapia (Oreochromis sp.) Fed Black Soldier Fly (Hermetia illucens) Larvae and Probiotics

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Received: February 30, 2023; Accepted: April 16, 2023

Abstract

This study aimed to determine the effect of probiotics on the intestinal digestive enzyme, morphology and total bacteria of red tilapia (Oreochromis sp.) fed maggot BSF (Hermetia illucens) larvae. The research was conducted using randomized design of two treatments (with probiotics and without probiotics) in three replications. Tilapia (average weight 70 g) were reared for 60 days with diet composition of commercial feed and black soldier fly (Hermetia illucens) larvae at 7:3, respectively. Oral probiotics (Lactococcus sp. JAL 37 and Bacillus sp. PCP 1 (10^5 cell/g feed) and water probiotic (Klebsiella sp. A2, 10^3 cell/ml) were administered in seven days interval. We found that administration of maggot BSF (Hermetia illucens) larvae provide no defects on the fish gut morphology. The probiotics application did not change the intestinal cellulose enzyme activity, as well as the intestinal histology parameters, such as villi length, muscular layer thickness and amount of goblet cells. However, the probiotic application significantly enhanced protease and lipase activity (p<0.05), reduced total number of Aeromonas and enhanced total number of lactic acid bacteria (p<0.05).

Keywords: tilapia, probiotic, maggot BSF, intestine, enzyme, bacteria.

Introduction

Tilapia is popular fish among consumers because it has a thick meat and high nutrient, and is inexpensive (Dailami et al., 2021). Other advantages of tilapia include its simplicity of cultivation, rapid growth, resistance to illness, and ability to live in a wide range of salinity (Andriani, 2018). Red tilapia (Oreochromis sp.) is a popular hybrid strain in Indonesia. Increased aquaculture production necessitates an increase in the demand for fish feed. Alternate feeds are required to support tilapia aquaculture activities (Nikhiali et al., 2022). Maggot can be utilized as an alternative feed because it meets the parameters for feed ingredients such as harmless to fish, high in nutrition, easy to get, easy to process, and cheap (Pratiwi, 2022). The larva of black soldier fly (Hermetia illucens) has a high protein content (40-50%), a long life-span (about 4 weeks), does not require high technology for production (Bokau & Basuki, 2020), and available in various sizes (Fahmi et al., 2009). Even though maggot includes a variety of nutrients that are beneficial to fish, it can only be used as a feed partial substitution due to the high content of fat (9-28% of dry weight). The excessive use of BSF larvae (>33%) not only inhibits fish development performance but also affects protein digestibility in the digestive process of fish (Barragan-Fonseca et al., 2017). Gut is a vital digestive organ that is directly associated with nutrition absorption. Intestinal histomorphology, such as the length of the villi, can reveal fish’s ability to absorb nutrients as well as their intestinal health status, according to Buyukdeveci et al. (2023). Villi lengthening has been shown to improve food absorption and fish development (Dewanti et al., 2022).

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Probiotics are helpful bacteria that improve water quality, the immune system, fish survival, growth, and feed digestion. Probiotic bacteria commonly used in aquaculture include Bacillus sp. and lactic acid bacteria (Lactobacillus sp., Lactococcus lactis, Pediococcus acidilactici) (Kuebutherland, 2020). Lactococcus sp. JAL 37, Bacillus sp. PCP1 (Dewanti, 2022), and Klebsiella sp. A2 (Aswiyanti et al., 2021) are among the probiotic bacteria strains created for aquaculture. Lactococcus sp. JAL 37 and Bacillus sp. PCP1 probiotics in eel feed at 3x107 cfu/gr of feed balances the number of intestinal bacteria while having no effect on eel intestine histology such as intestinal diameter, villi length, and thickness of the intestinal wall muscle layer (Dewanti et al., 2022). Meanwhile, Klebsiella sp. A2 is simulant nitrification/denitrification (SNNDN) bacterium that improves aquaculture water quality and is safe to use in tilapia farming at a level of 10⁵ cfu/ml (Aswiyanti et al., 2021). Klebsiella sp. may produce hydroxylamine oxidase (HAO), periplasmic nitrate reductase (Nap), and nitrite reductase (NIR), all of which are required for heterotrophic nitrification and aerobic denitrification (Pal et al., 2015).

Lu et al. (2020) investigated maggot as fish feed, examining the effect of maggot flour substitution on the intestinal histology of koan fish (Ctenopharyngodon idellus). As a result, as the maggot application dose increases, the length of the koan's intestinal villi decreases. Similarly, Li et al. (2017) observed that replacing maggot flour in carp (Cyprinus carpio var. Jian) can reduce intestinal villi performance by more than 50%. The use of maggot at 15% of food portion in rainbow trout farming affects the gut bacteria (Rimoldi et al., 2021). Research on the application of probiotics to the digestive tract conditions of tilapia fed BSF maggot is still limited. This study aims to determine the effect of probiotics on the digestive tract conditions of tilapia fed BSF maggot feed.

**Material and Methods**

The study used a randomized design with two treatments and three replications. Red tilapia (Oreochromis sp., 70 g) population was acclimatized for one week in cylinder tanks with the density of 50 fish/tank, followed by treatment as follows:

A: feed a mixture of 30% maggots and 70% commercial pellets with probiotics added.
B: feed a combination of 30% maggots and 70% commercial pellets without probiotics.

The probiotic bacteria employed were oral probiotics (Bacillus sp. PCP1 and Lactococcus sp. JAL 37 at 10⁷ cells/g feed) (Dewanto et al., 2022), and water probiotics (Klebsiella sp. A2 at 10⁶ cells/mL) (Aswiyanti et al., 2021). Maggot was supplied by PT. Trimitra Bumi Lestari in Yogyakarta. Maggot was housed in a box made of plastic. The maggot utilized in the present study was fresh baby maggot that was 5 to 6 days old and was replenished once a week.

**Preparation of probiotics**

Oral probiotics (Bacillus sp. PCP1, Lactococcus sp. JAL37), and water probiotics (Klebsiella sp. A2) were activated by introducing 10 µl each into 10 mL of Tryptone Soya Broth (TSB) medium and incubating for 24 hours at room temperature. The bacteria were then cultured for 24 hours at 30°C on Trytome Soya Agar (TSA) medium. Bacteria were extracted using a sterile Phosphate Buffer Saline (PBS) solution, and density was determined. Bacterial density was measured using a spectrophotometer (625 nm) and a McFarland standard solution. The harvested bacteria were blended with the pellets using a sprayer. Probiotic feed was supplied twice a day, in the morning and evening, every seven days.

**Gut histology analysis**

Tilapia were dissected, and 2 cm of the inner intestine was removed before being fixed in a 10% neutral buffer formaline (NBF) solution. The Pathology and Anatomy Laboratory, Faculty of Medicine and Public Health, Gadjah Mada University, performed further tissue processing. Tissue dehydration was accomplished by immersing the samples in row ethanol (70% to 100% ethanol). Following that, the tissue samples are soaked in liquid paraffin in an oven at 58 - 60°C until the solvent entirely evaporates, after which they are immersed in xylol solution till they become translucent. A microtome was used to cut the material with a thickness of 1-10 m for staining with hematoxylin and eosin. Morphological data such as villi length and goblet cell number were measured with a microscope and evaluated with image raster software.
**Enzymes activity test**

Clear zones on SMA medium (skim milk agar), tween 80 agar, and carboxymethyl cellulose (CMC) were employed to test protease, lipase, and cellulase enzyme activity. The tilapia was dissected, and the intestinal organs were cut to 1 cm length before being crushed and centrifuged to collect the supernatant. The centrifuge was set to 4000 rpm and 4°C. A total of 20 l of supernatant was dripped onto a paper disc for protease activity, tween 80 agar medium for lipase activity, and CMC medium (carboxymethyl cellulose) for cellulase activity. The samples were then incubated for 24 hours at 30°C. The enzyme activity of cellulase was tested by adding 1% congo red solution to the CMC medium. The presence of enzyme activity was indicated by the formation of a clear zone in the media around the paper disc. Protease activity was indicated by the presence of a clear zone around the paper disc indicating that the casein contained in the skim milk agar media had been hydrolyzed into peptide compounds and amino acids which were soluble in the medium. Lipase is an enzyme that catalyzes the hydrolysis of triglycerides into glycerol and fatty acids (Assan et al., 2022). Lipase enzyme activity was indicated by a clear zone in 1% tween 80 medium. The clear zone is formed due to the hydrolysis of a combination of free fatty acids and Ca2+ ions (García-Bernal et al., 2015). The enzymatic hydrolysis index was calculated by dividing the diameter of the clear zone by the diameter of the paper disc (Hastuti et al., 2017, Istiqomah et al., 2019).

**Analysis of cultivable bacteria**

This experiment was carried out to investigate the number of cultivable bacteria in the intestines of tilapia. The tilapia's anterior gut was removed and cleaned with a PBS (phosphate buffered saline) solution. After crushing the intestinal samples with a mortar and pestle until smooth, they were placed in a sterile 1.5 mL microtube with 1 mL PBS solvent and weighed. Following that, 100 l of sample was vortexed and diluted to 10⁶ dilutions in 900 l of sterile PBS before being cultured in 10 l of medium. Total bacteria were counted using TSA medium (tryptone soya agar), while Aeromonas were counted using GSP medium (starch phenyl glutamate agar). Total bacteria were counted using TSA medium (tryptone soya agar), Aeromonas bacteria were counted using GSP medium (starch phenyl glutamate agar), and lactic acid bacteria were counted using MRSA (De Man, Rogosa, Sharpe Agar). Bacteria were cultured for 24 hours at 30°C. Calculation of bacteria in the intestine was carried out using the total plate count method with cfu/g intestine units.

**Water quality analysis**

Physical water quality parameters such as temperature, pH and dissolved oxygen (DO) were measured using a water quality checker. Chemical water quality parameters such as ammonia, nitrite and nitrate were measured according to the guidelines of SNI 06-6989.1-2004 (2004) using the spectrophotometer method.

**Data analysis**

The data was analyzed using parametric statistical analysis with an independent sample T test one tail using SPSS IBM 28 software. The data was assessed for homogeneity and normalcy using classical assumptions. The significance value (sig.) was used to make decisions on homogeneity and normality testing. The data that did not meet the classical assumption test were analyzed using non-parametrical test (the Mann Whitney Test).

**Result**

**Gut morphology**

Figure 1. Tilapia gut morphology following maggot feeding (Magnification = 20x; SG = goblet cells, V = villi length, O = thickness of the gut wall's muscular layer). Scale bar = 100 µm.
Figure 2. Tilapia gut morphology following maggot feeding and probiotics administration (Magnification = 20x; SG = goblet cells, V = villi length, O = thickness of the gut wall's muscular layer). Scale bar = 100 µm.

Table 1. The intestine morphology of red tilapia in the present study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Length of phili (µm)</th>
<th>Thickness of muscular layer (µm)</th>
<th>Number of goblet cells (cells/villi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotics</td>
<td>318 ± 20a</td>
<td>55 ± 14a</td>
<td>16 ± 7a</td>
</tr>
<tr>
<td>Non probiotics</td>
<td>275 ± 50a</td>
<td>47 ± 12a</td>
<td>8 ± 3a</td>
</tr>
</tbody>
</table>

The average of three replicates per treatment was used to represent the data. Same superscripts in the same column means no significant difference (p>0.05).

There were no defects on the fish gut morphology of all groups. The average length of the intestinal villi in the control fish (non-probiotic treatment) was 275.37 µm. The length of the intestinal villi in the probiotic treatment was 318.41 µm.

Supplementation of probiotics did not affect the number of goblet cells in the tilapia intestine. The number of goblet cells in the probiotic treatment was 16.2 cells/villi while in the non-probiotic treatment it was 8.3 cells/villi.

Addition of probiotics did not affect the thickness of the muscle layer of the tilapia intestinal wall. The probiotic treatment had a thickness of the muscle wall layer of 54.8 µm, while the thickness of the intestinal wall muscle layer in the probiotic treatment was 46.9 µm.

Protease activity

The probiotic treatment had stronger protease enzyme activity than the non-probiotic treatment on the 30th and 60th days (Table 2). The average protein hydrolysis index for the probiotic treatment was 3.3 on the 30th day, while it was 2.4 for the non-probiotic treatment. On the 60th day, however, the activity of the protease enzyme differed significantly between the probiotic and non-probiotic treatments. When compared to the non-probiotic treatment, the average protein hydrolysis index improved significantly by 4.2 in the probiotic therapy.

Lipase activity

In fish given probiotics, the lipase index increased on day 30, earlier than the control groups. The probiotic and non-probiotic treatments had significantly different average lipase indexes on day 30, with the probiotic treatment having a lipase index of 2.2 and the non-probiotic treatment having a lipase index of 1.7 (Table 2). There was no significant difference in lipase enzyme activity between the probiotic and non-probiotic treatments on the 60th day.

Table 2. Enzymatic index in the intestine of red tilapia from each treatment.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Group</th>
<th>Day-30</th>
<th>Day-60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>3.3 ± 0.66a</td>
<td>4.2 ± 0.25a</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>2.4 ± 0.36a</td>
<td>3.1 ± 0.13b</td>
</tr>
<tr>
<td>Lipase</td>
<td>P</td>
<td>2.2 ± 0.25a</td>
<td>2.4 ± 0.33a</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>1.7 ± 0.17b</td>
<td>2.4 ± 0.44a</td>
</tr>
<tr>
<td>Cellulase</td>
<td>P</td>
<td>1.4 ± 0.17a</td>
<td>1.4 ± 0.16a</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>1.4 ± 0.21a</td>
<td>1.5 ± 0.16a</td>
</tr>
</tbody>
</table>

The average of three replicates per treatment was used to represent the data. Different superscripts in the same column differ significantly (p<0.05). P: probiotics, NP: Non probiotics

Cellulase activity

The average cellulolytic index on day 30 was 1.36, which did not differ significantly between the probiotic and non-probiotic regimens (Table 2). Cellulase enzyme activity rose on the 60th day, however the difference between probiotic and non-probiotic treatments was not significant (p>0.05). The average cellulolytic index in the probiotic therapy was 1.4 while in the non-probiotic treatment was 1.5.

The number of total bacteria

The probiotic therapy similar average number of total bacterial colonies forming units in the stomach of tilapia to the non-probiotic treatment (Table 3). The average total bacterial count in the probiotic treatment was 1.7 x 10⁸ cfu/g intestine, while the non-probiotic was 2.2 x 10⁸ cfu/g.
The digestive tract is crucial in the process of digesting nutrition. The digestive organs are

intestine. The total bacterial count increased on the 60th day, however the difference was not significant between the probiotic and non-probiotic therapies. The average total bacterial count increased to 2.8 x 10^5 cfu/g intestine in the probiotic therapy and 1.9 x 10^5 cfu/g intestine in the non-probiotic treatment.

Table 3. The average number of bacteria in the intestine of tilapia.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Group</th>
<th>Day-30 (x 10^5 cfu/g intestine)</th>
<th>Day-60 (x 10^5 cfu/g intestine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacteria</td>
<td>P</td>
<td>1.7 ± 0.42^a</td>
<td>2.8 ± 1.38^a</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>2.2 ± 0.74^b</td>
<td>1.9 ± 0.57^b</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>P</td>
<td>11.2 ± 3.98^a</td>
<td>8.1 ± 4.05</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>19.8 ± 4.61^b</td>
<td>10.3 ± 3.34</td>
</tr>
<tr>
<td>LAB</td>
<td>P</td>
<td>0 ± 0</td>
<td>5.8 ± 3.1^a</td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

The average of three replicates per treatment was used to represent the data. Different superscripts in the same column differ significantly (p<0.05). P: probiotics, NP: Non probiotics

The number of Aeromonas

The probiotic treatment had fewer presumptive Aeromonas than the non-probiotic treatment (Table 3). There was a substantial difference in the amount of Aeromonas on the 30th day between the probiotic and non-probiotic treatments. Aeromonas in the probiotic groups were 11.2 x 10^5 cfu/g intestine, while Aeromonas bacteria in the placebo treatment were 19.8 x 10^7 cfu/g intestine. On the 60th day, the quantity of Aeromonas dropped but not considerably between probiotic and non-probiotic treatments, with the probiotic treatment having 8.1 x 10^5 cfu/g intestine and the non-probiotic treatment having as much as 10.3 x 10^7 cfu/g intestine.

The Number of Lactic Acid Bacteria

On the 30th day, neither probiotic nor non-probiotic treatments had any presumptive lactic acid bacteria growing in the medium (Table 3). On the 60th day, the quantity of the presumptive lactic acid bacteria in the probiotic therapy increased to 5.8 x 10^6 cfu/g intestine, but no colonies grew in the non-probiotic treatment. The Mann Whitney Test results revealed a significant difference between the probiotic and non-probiotic treatments on the 60th day, indicating that probiotics could influence the quantity of lactic acid bacteria in the digestive tract of tilapia.

Water Quality

Water quality parameters such as pH, temperature, and dissolved oxygen (DO) were found to be excellent in both probiotic and non-probiotic treatments (Table 8). The pH level of the water in the probiotic treatment was 7, the temperature was 28.3°C, and the dissolved oxygen level was 4.1 ppm. The water quality in the non-probiotic treatment has a pH of 7, a temperature of 27.7°C, and a dissolved oxygen content of 4.2 ppm. Daily water quality during the study remained consistent with SNI (2009) water quality standards for raising tilapia culture, namely temperatures ranging from 25 to 32 °C, pH 6.5 to 8.5, and dissolved oxygen (DO) >3 ppm.

Total ammonia nitrogen (TAN) levels in the probiotic therapy were lower than in the non-probiotic treatment. (Table 8). The T test findings showed that there was no significant average difference in TAN levels between the probiotic and non-probiotic treatments (P>0.05), indicating that the addition of probiotics had no effect on TAN levels in the tilapia rearing medium. The probiotic treatment had a TAN ammonia level of 0.00074 ppm, whereas the non-probiotic treatment had a TAN ammonia level of 0.00266 ppm. There was no difference in nitrite and nitrate levels between treatments in this study.

Table 8. Water quality during red tilapia culture.

<table>
<thead>
<tr>
<th>Group</th>
<th>Probiotics</th>
<th>Non probiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>28.3 ± 0.1</td>
<td>28.0 ± 0.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.0 ± 0.0</td>
<td>7.0 ± 0.0</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/l)</td>
<td>4.1 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>TAN (mg/l)</td>
<td>0.05 ± 0.01</td>
<td>0.3 ± 0.04</td>
</tr>
<tr>
<td>Ammonium (mg/l)</td>
<td>0.049 ± 0.000</td>
<td>0.29 ± 0.000</td>
</tr>
<tr>
<td>Ammonia (mg/l)</td>
<td>0.0007 ± 0.00054</td>
<td>0.0027 ± 0.0025</td>
</tr>
<tr>
<td>Nitrite (mg/l)</td>
<td>0.7 ± 0.10</td>
<td>0.9 ± 0.25</td>
</tr>
<tr>
<td>Nitrate (mg/l)</td>
<td>15.6 ± 1.74</td>
<td>13.0 ± 1.14</td>
</tr>
</tbody>
</table>

The average of three replicates per treatment was used to represent the data. Different superscripts in the same column differ significantly (p<0.05).

Discussion

The digestive tract is crucial in the process of digesting nutrition. The digestive organs are
extremely sensitive to the nutrients in the feed, which can result in direct changes in the activity of digestive enzymes, which eventually reflect health status and affect fish growth (Sankar et al., 2017). The purpose of this study is to see how probiotics affect intestine histology, enzyme function, and microbial count in red tilapia (Oreochromis sp.) fed BSF maggot larvae. Because the morphological structure of the gut can change rapidly in response to feed input, it serves as an indicator of fish health. Changes in intestinal shape might affect food absorption, hence affecting fish development and production. Intestinal morphological measures such as villi length and muscle layer thickness of the intestinal wall can serve as indicators of fish gut health (Han et al., 2015). Histology investigation revealed that the tilapia intestine is in good health. The use of probiotics did not affect the intestinal villi, walls thickness, and goblet cells number in red tilapia intestine. Longer intestinal villi can increase absorption surface area and feed nutrient utilization, resulting in improved fish development performance (Ramos et al., 2017). According to Chen et al. (2020), the increase in villi length is connected to the ability of ingested probiotic bacteria to colonize the epithelium because probiotic bacteria attached to the epithelium may consume feed carbohydrates and create short chain fatty acids.

The thickness of the intestinal wall’s muscular layer indicates the strength and weakness of intestinal contractions. Increasing the thickness of the intestinal wall muscle layer strengthens intestinal contractions during feed digestion (ShaoWei et al., 2016; Shi et al., 2019). The thickness of the intestinal wall muscle layer was similar between treatment in the present study. These findings are consistent with previous research (Dewanti et al., 2022), which found that the probiotic bacteria Bacillus sp. PCP 1 and Lactococcus sp. JAL 37 had no effect on the villi length or muscle layer thickness of the eel intestinal wall. According to Han et al. (2015), the application of the probiotic Bacillus licheniformis at doses of 10^6 and 10^7 cfu/g feed did not show a significant difference in the length of the villi and the thickness of the muscle wall layer of the tilapia intestine between the probiotic and control treatments. This can be modified by a variety of factors such as fish type, bacteria species used, feeding duration, and environmental factors.

Goblet cells are specialized epithelial cells that play a function in innate resistance in the gut. Mucus is produced by goblet cells, particularly mucin, which contains glycoproteins that can inhibit pathogens from entering the intestinal epithelium. The use of probiotics in this study boosted the number of goblet cells, albeit only marginally (P>0.05). Similar findings were reported in a study (Kuebutornye et al., 2020) that demonstrated no significant variation in the number of goblet cells in the intestines of tilapia with the application of the probiotic Bacillus sp. 108. An increase in the number of goblet cells maybe associated to a rise in probiotic supplementation, which can result in an increase in mucin secretion (Buyukdeveci et al., 2023). Goblet cells work as intestinal guardians by producing bactericidal mucus, which can reduce infection and fight pathogens (Hossan et al., 2022). Because lactic acid bacteria can produce mucus, the number of goblet cells is proportional to the number of lactic acid bacteria (Ruiz et al., 2020). Mucus forms a gel that attaches to the intestinal epithelium and serves as the intestine’s first line of defense against infections and intestinal damage caused by pathogens and enzymes that can affect the intestinal epithelium’s performance.

Digestive enzymes help to convert macronutrients in feed into particles that are easier for the body to absorb. The presence of enzymes that match the feed composition determines fish’s capacity to break down nutrients in feed. Protease, lipase, and amylase are the three main digestive enzymes. There are other supporting hydrolysis enzymes like cellulase (similar to amylase enzymes). Because they are related to the feed consumed, these enzymes play an important role in the digestive system of omnivorous and herbivorous fish (Assan et al., 2022).

Proteases are importance digestive enzymes that hydrolyze protein peptide bonds into amino acids (Assan et al., 2022), while lipase plays an important role in breaking down fats, especially triacylglycerols into fatty acids and glycerol. The results showed that the activity of the protease and lipase enzyme in the probiotic treatment was higher than in the non-probiotic treatment. The findings of this study support previous studies that probiotic bacteria can boost the secretion of endogenous digestive enzymes, which is connected with improved fish development and metabolism. Increased enzyme activity indicates
increased fish digestibility because probiotic bacteria may break down macromolecules into smaller one allowing fish to absorb more nutrients (Hortillosa et al., 2021). Despite an increase on day 30, lipase activity on day 60 was consistent across treatments. This phenomenon regarding not increasing lipase activity is consistent with previous probiotic studies in grouper Epinephelus coioides (Sun et al., 2012), Oreochromis mossambicus (Sankar et al., 2017), Tor grpus (Mohammadian et al., 2017), and rainbow trout Oncorhynchus mykiss (Mohammadian et al., 2019).

Gastrointestinal microbiota contributes to host animal health by increasing nutritional digestibility, immunity, pathogenic bacteria inhibition, and energy homeostasis (Welker and Lim, 2011). Fish gut microbiota is responsive to rearing environment, seasonal changes, and nutritional changes, including probiotic treatment (Standen et al., 2015).

In the present study, the probiotic treatment had a lower average total bacterial count in the gut of tilapia than the non-probiotic treatment (Table 6). This study’s findings are comparable to those of previous studies on eel (Dewanti et al., 2022), catfish (Afrilasari et al., 2016), and rohu, Labeo rohita (Mohapatra et al., 2012). This is possible because the culture process still employs the agar plate approach, resulting in suboptimal outcomes. According to Dewanti (2022), the total number of bacteria in the intestine calculated using the TPC method solely indicates the number of bacteria in the media utilized. only a small part of the total bacteria in the intestine can be cultivated in the laboratory using the agar plate method. This can also be altered by the method utilized, specifically the agar plate approach, which does not represent the true amount of lactic acid bacteria. According to Standen et al. (2015), only a small part of the total bacteria in the intestine can be cultivated in the laboratory using the agar plate method. The detection of lactic acid bacteria in probiotic treated groups indicating that the use of probiotics could affect the quantity of lactic acid bacteria in the digestive tract of tilapia (P0.05).

The probiotic bacteria utilized were very efficient in enhancing tilapia digestibility and immunity, as evidenced by increased enzyme activity and a decrease in the quantity of Aeromonas bacteria in the tilapia intestine. Lactic acid bacteria help to keep harmful bacteria in check in the intestine. Lactic acid produced by lactic acid bacteria has a bactericidal effect, balancing intestinal microbes and lowering intestinal pH to 3-4.5, inhibiting the growth of other bacteria, including putrefactive bacteria (Setyawan et al., 2014).

Throughout the study, daily water quality parameters remained within optimal limits This is based on the water quality requirements specified in SNI (2009), which are temperatures ranging from 25 to 32 degrees Celsius, pH 6.5 to 8.5, dissolved oxygen (DO) 3 ppm, and ammonia concentration (NH3) 0.02 ppm. In this investigation, the use of feed and water probiotics had no discernible effect on water quality.

The use of probiotics in feed and water can improve the digestive tract condition of tilapia fed BSF maggot. Improving the condition of the digestive tract indicates higher growth and immunity in fish. More research is needed on the application of probiotic bacteria to the digestive tract of tilapia at various doses, both low and high. Synbiotic treatments can also be utilized to boost the efficiency of probiotic microorganisms. Synbiotics are a cross between probiotics and prebiotics. Cavalcante et al. (2020) discovered that using synbiotics (a combination of probiotic bacteria and prebiotic mannan oligosaccharides (MOS)) could boost tilapia immunity to Aeromonas hydrophila infection without decreasing tilapia development.

The use of probiotics in feed and water in the present study enhances the condition of the intestine of red tilapia fed BSF maggot, particularly in terms of enzymatic activity, decrease the Aeromonas growth, increase the population of lactic acid bacteria, and maintain
gut morphology. Improving the condition of the digestive tract indicates higher growth and immunity in fish. More research is needed on the application of probiotic bacteria to the digestive tract of tilapia at various doses, both low and high. Synbiotic treatments can also be utilized to boost the efficiency of probiotic microorganisms. Another study found that utilizing synbiotics (a combination of probiotic bacteria and prebiotic mannann oligosaccharides (MOS)) could increase tilapia immunity to Aeromonas hydrophila infection without reducing tilapia development (Cavalcante et al., 2020).

Acknowledgments

The authors would like to acknowledge Mr Kusmanto, Mr Aditya Arif, Ms Lubna Tanti P and Ms Hamda Raihana Fatin for their technical support in handling of red tilapia and MSF maggot during the experiments.

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