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Maggot and probiotic feed supplementation on growth performance and intestinal microbiota in *Anguilla bicolor bicolor*

Suplementasi pakan maggot dan probiotik terhadap performa pertumbuhan dan mikrobiota usus pada *Anguilla bicolor bicolor*

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ABSTRACT

The purpose of this study was to determine the effectiveness of probiotic and maggot flour supplementation on production performance, water quality, and eel gut microbiota. There were five treatment groups of maggot flour doses (0, 25, 50, 75, and 100%) each with three replications. The eel seeds used were three months old (average weight 6.11 ± 4.28 g, average length 14.3 ± 3.22 cm) as many as 15 fish/aquarium which were fed until full twice a day for 60 days. Growth performance measurements were carried out every two weeks and at the end of the study, survival rate, feed digestibility, water quality, and the abundance of water and eel gut bacteria. The growth rate of group P1 (0% maggot flour) of 1.222 ± 0.662 g/day was significantly higher (P≤0.05) than group P5 (100% maggot flour) of 0.223 ± 0.094 g/day. The best FCR value was shown by treatment P1 of 2.576 ± 0.598. The highest protein digestibility was shown by group P5 at 75.90%, while the lowest protein digestibility was shown by group P1 at 62.41%. Low digestible protein and high fecal protein were shown by group P5 of 15.15% and 28.77%, respectively. The abundance of bacteria in the *Bacillus* sp. and *Lactobacillus* sp. groups dominated in groups P2 and P5. Based on the research results, it can be concluded that the use of commercial probiotics and 100% dose of maggot flour is not yet effective in improving growth performance, but can increase the abundance of good bacteria in the intestines of eels.

Keywords: Anguilla bicolor, growth parameters, gut microbiota, maggot flour, probiotic

ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui efektivitas suplementasi probiotik dan tepung maggot terhadap performa produksi, kualitas air, dan mikrobiota usus sidat. Terdapat lima kelompok perlakuan dosis tepung maggot (0, 25, 50, 75, dan 100%) masing-masing dengan tiga kali ulangan. Benih sidat yang digunakan berumur tiga bulan (berat rata-rata 6.11 ± 4.28 g, panjang rata-rata 14.3 ± 3.22 cm) sebanyak 15 ekor/akuarium yang diberi pakan sampai kenyang sebanyak dua kali sehari selama 60 hari. Pengukuran performa pertumbuhan dilakukan setiap dua minggu sekali dan akhir penelitian, tingkat kelangsungan hidup, daya cerna pakan, kualitas air, serta kelimpahan air dan bakteri usus belut. Laju pertumbuhan kelompok P1 (0% tepung maggot) sebesar 1,222 ± 0,662 g/hari lebih tinggi secara signifikan (P≤0,05) dibandingkan kelompok P5 (100% tepung maggot) sebesar 0,223 ± 0,094 g/hari. Nilai FCR terbaik ditunjukkan oleh perlakuan P1 sebesar 2,576 ± 0,598. Daya cerna protein tertinggi ditunjukkan oleh kelompok P5 sebesar 75,90%, sedangkan daya cerna protein terendah ditunjukkan oleh kelompok P1 sebesar 62,41%. Protein cerna rendah dan protein feses tinggi ditunjukkan oleh kelompok P5 masing-masing sebesar 15,15% dan 28,77%. Kelimpahan bakteri pada kelompok *Bacillus* sp. dan *Lactobacillus* sp. mendominasi pada kelompok P2 dan P5. Berdasarkan hasil penelitian dapat disimpulkan bahwa penggunaan probiotik komersial dan tepung maggot dosis 100% belum efektif dalam meningkatkan kinerja pertumbuhan, tetapi mampu meningkatkan kelimpahan bakteri baik dalam usus dari ikan sidat.

Kata kunci: Anguilla bicolor, mikrobiota usus, parameter pertumbuhan, probiotik, tepung maggot

INTRODUCTION

Eel is a catadromous fish species that spends most of its life in freshwater, and they complete their spawning activities by migrating from freshwater to seawater environments (Tsukamoto *et al.,* 2011). Eel farming has long been a global aquaculture sector focusing on growth performance to reach commercial size (Liang *et al.,* 2023). Tropical anguilla eels, such as the shortfin eel (*Anguilla bicolor*) are one of the most commercially important species occurring in Southeast Asia (Arai *et al.,* 1999). It is because its texture and taste are similar to *A. japonica*, making it commercially important in terms of market demand (Arai, 2014). Eel is very rich in essential fatty acids that are very useful for meeting nutritional value in society, the content of nutrients such as EPA and DHA eel was 742 mg/100 g and 1,337 mg/100 g, this content was higher than salmon at 492 mg/100 g and 820 g/100 g (Arief *et al.,* 2016).

A. bicolor is known to inhabit most tropical to subtropical environments, especially in the waters around the Indonesian archipelago (Aoyama *et al.,* 2001; Minegishi *et al.,* 2012). Poor growth rates and high mortality rates because of the disease are significant obstacles in *A. bicolor* cultivation activities. Given this, *A. bicolor* cultivation practices must be carried out by utilizing the right feed, so as to produce growth performance and efficiency (Luchiari & Pirhonen, 2008). Fishmeal (FM)-based feed has contributed as the main protein source in aquaculture feed production due to its nutritional content and palatability that stimulates the appetite of farmed animals (Hussain *et al.,* 2024). However, with the global aquaculture consumption demand expected to increase fourfold by 2050, alternatives to replace FM as a raw material for aquaculture feed are very important (Boyd *et al.,* 2022).

Insect meal has been described as a rich source of protein and can be used to replace FM (Carvalho *et al.,* 2023). Insects are the third generation of protein sources in the world, and have been promoted as a sustainable alternative to replace fishmeal while reducing farmers' dependence on imported feed supplies (Chia *et al.,* 2019; Raman *et al.,* 2022), especially in Indonesia. Meal made from black soldier fly (BSF) larvae known as "maggots" has a strong enzymatic system, better bioconversion, and relatively fast reproduction strongly supports the use of this flour as a potential source of animal protein in aquaculture animals (Siddiqui *et al.,* 2024). Maggot flour has a relatively cheap price of Rp. 3,000.00 per kg with a high nutritional value of around 42-60 g/kg of crude protein (Aldis *et al.,* 2024). This nutritional value is ideal as a conventional protein source in freshwater aquaculture efforts, including eel.

Maggot flour supplements have been reported to be able to improve gut microbiota, growth, and immunity in swamp eels *Monopterus albus* (Xiang *et al.,* 2020). Although previous studies have reported the potential for substituting fish meal with maggot flour in farmed fish species, most have only focused on the effects of maggot flour as a single alternative ingredient. Until now, reports of maggot flour supplementation with probiotics in eel feed have not been reported. Probiotic is a class of microorganisms that consumed in sufficient amounts may provide health benefits to their hosts (Swanson *et al.,* 2020). Lactic acid bacteria (LAB) are well-known probiotics that live mostly in the small intestine and are resistant to acidic and bile-rich environments (Al-Fakhrany & Elekhnawy, 2024).

Previous studies have revealed the effectiveness of *Bacillus subtilis* natto NTU-18 in feed to improve growth performance, increase immune-related gene expression, and increase the diversity of gut microbiota of *A. japonica* (Lin *et al.,* 2024). The metagenomic technology approach has also successfully documented the effectiveness of probiotic supplementation in Pacific white shrimp (*Litopenaeus vannamei*) with three probiotic candidates that can inhibit the growth of *V. parahaemolyticus* Vp5 (Sutanti *et al.,* 2024). Likewise, the addition of *L. plantarum* MH079448 strains in Japanese eel feed had been shown to improve production performance, immune-related gene expression in the gills and liver, and increase the abundance of gut microbiota that are predicted to play a role in energy metabolism, nutrient absorption, and secondary metabolite production (Guo *et al.,* 2025). Based on this, the purpose of this study was to determine the effect of maggot flour and probiotic supplementation on production performance, water quality, and microbiota abundance of elver phase eel (*A. bicolor*). The results of this study will contribute significantly to a better understanding of maggot flour and probiotic supplementation in feed on increasing the role of intestinal microbiota and water quality to support the production performance of elver phase eel. In addition, it can provide valuable information for utilizing maggot flour and probiotics in eel cultivation.

MATERIALS AND METHODS

Ethical approval

The implementation of this research has obtained a permit stipulated by the Center for Limnology and Water Resources Research, National Research and Innovation Agency (BRIN) with Number: B-30326/III.4.4/KP/13.00//2022.

Study period and location

This research was conducted from January to March 2022 at the Aquatic Laboratory, Limonology and Water Resources Research Center, National Research and Innovation Agency (BRIN) Cibinong, Bogor, West Java. The maggot flour used was collected from Maggot farmers in Bogor City. Elver phase eel seeds were obtained from PT. Laju Banyu, Ciampea, Bogor Regency. Proximate analysis of eel feed was carried out at the PAU (Inter-University Center) Laboratory, IPB University, Bogor.

Diet preparation

The probiotic used was commercial Effective Microorganism 4 (EM4) with a product volume of 1 liter. Probiotics contained a consortium of microbes such as *Bacillus* spp. and *Lactobacillus* spp. The maggot flour used had a protein content of 45%, fat 10.12%, crude fiber 8.23%, ash 5%, NFE 10%, and water content 7.31%. Commercial feed in the form of eel feed which was generally used by eel farmers obtained from PT. Japfa.

In the first four weeks, eel seeds were given commercial flour feed with feed code PIS-2 with the criteria for eel seeds weighing 1.5 ± 0.13 g/individual or equivalent to have a protein content of 50-52%, fat of 6%, crude fiber of 2%, ash content of 14% and water content of 10%. Feeding for biomass trial purposes was taken for ten days according to feed requirements with a weight of 5% of the weight of eel biomass.

Management of eel fry

The experimental design used in this study was a Completely Randomized Design (CRD). The eel seed maintenance containers used were 15 aquariums measuring 60×40×40 cm^3 equipped with a filter system. The aquariums were arranged in tiered parallel and siphoning was carried out periodically to maintain water quality. Eel seeds aged three months with an average weight of 3 g and an average length of 14 cm were used as model animals in this study. A total of 20 acclimatized eel seeds were then taken randomly and transferred to each aquarium. Feeding was carried out programmatically twice a day that was adjusted to the treatment group. Experimental feeding was carried out twice a day as much as 5% of the eel biomass. The eel maintenance period was carried out for 60 days. The feed treatment groups used in this study were as follows:

- P1: 15 mL probiotics + 0% maggot flour + 100% commercial feed
- P2: 15 mL probiotics + 25% maggot flour + 75% commercial feed
- P3: 15 mL probiotics + 50% maggot flour + 50% commercial feed
- P4: 15 mL probiotics + 75% maggot flour + 25% commercial feed
- P5: 15 mL probiotics + 100% maggot flour + 0% commercial feed

Proximate analysis of eel feed

Proximate analysis used the AOAC analysis standard for animal feed in this study that was carried out on feed pellets containing probiotics, maggot flour, and commercial feed (Ahn *et al.*, 2014). These feed ingredients were developed by mixing according to the proportion of the treatment group with the purpose to increase the proportion of crude protein needed by eels while reducing excessive fiber content converted by EM4 probiotics.

Observation of eel growth

The length and weight of eels can be determined by sampling every 14 days during the 60-day trial period. In addition to the length and weight parameters, other growth parameters measured include the eel specific growth rate, feed efficiency level (average daily gain (ADG/day), condition factor (FK), and feed conversion ratio (FCR), feed digestibility, and survival rate.

SGR measurements on eels were carried out at each phase starting from stage 1 to stage 4. The equation used to measure the specific growth rate (SGR) of eels is as follows (Muchlisin *et al.*, 2020):

$$SGR = \left(\frac{\text{Ln Wt} - \text{Ln W0}}{t}\right) \times 100$$

Note:

SGR = Specific growth rate (%/day)
W0 = Average initial weight of maintenance (g)
Wt = Average final weight of maintenance (g)
t = Maintenance time (days)

The equation used to measure the average daily weight gain (ADG) of eels is as follows (Muchlisin *et al.,* 2020):

$$ADG = \frac{Wt - W0}{2 \times T}$$

Note:
ADG = Average daily weight gain (g/day)
Wt = Final maintenance weight (g)
W0 = Initial maintenance weight (g)
T = Maintenance time (days)

The equation used to measure the feed conversion ratio (FCR) of eels is as follows (Muchlisin *et al.,* 2020):

$$FCR = \frac{F}{(Wt + D) - W0}$$

Note:
FCR = Feed conversion ratio (kg)
F = Amount of feed given (g)
W0 = Total biomass at the beginning of maintenance (g)
Wt = Total biomass at the end of maintenance (g)
D = Total weight of dead fish (g)

The equation used to measure the condition factor (CF) of eels is as follows (Indrayani *et al.,* 2023):

$$FK = W / (L^3)$$

Note:
FK = Condition factor (g/cm)
W = Fish weight (g/fish)
L = Fish length (cm/fish)

Eel feces were collected daily on the tenth day after administration of chromium oxide (Cr_2O_3), which was used as a feed digestibility. Briefly, 10 g of dry samples were dried and weighed for proximate testing and Cr_2O_3 content in the feces using the following equation (Siddik *et al.,* 2024):

$$DA = 100 - \left\{ 100 \times \frac{IP}{IF} \times \frac{NP}{NF} \right\}$$

Note:
DA = Feed Digestibility (%)
IP = Percentage of Indicator in Feed
IF = Percentage of Indicator in Feces
NP = Percentage of Nutrient in Food
NF = Percentage of Nutrient in Feces

The equation used to measure the survival rate (SR) of eels is as follows (Yang *et al.,* 2025):

$$SR = \frac{\text{Final number of fish}}{\text{Initial number of fish stocked}} \times 100$$

Bacterial abundance analysis

The abundance of bacteria in maintaining water and intestines of eels was analyzed using the counting plate method with sample dilutions of 10^{-3} and 10^{-4} and sampling at the end of the maintenance period. Analysis of bacterial abundance was carried out with the following equation (Kamaruddin *et al.,* 2021):

$$\text{Bacterial abundance} = \frac{\text{inoculation volume} \times}{\sum \text{colony} \times \sum \text{dilution}}$$

Water quality measurement

Measurement of water quality parameters was carried out once a week in each treatment group. Water quality test parameters and tools used in this study include: pH was measured using a pH meter, dissolved oxygen (DO) was calculated using a DO meter with units of mg/L, temperature was measured using a thermometer with a Celsius scale (°C), ammonia was measured using an Ammonium test kit (Merck, UK) with units of mg/L, and alkalinity was measured using sulfuric acid equipped with a digital titrator with units of mg/L.

Data analysis

This study used a completely randomized design with three repetitions of the experiment for growth parameters and five repetitions for water quality parameters. The data obtained were tabulated in Microsoft Excel (Microsoft, USA) and then analyzed statistically for variance using Statistical Package for the Social Sciences software version 23.0 (IBM Corp., NY, USA). One Way ANOVA analysis was used to determine the mean variation of each treatment group and Duncan's post hoc test was performed to determine significant differences between treatment groups ($P \leq 0.05$). The data that had been analyzed statistically were then displayed in the form of tables, graphs, and images.

RESULTS AND DISCUSSION

Result

Proximate content analysis in eel fry feed

The results of the proximate analysis of the eel fry feed treatment groups were shown in Table 1. The highest protein content was shown in the P5 treatment (15 ml probiotics + 100% maggot flour + 0% commercial feed) which was 43.93%. The protein content in the P5 treatment was not significantly different from the P2 group (15 ml probiotics + 25% maggot flour + 75% commercial feed) which was 43.92%. The lowest protein content was shown by the P3 treatment (15 ml probiotics + 50% maggot flour + 50% commercial feed) which was 42.37%. However, the fat and crude fiber content produced by the P5 group tended to be high, namely 10.80% and 9.95%, respectively, when compared to other feed groups in this study. The addition of 15 ml probiotics and 100% maggot flour had been shown to increase protein, fat, and crude fiber levels in the eel fry feed model.

Eel fry growth performance

The survival rate of eel fry in this study was 100% in all treatment groups. However, there were differences in the results of other growth performance parameters. Treatments P3 and P2 with the addition of 50% and 25% maggot flour showed a total growth of 0.926 ± 0.253 g/day and 0.924 ± 0.313 g/day, significantly different (P≤0.05) when compared to group P1 (control) with 100% using commercial eel feed, which was 1.222 ± 0.662 g/day a significant difference was also shown by treatment P5 (100% maggot flour) of 0.223 ± 0.932 g/day (Table 2). The highest ADG value observed on the 60th day was shown in group P2, which was 2.50 ± 1.43 g/day, significantly higher when compared to group P5, which was 0.26 ± 0.28 g/day (Table 3). The ADG value in group P5 experienced a significant decrease (P≤0.05) when compared to the previous observation (day 45), which was 0.42 ± 0.45 g/day. These results indicate that providing feed made from maggot flour up to 100% was unable to increase the ADG value in eel without the addition of commercial feed.

The SGR value of elver phase eels given a variety of feeds containing probiotics and maggots showed varying results. Group P2 had a significantly higher SGR value (1.80 ± 0.84%/day) when compared to group P5 (0.41 ± 0.45%/day) on the 60th day of observation. Providing feed based on 100% maggot flour and EM4 probiotics has not been able to maximize the growth of eels in this study. At least, the addition of maggot feed must be combined with 75% commercial feed and probiotics to produce optimal SGR values (Table 4). The condition factor (CF) value of eel can be seen from the increase in length and weight of the fish after being given a variety of maggot-based feed for 60 days (Table 5).

Table 1. Proximate feed in each treatment group.

No	Sample	Water Content %	Ash %	Fat %	Protein %	Crude fiber %
1	P1	7.89	12.20	5.25	44.11	0.00
2	P2	7.46	12.29	7.47	43.83	1.83
3	P3	7.84	12.38	8.37	42.37	2.81
4	P4	6.90	13.42	9.48	43.13	6.60
5	P5	6.07	12.66	10.80	43.92	9.95

Note: P1= 15 ml probiotics + 0% maggot flour + 100% commercial feed, P2 = 15 ml probiotics + 25% maggot flour + 75% commercial feed, P3 = 15 ml probiotics + 50% maggot flour + 50% commercial feed, P4 = 15 ml probiotics + 75% maggot flour + 25% commercial feed, P5 = 15 ml probiotics + 100% maggot flour + 0% commercial feed.

Table 2. Eel specific growth rate (g/day) every two weeks and completed 60 days of research.

Treatment	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Overall**
P1	0.238 ± 0.086[a]	1.152 ± 0.602[c]	2.070 ± 1.163[a]	2.653 ± 1.558[c]	1.222 ± 0.662[e]
P2	0.300 ± 0.359[a]	0.592 ± 0.477[ab]	2.144 ± 0.931[a]	1.582 ± 0.349[ab]	0.924 ± 0.313[cd]
P3	0.521 ± 0.373[a]	1.007±0.432[c]	1.598 ± 1.048[a]	1.505 ± 0.671[ab]	0.926 ± 0.253[cd]
P4	0.066 ± 0.027[a]	0.190±0.168[a]	0.712 ± 0.650[a]	0.818 ± 0.456[a]	0.357 ± 0.094[ab]
P5	0.094 ± 0.056[a]	0.146±0.049[a]	0.558 ± 0.524[a]	0.316 ± 0.218[a]	0.223 ± 0.932[a]

Note: P1 = 15 ml probiotics + 0% maggot flour + 100% commercial feed, P2 = 15 ml probiotics + 25% maggot flour + 75% commercial feed, P3 = 15 ml probiotics + 50% maggot flour + 50% commercial feed, P4 = 15 ml probiotics + 75% maggot flour + 25% commercial feed, P5 = 15 ml probiotics + 100% maggot flour + 0% commercial feed. Letter notation in the same column showed no significant difference between treatments (P≥0.05) on the growth of eels.

A significant increase was shown by group P2 which during the 60-day maintenance period experienced an increase, namely day 14 (9.32 ± 0.82 g/cm), day 30 (10.12 ± 1.21 g/cm), day 45 (12.16 ± 1.52 g/cm), and day 60 (14.86 ± 2.62 g/cm). However, there was no significant increase in other groups, especially in group P5 in this study. The best FCR value was shown in group P2, which was 1.53 ± 0.38 kg on the 60th day of maintenance. This value was not significantly different when compared to groups P1, P3, and P4, but was significantly different from group P5. The FCR value in group P5 tended to be high from the beginning of the maintenance period and increased until the 60th day. These values were consecutively in group P5 on day 14 (6.45 ± 1.15 kg), day 30 (5.39 ± 1.92 kg), day 45 (6.66 ± 0.56 kg), and day 60 (7.20 ± 2.19 kg) (Table 6).

Table 3. Average daily gain (ADG) with appropriate feed variations.

Treatment	Days of culture -			
	14	30	45	60
P1	0.22 ± 0.24^{a}	0.53 ± 0.38^{a}	2.22 ± 1.27^{a}	0.79 ± 0.79^{ab}
P2	0.75 ± 0.77^{a}	0.74 ± 0.48^{a}	2.12 ± 0.36^{a}	2.50 ± 1.43^{a}
P3	0.33 ± 0.39^{a}	0.83 ± 0.46^{a}	2.62 ± 0.38^{a}	1.67 ± 1.28^{ab}
P4	0.24 ± 0.23^{a}	0.39 ± 0.43^{a}	1.27 ± 1.10^{b}	1.61 ± 0.51^{ab}
P5	0.13 ± 0.16^{a}	0.11 ± 0.04^{a}	0.42 ± 0.45^{b}	0.26 ± 0.28^{b}

Note: P1 = 15 ml probiotics + 0% maggot flour + 100% commercial feed, P2 = 15 ml probiotics + 25% maggot flour + 75% commercial feed, P3 = 15 ml probiotics + 50% maggot flour + 50% commercial feed, P4 = 15 ml probiotics + 75% maggot flour + 25% commercial feed, P5 = 15 ml probiotics + 100% maggot flour + 0% commercial feed. Letter notation in the same column showed no significant difference between treatments (P≥0.05) on the growth of eels.

Table 4. The specific growth rate (SGR) with appropriate feed variations.

Treatment	Days of culture -			
	14	30	45	60
P1	0.47 ± 0.59^{a}	0.81 ± 0.40^{ab}	2.72 ± 1.22^{a}	0.68 ± 0.47^{ab}
P2	1.10 ± 1.08^{a}	0.88 ± 0.47^{ab}	2.14 ± 0.08^{ab}	1.80 ± 0.84^{a}
P3	0.58 ± 0.71^{a}	1.25 ± 0.65^{b}	2.95 ± 0.21^{a}	1.30 ± 0.96^{ab}
P4	0.36 ± 0.38^{a}	0.52 ± 0.57^{ab}	1.49 ± 1.25^{ab}	1.53 ± 0.31^{ab}
P5	0.24 ± 0.28^{a}	0.21 ± 0.06^{b}	0.67 ± 0.66^{b}	0.41 ± 0.45^{b}

Note: P1 = 15 ml probiotics + 0% maggot flour + 100% commercial feed, P2 = 15 ml probiotics + 25% maggot flour + 75% commercial feed, P3 = 15 ml probiotics + 50% maggot flour + 50% commercial feed, P4 = 15 ml probiotics + 75% maggot flour + 25% commercial feed, P5 = 15 ml probiotics + 100% maggot flour + 0% commercial feed. Letter notation in the same column showed no significant difference between treatments (P≥0.05) on the growth of eels.

Table 5. The condition factor (CF) (g/cm) with appropriate feed variations.

Treatment	Days of culture -			
	14	30	45	60
P1	7.57 ± 1.23^{ab}	8.23 ± 1.56^{ab}	10.66 ± 2.25^{a}	11.47 ± 3.34^{ab}
P2	9.32 ± 0.82^{c}	10.12 ± 1.21^{b}	12.16 ± 1.52^{a}	14.86 ± 2.62^{b}
P3	8.08 ± 0.23^{abc}	8.91 ± 0.62^{ab}	11.93 ± 0.93^{a}	13.58 ± 2.11^{b}
P4	8.92 ± 0.18^{bc}	9.26 ± 0.83^{b}	10.26 ± 1.18^{ab}	12.23 ± 1.59^{ab}
P5	7.10 ± 0.88^{a}	7.07 ± 0.79^{a}	7.61 ± 1.44^{b}	7.86 ± 1.48^{b}

Note: P1 = 15 ml probiotics + 0% maggot flour + 100% commercial feed, P2 = 15 ml probiotics + 25% maggot flour + 75% commercial feed, P3 = 15 ml probiotics + 50% maggot flour + 50% commercial feed, P4 = 15 ml probiotics + 75% maggot flour + 25% commercial feed, P5 = 15 ml probiotics + 100% maggot flour + 0% commercial feed. Letter notation in the same column showed no significant difference between treatments (P≥0.05) on the growth of eels.

These results indicate that the higher FCR value in group P5 triggered lower fish growth, increased the amount of wasted feed, and triggered a decrease in water quality due to the accumulation of residual feed.

The highest feed protein digestibility in the P5 treatment was 75.90% with digestible protein of 15.15% of the total feed protein of 43.92%, and the FCR in the P5 treatment was very high but the daily growth of eel fish was small, this was due to the possibility that the nutritional balance in maggot meal was not right, such as amino acids and enzymes for eel fish. Growth data shows that the greater the use of magot flour, the smaller the percentage of growth in eel fish. The protein digestibility of the P1 treatment was 62.41% with the digested protein reaching 20.90% or higher when compared to the other groups. The feces protein in the P1 group was the lowest at 23.21%, so a lot of protein was digested in the body of the eel fish resulting in the highest growth in each treatment. These results show that giving maggot flour and probiotics to eel seed feed can increase protein digestibility with a low percentage of digested protein and slow growth so that maggot flour cannot be used as an alternative feed for eel feed without a combination of other feeds (Table 7).

Water Quality

The pH parameters were not significantly different in each pH treatment 7.45-7.66, DO was not significantly different ranging from 4.17-4.68 mg/L, temperature ranging from 29.58-30.97°C that there was a significant difference in treatments P2 and P4. Ammonia ranging from (0.032-0.085 mg/L) in treatment P5 or significantly different from other treatments and for alkalinity ranging from (60.902-82.240) mg/L which was significantly different for each treatment group (Table 8).

Abundance of bacteria

The abundance of bacteria in the water for maintaining eel seeds was found to be 10 types, including *Bacillus* sp., *Enterococcus* sp., *Escherichia coli*., *Nitrosomonas* sp., *Nitrobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Aeromonas* sp., and *Streptococcus* sp. The highest abundance was found in treatments P2 and P3 that were dominated by *Lactobacillus* sp. and *Bacillus* sp. Several types of bacteria that

Table 6. Feed conversion ratio (FCR) (kg) with appropriate feed variations.

Treatment	Days of culture -			
	14	30	45	60
P1	2.93 ± 1.30[a]	1.76 ± 1.21[a]	1.76 ± 1.21[a]	2.96 ± 0.56[b]
P2	1.94 ± 0.27[a]	1.86 ± 0.08[a]	1.86 ± 0.08[a]	1.53 ± 0.38[b]
P3	3.34 ± 0.45[a]	1.27 ± 0.11[a]	1.27 ± 0.11[a]	2.13 ± 0.13[b]
P4	3.22 ± 0.20[a]	2.78 ± 1.36[a]	2.78 ± 1.36[a]	2.80 ± 0.67[b]
P5	6.45 ± 1.15[a]	5.39 ± 1.92[b]	6.66 ± 0.56[b]	7.20 ± 2.19[a]

Note: P1 = 15 ml probiotics + 0% maggot flour + 100% commercial feed, P2 = 15 ml probiotics + 25% maggot flour + 75% commercial feed, P3 = 15 ml probiotics + 50% maggot flour + 50% commercial feed, P4 = 15 ml probiotics + 75% maggot flour + 25% commercial feed, P5 = 15 ml probiotics + 100% maggot flour + 0% commercial feed. Letter notation in the same column showed no significant difference between treatments ($P \geq 0.05$) on the growth of eels.

Table 7. Digestibility of eel fry feed in this study.

Treatment Group	Cr_2O_3 of Feed (%)	Cr_2O_3 of Feces (%)	Feed Protein (%)	Fecal Protein (%)	Digested Protein (%)	Protein Digestibility (%)
P1	0.5	1.79	44.11	23.21	20.90	62.41
P2	0.5	2.27	47.83	26.79	21.04	65.82
P3	0.5	2.50	45.37	24.7	20.67	63.26
P4	0.5	2.57	43.13	28.53	14.60	69.24
P5	0.5	3.17	43.92	28.77	15.15	75.90

Note: P1 = 15 ml probiotics + 0% maggot flour + 100% commercial feed, P2 = 15 ml probiotics + 25% maggot flour + 75% commercial feed, P3 = 15 ml probiotics + 50% maggot flour + 50% commercial feed, P4 = 15 ml probiotics + 75% maggot flour + 25% commercial feed, P5 = 15 ml probiotics + 100% maggot flour + 0% commercial feed.

were considered to have pathogenic properties such as *Enterococcus* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Aeromonas* sp., and *Streptococcus* sp., were also found in each treatment group with lower abundance (Figure 1). The abundance of bacteria in the intestines was found to be five types of bacteria including *Bacillus* sp., *Nitrosomonas* sp., *Lactobacillus* sp., *Nitrobacter* sp., and *Staphylococcus* sp with varying abundance values in each treatment. The abundance of *Bacillus* sp. in the intestines of eel group P5 showed the highest number compared to other groups that was 197,62×10^5 CFU/100gr. Likewise, the abundance of *Lactobacillus* sp. in group P5 was 88,97×10^5 CFU/100 gr. The presence of an abundance of pathogenic bacteria in the intestines of eel was also found in this study, but had a lower abundance in all treatment groups (Figure 2).

Discussion

Fish is an important food source for human health. Eels contain high protein, fat, and essential micronutrients. Moreover, eels are a good source of protein with lower calorie density than land animals because of high content of omega-3 long-chain polyunsaturated fatty acids (PUFAs) (Tacon & Metian, 2013). In order to continuously increase and maintain the protein content in fish meat, the addition of supplements to their feed is the right choice. The nutritional value of farmed fish varies greatly because it is related to the life cycle of the fish, temperature, salt content, and the proximate content of the feed given. The protein content of maggot flour and probiotic feed may increase the protein content of eel feed.

Proximate content is a measure of the percentage of body weight of each major biochemical component that may form the body mass of fish, namely water, protein, lipid, carbohydrate, and ash content. The proximate of Chinook Salmon (*Oncoryhnchus tshawytscha*) feed supplemented with fish meal (BioVita) and alternative diets has been shown to increase the content of essential amino acids in feed such as

Table 8. Results of water quality measurements for each treatment.

Treatment	pH	DO (mg/L)	Temperature (°C)	Ammonia (mg/L)	Alkalinity (mg/L)
P1	7.45 ± 0.14^a	4.26 ± 0.51^a	30.97 ± 0.39^a	0.045 ± 0.02^a	73.43 ± 1.75^b
P2	7.50 ± 0.14^a	4.34 ± 0.28^a	30.50 ± 0.19^b	0.032 ± 0.01^a	82.24 ± 2.46^c
P3	7.66 ± 0.19^a	4.27 ± 0.40^a	29.58 ± 0.32^c	0.057 ± 0.01^a	75.77 ± 3.79^{bc}
P4	7.62 ± 0.11^a	4.17 ± 0.37^a	30.34 ± 0.33^b	0.085 ± 0.01^b	60.90 ± 4.92^a
P5	7.48 ± 0.80^a	4.68 ± 0.18^a	30.47 ± 0.30^b	0.038 ± 0.01^a	69.69 ± 8.95^b

Note: P1 = 15 ml probiotics + 0% maggot flour + 100% commercial feed, P2 = 15 ml probiotics + 25% maggot flour + 75% commercial feed, P3 = 15 ml probiotics + 50% maggot flour + 50% commercial feed; P4: 15 ml probiotics + 75% maggot flour + 25% commercial feed, P5 = 15 ml probiotics + 100% maggot flour + 0% commercial feed. Letter notation in the same column indicates had no significant difference between treatments (P≥0.05) on the growth of eel fry.

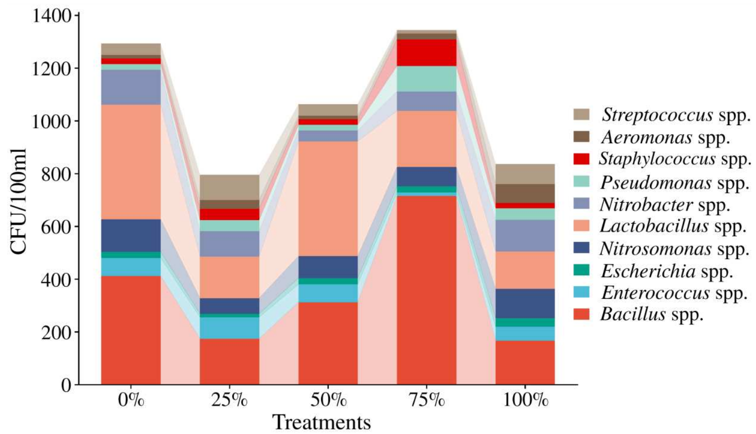

Figure 1. Abundance of bacteria in the water of eel fry maintenance in each feed treatment group. P1: 15 ml probiotics + 0% maggot flour + 100% commercial feed; P2: 15 ml probiotics + 25% maggot flour + 75% commercial feed; P3: 15 ml probiotics + 50% maggot flour + 50% commercial feed; P4: 15 ml probiotics + 75% maggot flour + 25% commercial feed; P5: 15 ml probiotics + 100% maggot flour + 0% commercial feed.

phenylalanine, leucine, and isoleucine (Rogers *et al.,* 2019). Supplementation of *Bacillus subtillis* and *Lactobacillus casei* at a dose of 15% in catfish (*Clarias gariepinus*) feed had been shown to increase protein content by 12.7%, fat by 5.3%, crude fiber by 5.02%, and BETN by 52.74% (Aini *et al.,* 2024). The increase in proximate feed, especially on protein content, it can be caused by the performance of probiotics and maggot flour added to the feed.

Probiotics are single cultures or combinations of microbial communities that in sufficient quantities can help improve the nutrition and development of fish. The probiotic used in this study was EM4 that had been marketed and sold as a supplement to maintenance water and feed additives because of its unique properties and health benefits (El-Saadony *et al.,* 2021; Yilmaz *et al.,* 2022; Monier *et al.,* 2023). In addition to probiotic, the addition of maggot flour that has an impact on increasing proximate levels. Previous research using maggot flour given with palm kernel cake substrate was able to increase feed protein by 15.98%, carbohydrates by 30.76%, fat by 10.35%, and ash content by 3.69% (Syahrizal *et al.,* 2022). The formulation of the tilapia feed ratio with fish meal: maggot flour (0:100) was able to increase proximate levels by 38.81% protein, 12.19% fat, 3.49% crude fiber, 11.51% ash content, 5.97% water content, and 35.52% carbohydrates (Prajayati *et al.,* 2020).

The growth rate of eel seeds given a combination of probiotic and maggot flour formulations was still not optimal when compared to commercial feed. This may be due to the high levels of crude fiber contained in the probiotic and maggot flour feed in this study. Feed fiber has been associated with production performance (Kamarudin *et al.,* 2018; Zhong *et al.,* 2020), nutrient digestibility (Nafees *et al.,* 2023; Nguyen *et al.,* 2024), intestinal histomorphology (Adorian *et al.,* 2016; Tran-Ngoc *et al.,* 2019), and the immune system of farmed animals (Mo *et al.,* 2015; Zhang *et al.,* 2024b). Feed products that are still rich in fiber must be supplemented with fat when formulated, so that they can increase feed digestibility by farmed animals. Previous studies had shown that animals given feed containing high crude fiber with lower growth performance compared to fiber-rich side feeds with added fat (Agyekum & Nyachoti, 2017). Maggots given by-product-based feed in the form of fiber may be the reason for the high fiber levels in this study and affect the digestibility of other nutrients by fish.

Similar results were also shown in production performance with weight parameters, condition factors, and length of eels given probiotic and maggot treatment diets, significantly lower when compared to commercial feed. However, P2 and P3 feed treatments affected the weight, CF, and length of eels higher than P5. We suspect that the feed formulation with a dose of 100% maggot flour without the addition of commercial feed to the feed was not optimal in increasing the production performance of eels. The addition of commercial feed to maggot flour + probiotics can complement the daily needs of eels. However, all eels given probiotic and maggot flour feed had a survival rate of 100%.

Previous studies reported that the addition of fermented maggots to commercial feed can significantly increase the survival rate and weight gain of *L. vannamei* (Junming *et al.,* 2012). The condition factor for eels with an elongated shape

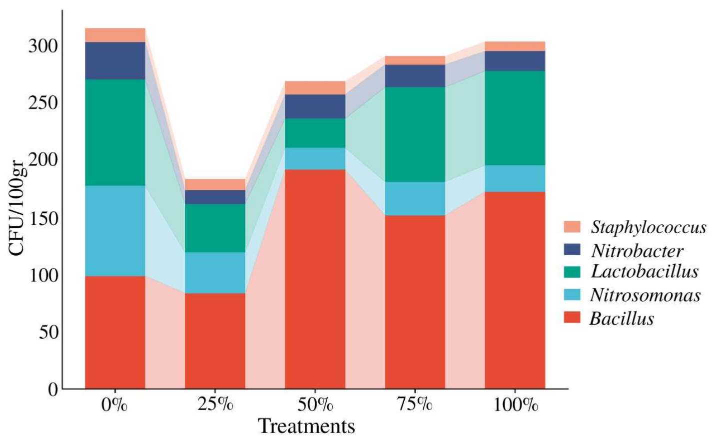

Figure 2. Abundance of bacteria in the intestines of eels in each feed treatment group. P1 = 15 ml probiotics + 0% maggot flour + 100% commercial feed, P2 = 15 ml probiotics + 25% maggot flour + 75% commercial feed, P3 = 15 ml probiotics + 50% maggot flour + 50% commercial feed, P4 = 15 ml probiotics + 75% maggot flour + 25% commercial feed; P5: 15 ml probiotics + 100% maggot flour + 0% commercial feed.

above 0.80 showed a normal growth category. It is in accordance with previous research which revealed that the condition factor (FK) value ranging from 0.83–1.31 g/cm showed a good growth category for eels (Datta *et al.,* 2013). The use of probiotics + 100% maggot flour as eel feed has not been effective in increasing protein digestibility, so that the percentage of digested protein is still low, at 15.15% with protein lost in feces of 43.92%. The low digestibility of feed in the P5 group can be caused by the amino acid and maggot oil content with the damaged due to the refining process. Likewise, the relatively high crude fiber in the P5 treatment can complicate the digestive activity of eels.

The digestibility of protein and amino acids in feed in the intestine is a very important parameter for evaluating the success of feed formulation in fisheries cultivation (Hamre *et al.,* 2013; Gilannejad *et al.,* 2019; Aragão *et al.,* 2022). Not optimal Protein digestibility can be caused by the composition of certain feed ingredients (for example, protein, fiber, antinutritional factors) that are generally not considered in feed formulation (Zhang *et al.,* 2024a). It is also supported by previous research that feed with low fiber content (55 g crude fiber/kg) can produce better fattening results (Dobos *et al.,* 2019). Furthermore, the addition of commercial feed should also be done in addition to maggot flour and probiotics because feed with a compound composition has been proven to be better in terms of protein digestibility, digestible protein, and lower percentage of protein in feces (Barragan-Fonseca *et al.,* 2017; Lee *et al.,* 2023).

The water quality of eel maintenance in this study had almost the same range between treatment groups. Previous studies had explained that the water quality that can support the growth of eel is temperature (29.8–31.730C), pH (7.4–8.1), dissolved oxygen (DO) (4.7–5.57 mg/L), nitrite (0.10–0.78 mg/L), and ammonia (0.0008–0.0281 mg/L) (Suryono & Badjoeri, 2013). The ammonia levels found during the eel maintenance period in this study tended to be high in all treatment groups, or ranged from 0.038–0.085 mg/L. Group P5 resulted the highest ammonia levels compared to other groups.

Ammonia content in ponds is one of the most important indicators of water quality to consider (Xu *et al.,* 2020). Ammonia (NH_3) is harmful nitrogen molecule to fish if found in high amounts in maintenance ponds. Ammonia levels in freshwater such as in this study can come from various sources, including uneaten feed residues and metabolic waste from fish (Xue *et al.,* 2021). Ammonia levels of 0.2 mg/L are considered the threshold for freshwater aquaculture (Cong *et al.,* 2017; Almomani *et al.,* 2020), including eels. The high levels of ammonia in this study may be caused by the high levels of feed residue from eels containing organic materials, including protein. Commercial probiotics added to the feed have not optimally maintained ammonia levels in the maintenance pond. Increased ammonia levels in maintenance water are positively correlated with decreased oxygen levels (Akinnawo, 2023).

Aquatic animal health is greatly influenced by the composition of the microbial community both in living creatures and in the aquatic environment (Rostagno, 2022). As aquatic organisms, fish will continuously come into contact with water, thus increasing their susceptibility to various pathogens, especially bacteria. The abundance of bacteria in eel maintenance water was dominated by *Bacillus* sp. and *Lactobacillus* sp., specifically in the P2 and P5 treatment groups. The abundance of bacteria in water regulates the gill and intestinal microbiota of fish that affects physiological activity and contributes to the health of its host (Egerton *et al.,* 2018). Previous studies explained that the bacterial community in catfish cultivation water was dominated by *Proteobacteria* and *Firmicutes* (Wijayanti *et al.,* 2021). The abundance of *Bacillus* spp. and *Lactobacillus* spp. in groups P2 and P5 can be caused by the application of probiotic bacteria, high organic content of maggot flour, and high levels of ammonia and low levels of dissolved oxygen (Wijayanti *et al.,* 2021).

The fish intestine is the main organ that plays a role in digesting food and absorbing nutrients. In addition, the intestine regulates electrolytes, endocrine function and the immune system of fish (Lynch & Pedersen, 2016). The microbiome in the intestine regulates these actions by triggering better fish growth and increasing fish immunity. Furthermore, most of the bacterial communities in the large intestine contribute to probiotics (Verschuere *et al.,* 2000). Previous studies had revealed that Firmicutes in the host intestine can provide enzymes for the breakdown, digestion, and absorption of feed nutrients (Colston & Jackson, 2016). The addition of feed containing a combination of synbiotics, *L. plantarum* L20 and *Sargassum polycystum* showed an abundance of beneficial bacteria in the intestine of Black tiger shrimp, *Penaeus monodon*, including

Lactobacillaceae and *Lactiplantibacillus* (Chin *et al.,* 2025). The addition of probiotics containing *Lactobacillus reuteri* of 10^{11} CFU/kg in tilapia (*Oreochromis niloticus*) feed increased the diversity of intestinal microbiota composition and improved the relationship between intestinal bacterial species (Li *et al.,* 2022).

CONCLUSION

The addition of probiotics and maggot flour is ineffective in improving production performance and water quality in this study. The low digestibility of protein and digestible protein and high fecal protein in the P5 treatment show that maggot flour given at a dose of 100% is still ineffective and needs to be combined with commercial eel feed. However, the addition of probiotics and maggot flour may increase the abundance of beneficial bacteria in the maintenance pond and the intestinal microbiota of eels dominated by *Lactobacillus sp.* and *Bacillus sp.* These results provide basic data for further research examining the effects of fermentation with probiotics on maggot flour, so it is expected to reduce crude fiber levels and increase the digestibility of feed protein by elver phase eels.

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