Performance of growth, nutrition value, total carotene, EPA, and DHA in eel (*Anguilla bicolor*) in the culture with enrichment of earthworm (*Lumbricus* sp.) flour

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Abstract. The slow growth in farmed eel is associated with low feed digestibility. The slow growth rate is determined mainly by the quality of the feed given. To increase the growth rate of eels, high animal protein feed is necessary because eels are carnivorous. Eel feed enrichment using earthworm (*Lumbricus rubellus*) flour is eco-friendly because it provides an alternative source of protein through cleaner technology. This earthworm flour is very potential as an eel feed enrichment because it contains essential fatty acids eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and contains high total carotene. This study aims to examine the effect of feed enrichment with the addition of earthworm flour and to determine the optimum dose in feed on growth performance, nutritional value, total carotene, EPA, and DHA of eel (*Anguilla bicolor*). This study used completely randomized design (CRD) consisted of four treatments and each treatment had quadruplicates. The treatment A was no earthworm flour is added in artificial feed. Meanwhile, for treatments B, C, and D were in the artificial feed was added with earthworm flour of 2.5, 5, and 7.5%, respectively. The density of eel used was 20 fish / container, with an average weight of 14.479±0.017 g, and the volume of the container was 40 L. The culture duration was 90 days. This study used commercial artificial feed and commercial earthworm meal. The feeding method used was a fixed feeding with a dose of 5% of the biomass at a frequency of 3 times a day. The results showed that feed enrichment with a different percentage of earthworm flour had a significant effect ($p < 0.05$) on growth performance, nutritional value, total carotene, EPA, and DHA, but there was no significant effect ($p > 0.05$) on survival rate of eel for 90 days of the culture. The addition of 5% earthworm flour in artificial feed gave the best result, where the optimum dose based on polynomial analysis was the addition of 5.52% earthworm flour.

Key Words: *Anguilla bicolor*, earthworm flour, feed enrichment, nutritional value.

Introduction. Eel (*Anguilla bicolor*) is a type of catadromous fish with a slow growth rate (Kerans & Retnoaji 2020). Growth of eel in one cycle of rearing from glass eel (0.09-0.12 g) to consumption size (250 g) takes nine months to two years, and some of them stop at size of 2-3 g (Nawir et al 2015). This relatively slow growth problem of eels is due to eels' low feed digestibility (Winata et al 2018). According to Kamil et al (2000), the slow growth rate factor is very much determined by the quality of the feed given, so that to push the growth of eels, high animal protein feed is needed because of its carnivorous nature.

Eel growth can be optimized by improving the feed quality, which is determined by the macro and micro nutrient content of the feed, including protein, fat, carbohydrates, vitamins, and minerals. According to Subekti et al (2011) and Nafsiyah et al (2018), protein, as one of the macronutrients that determine the quality of feed, has a significant role in the growth of eel because it is the largest body component of meat, which is around 65-75% of total dry weight, and has a function as a building block for body tissues. Apart from protein, the levels of essential fatty acids, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) affect the growth of eel seeds (Ahn et al 2015). Wijayanti & Setiyorini (2018) added that different total carotene content also provides diverse growth of eel. This shows that the nutritional content (protein, EPA, DHA, and total carotene) of the feed given will affect eels' growth.

Ashraf et al (2011) explained that the culture system provides high nutritional feed, in addition to the natural feed available in the pond. Eel culture requires nutrients that come from artificial feed. The feed given should be utilized properly by eels, so that it can grow optimally. One solution to accelerate the growth rate and nutritional quality of eel products is feed enrichment. The use of earthworms as an alternative protein source in feed is an opportunity to protect the environment through cleaner technology (Parolini et al 2020). Their research showed that the crude protein composition of earthworm meal (58-71%) was higher than fish meal (56-62%). This earthworm flour is also very potential as enrichment for eel feed because it contains essential fatty acids EPA and DHA and contains high total carotene (Ahn et al 2015). This is supported by the research of Chilmawati et al (2013), which showed that the addition of artificial feed combined with earthworms affected the growth, blood haemocyt and survival of African catfish (*Clarias gariepinus*). The purposes of this study were to examine the effect of enrichment of earthworm flour (*Lumbricus* sp.) on growth performance and nutritional content of eel (*A. bicolor*), and to determine the optimum dose of earthworm flour in eel feed which provides the highest growth and nutritional content.

Material and Method

General information. A total of 320 eels was used with a length of 20-25 cm and an average initial weight of 14.479±0.017 g fish^{-1}. The feed used was artificial feed with a protein dose of 40%. The feed was given in the form of a paste based on the study of Suminto et al (2011). This research used fresh water deposited for 1 to 2 days and which was aerated to supply oxygen to each container. This study used 16 aquaria in total as containers. The volume of each aquarium was 60 liters, which was filled with 40 liters of water. The density of each aquarium was one fish / 2L. The culture container was given a black net so the eels could adapt to the dark environment. The research was conducted from April to July 2020, with cultured period of 90 days, at the Siwarak Fish Seed Center; Service of Agriculture, Fisheries and Food; Semarang Regency, Nyatnyono Village, West Ungaran District; Semarang Regency.

The preparation of experimental feed. The preparation stage begins by preparing commercial eel feed with a protein content of 40%. Proximate analysis was conducted of the commercial earthworm flour used in this study before it was mixed with artificial feed. Total protein content was analyzed using the micro-Kjeldahl method, total fat content using the Soxhlet extraction method, and carbohydrate content using the difference method (AOAC 2005). Meanwhile, crude fiber content and moisture content were analyzed by referring to Indonesian National Standard (BSN 2009).

The artificial feed was then mixed with hot water, gradually, until it formed a paste and combined with earthworm flour with different doses according to the treatment. The process of preparing the feed was carried out every time a feed was given.

The feeding experiments. The eel was acclimatized for one week in a culture container to adapt to the environment and feed. The feeding experiment was carried out for three months. The feeding method used was a fixed feeding with a dose of 5% of the biomass. Growth measurements were done by weighing the biomass of eel once every ten days. Data collection on the amount of feed given, dead fish, and fish weight was carried out for data analysis at the end of the study. Growth analysis was performed by calculating the relative growth rate. The frequency of feeding was three times a day, i.e., morning (06.00 West Indonesia Time Zone or WITZ), afternoon (12.00 WITZ), and evening (18.00 WITZ).

A completely randomized design (CRD) with four treatments and each treatment being repeated four times was designed in this study. Treatment A was no earthworm flour was added in artificial feed. Meanwhile, treatments B, C, and D were artificial feed added with earthworm flour of 2.5%, 5%, and 7.5%, respectively.

Data collection and statistical analysis. The data collected included relative growth rate (RGR), feeding efficiency (FE), survival rate (SR), and nutritional content (proximate, total carotene, EPA, and DHA). Observation of water quality in the aquarium, i.e. water temperature, dissolved oxygen (DO), and pH were carried out every day.

Data were analyzed using the SPSS 16 software package with $p < 0.05$ (Ghozali 2006). Before further analyses, the data were analyzed for normality and homogeneity. The ANOVA was applied to understand each treatment's influence on the RGR, FE, and SR. A Least Significant Difference (LSD) test was applied to explain significant differences between median values in each treatment, while a polynomial orthogonal test was applied to determine the optimum growth. The water quality and nutrient content data were tabulated and analyzed descriptively.

The relative growth rate of eel. The relative growth rate was calculated using the Zonneveld et al (1997) formula:

$$RGR = \frac{Wt - W0}{W0 \times t} \times 100$$

where: RGR = relative growth rate (% day^{-1});
Wt = average weight of fish at the end of the study (g);
W0 = average weight of fish at the beginning of the study (g);
t = duration of the study (days).

Feeding efficiency. Feeding efficiency was calculated using the formula from Tacon (1987):

$$FE = \frac{Wt - W0}{F} \times 100$$

where: FE = feeding efficiency (%);
Wt = weight of fish biomass at the end of the study (g);
W0 = weight of fish biomass at the beginning of the study (g);
F = amount of fish feed given during the study (g).

Survival rate. Fish survival rate was calculated using a formula by Effendi (2003):

$$SR = \frac{Nt}{N0} \times 100$$

where: SR = survival rate (%);
N0 = number of fish at the beginning of the study (fish);
Nt = number of fish at the end of the study (fish).

Results. The study results included growth performance, survival rate and nutritional value of ell, and the water quality during the study. The protein content of earthworm flour used was relatively high, reaching 60.40% so it can accelerate eel growth. The protein levels of earthworm flour used in this study were comparable to those used in previous studies. The *L. rubellus* flour used in the research of Damayanti et al (2008) and Istiqomah et al (2009) contained 65.63 and 63.06% protein, respectively. The best growth performance, survival rate, and nutritional value were obtained in the addition of 5% earthworm flour in the artificial feed.

Proximate analysis of earthworm flour. The proximate analysis of earthworm flour was 60.40% protein; 3.03% crude fiber, 6.89% fat; 11.0% ash, and 10.76% extract material without nitrogen.

Relative growth rate. The ANOVA results showed that the enrichment of earthworm flour had a significant effect ($p < 0.05$) on the eel's average RGR. Figure 1 shows that

5% of enrichment feeding gave the highest RGR (1.751±0.03% d^{-1}) of eel compared to other treatments, while the lowest (0.920±0.06% d^{-1}) was treatment without enrichment (0%).

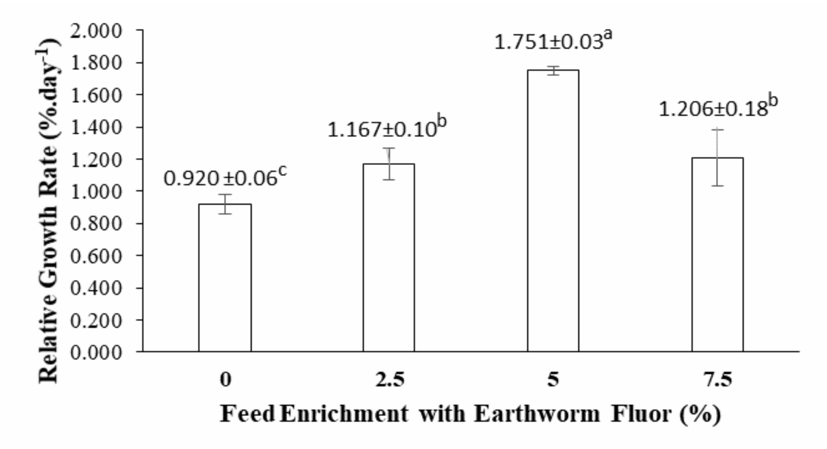

Figure 1. Relative growth rate of eel (*A. bicolor*) enriched with different percentages of earthworm flour.

Based on the orthogonal polynomial test in the RGR of eel at various percentages in the enrichment of earthworm flour feed (Figure 2), a cubic pattern relationship was obtained with the following equation:

$$y = -0.0156x^3 + 0.144x^2 - 0.1636x + 0.9201$$

with a value of $R^2 = 1$ and the optimum point for enriching earthworm flour at 5.52%.

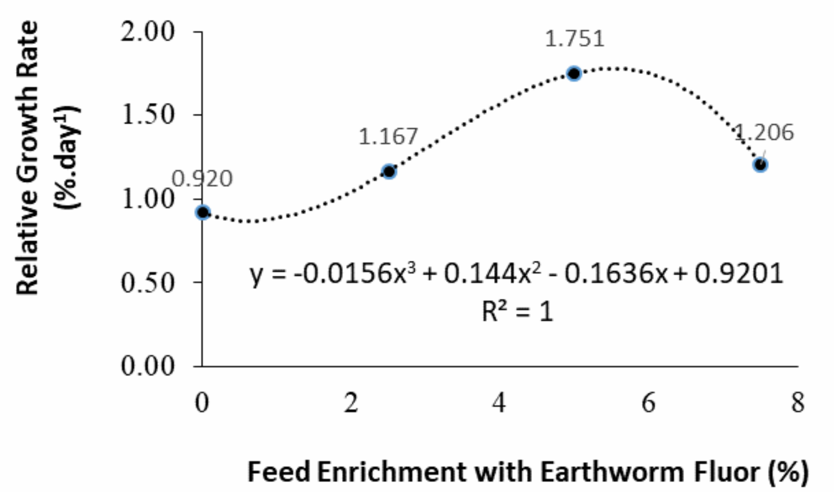

Figure 2. The relative growth rate response curve of eel (*A. bicolor*) on various percentages of earthworm flour enrichment in the feed.

Feeding efficiency. The ANOVA results showed that the enrichment of earthworm flour feed with different percentages had a significant effect ($p < 0.05$) on the average eel FE. Figure 3 shows that treatment C (addition of 5% earthworm flour) had the highest FE value (26.781±0.53%) and was significantly different from other treatments. On the other hand, treatment A (without earthworm flour) had the lowest FE value (12.150±0.98%).

Based on the orthogonal polynomial test in the efficiency of eel feed utilization at various percentages of added earthworm flour (Figure 4), a cubic pattern relationship was obtained with the following equation:

$$y = -0.3395x^3 + 3.2803x^2 - 4.9871x + 12.15$$

with a value of $R^2 = 1$ and the optimum point of enrichment for earthworm flour at 5.56%.

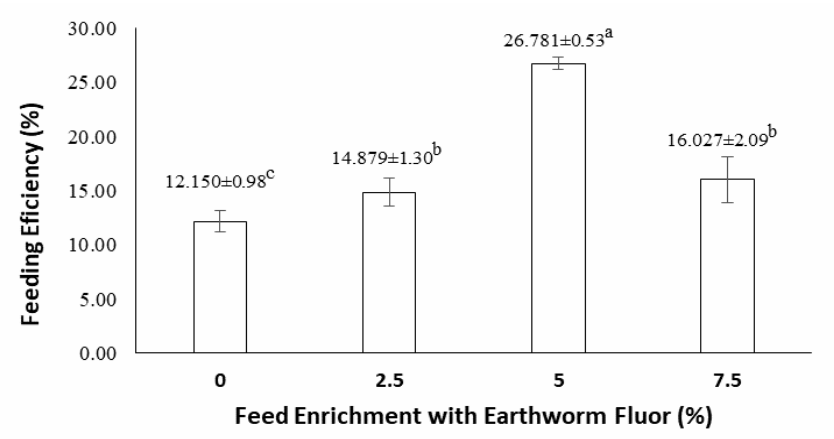

Figure 3. Feeding efficiency of eel (*A. bicolor*) enriched with different percentage of earthworm flour.

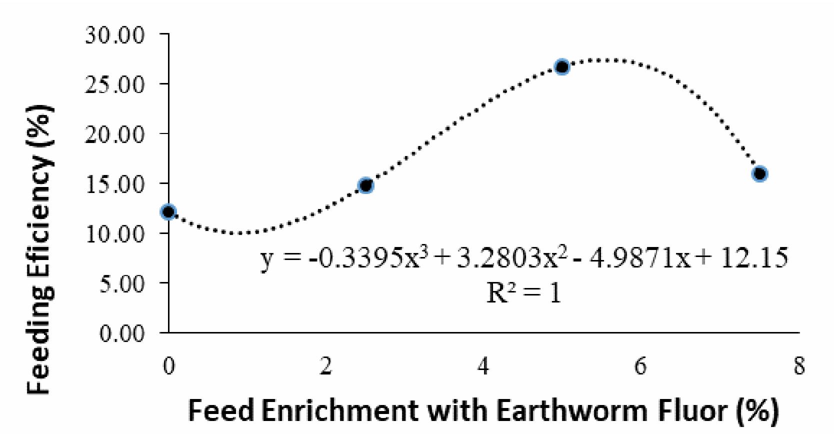

Figure 4. The feeding efficiency response curve of eel (*A. bicolor*) on various percentages of earthworm flour enrichment in the feed.

Survival rate of eel. The ANOVA results showed no significant effect ($p > 0.05$) on the average SR of eel. The SR of eel enriched with different percentages of earthworm flour was ranged between 98.75 and 100% (Figure 5).

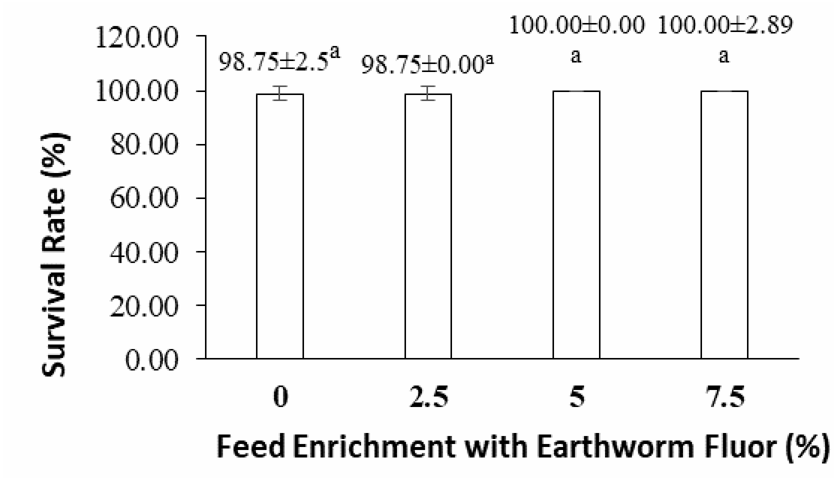

Figure 5. Survival rate of eel (*A. bicolor*) enriched with different percentages of earthworm flour.

Based on the orthogonal polynomial test in the survival rate of eel at various percentages of added earthworm flour (Figure 6), a cubic pattern relationship was obtained with the following equation:

$$y = -0.04x^3 + 0.4x^2 - 0.75x + 98.75$$

with a value of $R^2 = 1$ and the optimum point of enrichment for earthworm flour at 5.54%.

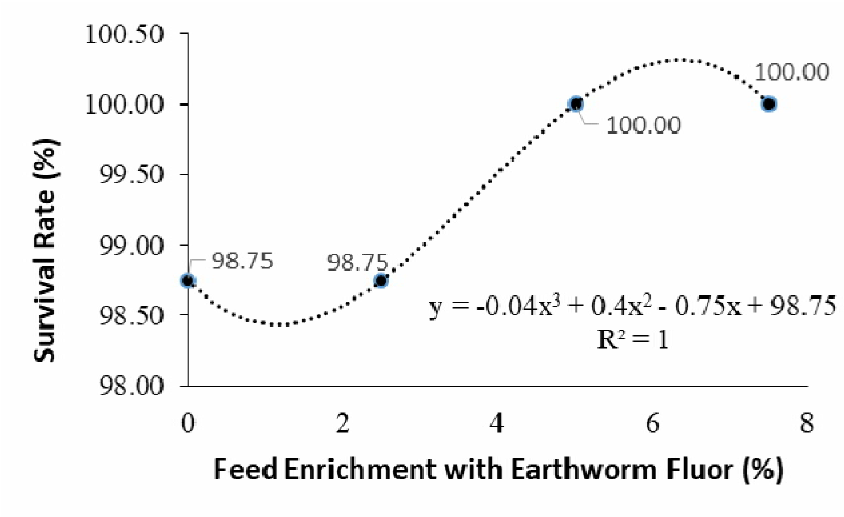

Figure 6. The survival rate response curve of eel (*A. bicolor*) on various percentages of earthworm flour enrichment in the feed.

Proximate analysis results and total carotene content. The proximate analysis of eel given enrichment of earthworm flour in feed with different percentages in each treatment can be seen in Table 1. It shows that the highest average protein and fat content was found in treatment C (feed enrichment with earthworm flour by 5%).

Table 1

Proximate analysis and total carotene content of eel (*A. bicolor*) enriched with different percentages of earthworm flour

Component	*A (0%)*	*B (2.5%)*	*C (5%)*	*D (7.5%)*
Water (%)	75.838±0.079	72.563±0.435	71.820±0.057	73.250±0.074
Ash (%)	1.082±0.019	1.124±0.002	1.153±0.024	1.179±0.021
Fat (%)	6.200±0.024	7.862±0.011	8.649±0.084	8.300±0.038
Protein (%)	16.106±0.034	16.591±0.034	17.026±0.035	16.985±0.011
Crude fiber (%)	0.159±0.010	0.231±0.027	0.282±0.001	0.151±0.008
Total carotene (mg/100 mg)	0.754±0.016	0.765±0.010	0.815±0.001	0.943±0.022

The highest total carotene content of eel was shown in the enrichment of earthworm flour with a 7.5% percentage of the feed given, i.e. 0.943±0.022 mg/100 g; followed by a 5% enrichment treatment (0.815±0.001 mg/100 g), then a 2.5% enrichment treatment (0.765±0.010 mg/100 g), and the lowest was 0% enrichment treatment (0.754±0.016 mg/100 g).

The EPA and DHA content. The EPA content of eel was ranged from 42.55 to 171.10 mg / 100 mg and DHA content were ranged from 226.35 to 1033.40 mg / 100 mg (Table 2). The highest EPA and DHA content were produced by eel which was fed with 5% enrichment of earthworm flour from the feed given.

Table 2

EPA and DHA content of eel (*A. bicolor*) at the end of the research (mg/100 mg)

Treatment	*EPA*	*DHA*
A	42.55	226.35
B	116.05	646.65
C	171.10	1033.40
D	141.15	731.45

Water quality of eel (A. bicolor) culture. The observation of water quality for eel culture during the study (Table 3) shows that the range of values was still suitable for eel life (Otwell & Rickards 1982; Herianti 2005; Samsudin & Nainggolan 2009).

Table 3

Water quality culture of eel *(A. bicolor)* during study

Treatment	*Range and mean value*		
	pH	*DO (mg L^{-1})*	*Temperature (°C)*
A	8.3-8.4 (8.35±0.07)	4.63-4.86 (4.25±2.28)	24.8-25.5 (25.15±0.49)
B	8.03-8.36 (8.20±0.23)	4.56-4.52 (4.04±0.51)	25.4-25.9 (2.56±0.36)
C	7.94-8.37 (8.16±0.30)	4.82-4.54 (4.18±0.51)	25.5-26.1 (25.8±0.42)
D	8.34-8.35 (8.35±0.01)	4.24-4.56 (3.40±0.23)	25.4-26.1 (25.7±0.49)

Discussion. The results showed that the highest RGR and FE values were achieved in treatment C (with 5% addition of earthworm flour), then followed by treatments D (7.5% addition of earthworm flour), B (2.5% addition of earthworm flour), and A (0% addition of earthworm flour). This is presumably because the addition of earthworm flour as much as 5% is the proper dosage and has better absorption of nutrients to increase the relative growth rate and efficiency of feed utilization in eel. The availability of feed, the ability to utilize feed, and the quality of water affect eels' growth (Diansyah et al 2014; Harianto et al 2014). Giving good quality feed will be very helpful in terms of growth and resistance to disease or parasites.

Furthermore, the results of the proximate analysis of eels (Table 1) also showed that treatment C (5%) had the highest protein content (17.026±0.035%), compared to treatments A (0%), B (2.5%) and D (7.5%) i.e. 16.106±0.034; 16.591±0.034; and 16.985±0.011%, respectively. This manner proves that good quality feed can stimulate fish growth. This opinion is in accordance with Tibbetts et al (2000), who reported that *A. rostrata* size 8 g achieved optimum growth when fed with energy content and protein energy ratios of 5.112 kcal kg^{-1} and 10.75%, respectively. Lovell (1989) stated that feed that contains too much energy could limit the amount of feed consumed so that the growth rate decreases. Furthermore, it was explained that growth is also influenced by the balance of nutrients present in the feed, where this balance will result in good growth. Conversely, food that is malnourished, unbalanced, or toxic can have a negative impact on fish life.

A high RGR value results in a high FE value. Feeding artificial feed with the addition of 5% earthworm flour to the eels gave a high FE value (26.781±0.53%). This is probably because the eels, in this condition, are able to absorb the nutritional content of the feed into the body so that the energy in the feed can be used efficiently. On the other hand, there was no difference in the average FE value between treatments B and D, i.e. 14.879±1.30 and 16.027±2.09%, respectively. This means that the addition of 2.5% and 7.5% earthworm flour gives the same FE value. Treatment A, where no earthworm flour was added, gave the lowest FE value (12.150±0.98%) compared to the other treatments. According to Fujaya (2004), the greater the value of FE, the more suitable the feed is given to support fish growth, and vice versa, the smaller the value of FE, meaning that the feed given is not effective in the process of nutrients absorption to become cells or fish body tissue, and eventually, fish growth slows down.

The high RGR value in treatment C refers to the results of the proximate analysis of feed, namely the amount of protein content of 60.40%, which is thought to be following the nutritional needs of the eel seeds themselves. Fadee's research (2012) showed that earthworms' content is 65% protein, 11% fat, 6% ash, and 19% nitrogen without extract. Earthworm protein content (72%) was higher than the fish meal protein (22.65%). In addition, worm flour is easier to digest and contains several amino acids higher than fish meal. Earthworms also contain beta carotene, ALE, i.e., linoleic fatty acids, linolenic fatty acids, EPA and DHA, and high omega-3 and omega-6. Earthworms can also be used as immunostimulants because they have active substances that are anti-bacterials (Julendra & Sofyan 2007).

The proximate results of eel showed that treatment C had a higher total protein content (17.03%) than treatments A, B, and D. Nafsiyah et al (2018) reported that *A. bicolor* has a protein content of 16.78%, 13.26% fat, 1.34% ash and 65.51% moisture content. This shows that giving earthworm flour affects the nutritional content of eel. The eel's body's protein content is influenced by the protein content in the feed given (Nawir et al 2015; Wijayanti & Setiyorini 2018). The composition of the ingredients following the needs of the fish will affect the resulting feed conversion value. The availability of the right protein in the feed will play an important role in eels' growth (Nawir et al 2015).

The results showed that the EPA content of eel, which was added with earthworm flour to the feed, was in the range of 116.05-171.10 mg/100 g; and the DHA content was in the range of 646.65-1,033.40 mg/100 g. This shows that the value of EPA and DHA content in eel with the addition of earthworm flour has increased. The results of research of Muhtiani et al (2020) regarding the EPA and DHA content in eels with different species, showed the EPA content in the range of 98.4-304 mg/100 g and DHA content of 144-445 mg/100 g. In addition, the research of Seo et al (2013) also showed that giving different eel feed formulas had a different effect on EPA and DHA content with a range of EPA content of 9.85-11.53% (equal to 458-634 mg/100 g) and DHA 15.73-20.86% (equal 731-1,147 mg/100 g). This shows that the addition of earthworm flour between 2.5 and 7.5% can increase the relatively high EPA and DHA content. Besides being rich in omega 3, eel is also rich in vitamin A (468 µg/100 g) and vitamin E (4.32 µg/100 g) (Suseno 2014).

According to Harianto et al (2020), the value of feed conversion becomes a benchmark for determining feed utilization level. The lower the feed conversion value, the higher the efficiency of feed used by eels, so that only a little protein is changed to meet energy needs and the rest is used for growth. Conversely, if the feed conversion value is high, the level of feed utilization efficiency is low. The feed conversion value on their study was 6.57.

The nutrient-rich feed is needed in fish farming in addition to natural productivity in the pond so that the high protein content of eel is due to high protein feed (Ashraf et al 2011; Wijayanti & Setiyorini 2018). Eel growth can be optimized through feed quality, including protein, fat, carbohydrates, vitamins, and minerals. Good quality feed will result in more efficient utilization of feed in the eel (Nawir et al 2015). Total carotene content in treatment C was the highest compared to other treatments. The total carotene content shows the antioxidants found in the combination of feed and the addition of earthworm flour as much as 5%.

The SR is not directly affected by feed. Eel mortality was thought to be due to stress during the study and an excess of uneaten feed that caused liver damage in eels. This is in accordance with Fekri et al (2014), who reported that excess feed causes the DO content to be lower and ammonia increases along with the amount of uneaten feed, causing death in eels. Biotic and abiotic factors influence the SR. Biotic factors consist of the fish's age and ability to adapt to the environment in which they live. Abiotic factors include food availability and quality of living media.

The water quality supports the SR in culture media, which is sufficient for the eel seeds' life, i.e., the water temperature of 25-26°C, water pH of 7.94-8.40, and DO of 4.24-4.86 mg L^{-1}. Those water quality values were in optimal range. The temperature is in line with the research of Otwell & Rickards (1982), which reported that eel appetite is good at temperatures of 24-28°C. The pH value is in the range with the research of

Samsudin & Nainggolan (2009), which reported that eels could live at pH of 4-11. The DO value in this study meets the criteria for eel maintenance, which is more than 3 mg L^{-1} (Herianti 2005).

Conclusions. The results showed that feed enrichment with a different percentage of earthworm flour had a significant effect on growth performance, nutritional value, total carotene, EPA, and DHA, but there was no significant effect on survival rate of eel for 42 days of the culture. The addition of 5% earthworm flour in artificial feed was the best treatment, where the optimum point was shown in the addition of 5.52% earthworm flour.

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